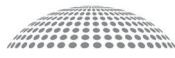


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Keynote Lectures



Keynote Lectures

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Gardner Lectureship Award

Accelerating genomics-based surveillance and biomedical innovation for disease-causing pathogens in Africa

Tulio de Oliveira, South Africa

Session 2
Global warming and the expanding frontiers of Arboviroses

Climate change and vector-borne diseases

Jan C. Semenza, Sweden

Climate change is an increasingly important driver of the emergence, transmission, and geographic expansion of vector-borne diseases. Climate-related health risks arise from the interplay of climatic hazards, exposure, and vulnerability, extending beyond human health to encompass animal and environmental health. Addressing these interconnected challenges therefore requires an integrated One Health–Climate risk approach. This is particularly relevant in Europe, where changing climatic conditions are creating increasingly favorable environments for the transmission and establishment of infectious diseases.

Gaps in climate change adaptation and preparedness can result in exposure and vulnerability to vector-borne diseases, creating significant health risks, if these gaps align in time and space. A One Health–Climate risk framework can help identify and address these emerging threats by integrating evidence across human, animal, and environmental domains.

This approach will be illustrated through selected examples of vector-borne diseases in Europe. Modelling transmission dynamics requires the integration of environmental and climatic monitoring with entomological and ornithological surveillance. Together, these sources can identify and delineate areas at heightened risk of transmission, supporting the targeted deployment of early warning systems, enhanced surveillance and vector control measures. Furthermore, projections under different climate change scenarios can identify regions where climatic conditions are likely to become increasingly suitable for vector-borne disease transmission. Anticipating these shifts provides an opportunity to strengthen preparedness before risks manifest themselves, enabling targeted interventions to reduce exposure and vulnerability to vector-borne pathogens and ultimately build more resilient health systems to climate change impacts.



Keynote Lectures

HEINE-MEDIN AWARD

Whole-Genome Sequencing and Clinical Metagenomics for Viral Infections in Immunocompromised Patients

Maud Salmona, France

Immunocompromised patients are at high risk of severe viral infections, prolonged viral replication, and infections caused by multiple or unexpected pathogens, making diagnosis and clinical management particularly challenging. The past decade has witnessed a paradigm shift in clinical virology, driven by the emergence of next-generation sequencing (NGS) technologies, which have expanded our ability to characterize viral pathogens beyond conventional molecular diagnostics.

Whole-genome sequencing (WGS) has substantially improved the characterization of clinically relevant viruses, particularly genetically diverse pathogens such as human adenoviruses, where frequent recombination limits conventional typing strategies. Complete genome analysis enables accurate genotyping, investigation of transmission chains, identification of viral determinants associated with pathogenicity or antiviral resistance, and characterization of intra-host viral diversity. Similar approaches have enhanced our understanding of BK polyomavirus and respiratory viruses, providing new insights into viral evolution, persistence, and genotype–phenotype relationships in transplant recipients.

Beyond pathogen-specific applications, NGS has enabled the implementation of clinical metagenomics as an unbiased diagnostic approach capable of detecting unexpected, emerging, or previously unrecognized viruses directly from clinical specimens. This strategy has expanded the diagnostic spectrum in complex infectious syndromes and contributed to the identification of rare human infections, including severe cases associated with pigeon avian paramyxovirus. In addition to pathogen detection, metagenomic sequencing provides new opportunities to explore the human virome and its role in health and disease.

Together, whole-genome sequencing and clinical metagenomics are reshaping the diagnosis and study of viral infections, improving outbreak investigation, surveillance, and precision management, particularly in immunocompromised patients.

Session 3

Viral respiratory infections: the ever-present challenge

RSV Across the Life Course: Clinical Burden and Real-World Performance of New Immunisation Tools

Harish Nair, United Kingdom

Respiratory syncytial virus (RSV) is increasingly recognised as a major cause of morbidity and mortality across the life course, extending well beyond its traditional characterization as a paediatric pathogen. This presentation will synthesise current evidence on RSV disease burden in infants and young children, adults with underlying comorbidities, and older adults, highlighting the substantial and often underappreciated impact in ageing populations and those with chronic cardiopulmonary and immunocompromising conditions.

In infants, RSV remains a leading cause of lower respiratory tract infection and hospitalisation globally, with disproportionate burden in the first months of life. In adults, particularly those with multimorbidity, RSV contributes to exacerbations of chronic disease and healthcare utilisation. Among older adults, RSV-associated hospitalisation and mortality rates are often substantially under-estimated. The adjusted / modelled rates approach those observed for influenza in some settings, underscoring its public health significance. Novel data from multi-specimen / vaccine probe studies support these findings.

The second part of the presentation will focus on the real-world performance of recently introduced RSV immunisation strategies, including long-acting monoclonal antibodies for infant protection and vaccines for older adults. Drawing on emerging data from early-adopting countries, we will examine effectiveness against severe outcomes, duration of protection, and programmatic considerations such as coverage, equity, and integration into existing immunisation platforms. Current evidence suggests substantial reductions in RSV-associated hospitalisations in infants receiving monoclonal prophylaxis, alongside promising effectiveness of vaccines in preventing severe disease in older adults.

Finally, the talk will consider implications for policy and future research, including optimisation of target groups, co-administration strategies, and the role of surveillance systems in evaluating long-term impact. Together, these data signal a transformative shift in RSV prevention, with important opportunities to reduce disease burden across all age groups.



Keynote Lectures

Session 4

Infants at risk: congenital, neonatal and pediatric viral infections

Congenital CMV Screening: Maternal, Universal Neonatal and Targeted Approaches

Ann Vossen, Netherlands

Congenital cytomegalovirus (cCMV) infection is a major cause of neonatal morbidity and long-term neurodevelopmental disorders, yet there is no consensus regarding optimal screening strategies. This presentation discusses recent developments in both maternal and neonatal CMV screening.

The first part briefly focuses on universal screening of pregnant women for primary CMV infection and the implementation of such programmes in several European countries. The potential benefits and limitations of maternal screening will be briefly addressed.

The second part covers neonatal screening for congenital CMV infection, including recent experiences with universal newborn screening programmes in the United States and Canada. Preliminary data on the yield of neonatal CMV screening will be presented and discussed in the context of targeted screening shortly after birth, such as that conducted within the NHS, and targeted screening based on clinical findings, such as fetal or neonatal growth restriction.

In addition, preliminary data on CMV screening practices in the Netherlands in recent years, including the number of infants identified, will be presented.

The presentation concludes with a discussion of the advantages and disadvantages of universal maternal screening, universal neonatal screening, and targeted CMV screening, as well as key questions to consider when developing future CMV screening policies.

Session 5

Viral infections in special populations and vulnerable hosts

Recent advances in the management of cytomegalovirus refractory and resistant patients

Sophie Alain, France

The recent consensus guidelines specifically addressed refractory and resistant CMV infections management, in both the stem cell and the solid organ transplant setting. They were reviewing the therapeutic possibilities in view of the availability of maribavir, for treatment of resistant or refractory CMV and of letermovir as primary or secondary prophylaxis. Since their publication, new algorithms were available and the need for quality genotyping results and harmonisation of practices was underlined. In addition, the place for immune testing and also for adjuvant therapeutics such as immunoglobulins or everolimus was discussed.

Since then, several surveys of maribavir efficacy and resistance in real life, and post hoc analyses from the phase III study have been completed, showing less resistance than expected, confirming in real life studies some risk factors such as a high viral load, albeit showing some benefits in terms of clearance from long-term maribavir treatment. Letermovir resistance was also surveyed, also in children. In parallel new approaches to better personalize the use of these new antivirals were developed on the basis of pharmacokinetic modelization.

After a first update on the genotyping methods, the recommendations for use, the available databases, and the genotyping interpretation problems, in link with the frequency of resistances, and the working groups in progress, we will review the results of real-life use of new antivirals, and the potential use of new markers such as drug dosage or immune testing to better manage refractory or resistant patients. Thanks to the Horus EU H2021 project, predictors for CMV non response or escape to prophylaxis should be clarified, and will help us to better manage these difficult-to-treat patients.



Keynote Lectures

Innovation Clinical Virology Award

New Horizons for Virus Diagnostics

Elisabeth Puchhammer-Stöckl, Austria

Expert virus diagnostics is the key to detect and judge reliably virus infections and is mostly based on direct virus detection and quantification via PCR methods, as well as on the identification of specific antiviral antibody profiles. However, actually we realize that virus diagnostic methods may be used for a much wider medical spectrum than just the identification of an acute or chronic virus infection.

So, it has been shown within the last few years that the detection and quantification of distinct non-pathogenic, commensal viruses reflects the level of immunosuppression in specific patient groups, and may be useful to optimize and guide immunosuppressive therapy schemes. As well, recently the detection of antiviral antibodies, directed against defined virus structures, but also cross-reactive to specific structures in the human organism has gained high importance. Newly developed antibody tests detecting and quantifying specifically such cross-reactive antibodies just emerge as promising tools identifying autoimmune processes and predicting a variety of human autoimmune diseases.

On the other hand, emerging data provide evidence that the severity of some virus infections may be better predicted, when the analysis of distinct human genomic variations of the patients is included in the diagnostics. Especially the testing for specific genetic variants affecting the antiviral immune response may be a new tool and enrichment of the virus diagnostic portfolio. Thus, virus diagnostics is currently on the way to new horizons and will become a significant enrichment for overall personalized medicine.

Session 6

Viral infections of the nervous and gastrointestinal system

Mapping the Mutational and Phenotypic Landscapes of cVDPV2 in Sewage

Rosa M Pintó, Spain

Since the launch of the Global Polio Eradication Initiative (GPEI), the incidence of poliomyelitis has declined dramatically. Despite this progress, the emergence and international spread of circulating vaccine-derived polioviruses type 2 (cVDPV2) continue to pose a significant threat to polio-free regions. On 16 September 2024, cVDPV2 was detected in wastewater from the metropolitan area of Barcelona, Spain, followed by genetically related detections in several European countries. In this study, we performed an in-depth characterization of the complete VP1 coding region mutant swarm present in Barcelona sewage using haplotype-resolved nanopore sequencing coupled with structural modelling to assess the impact of VP1 diversity on receptor binding, pocket factor interaction, and antibody recognition.

The cVDPV2 population comprised a single monophyletic cluster dominated by one haplotype, together with numerous low-frequency variants, including ancestral-like haplotypes closely related to the Nigerian NIE-ZAS-1 lineage. VP1 diversity was unevenly distributed across the protein, with the greatest variability concentrated in antigenic site 1, the multiple monoclonal antibody (mAb) binding region, and residues involved in receptor interaction.

To investigate the potential phenotypic consequences of VP1 variation, we applied Rosetta flex-ddG structural modelling to predict changes in binding affinity to the poliovirus receptor CD155, a representative pocket factor, and the neutralizing mAbs 9H2 and 10D2. While most haplotypes retained receptor and pocket factor binding, reduced antibody binding was commonly predicted, particularly for 10D2. Comparisons with Nigerian outbreak isolates and immunodeficiency-related VDPV2 (iVDPV2) sequences revealed distinct evolutionary trajectories, with iVDPV2 sequences exhibiting the broadest predicted antibody escape profiles.

Several low-frequency wastewater haplotypes displayed potentially advantageous phenotypes. Our findings demonstrate the utility of wastewater surveillance for the early detection of cryptic poliovirus variants.



Keynote Lectures

Session 7 Advances in HIV and viral hepatitis

HBV matters: Practical ART decision-making in HIV-HBV coinfection

Oana Săndulescu, Romania

The HIV antiretroviral therapy (ART) landscape is rapidly evolving, with increasing use of two-drug regimens, long-acting therapies, and tenofovir-sparing strategies. For people with HIV-HBV coinfection, however, these advances introduce an important additional consideration: optimizing HIV therapy must not come at the expense of effective HBV treatment. As ART becomes increasingly individualized, considering the patient's HBV status and maintaining adequate HBV-active therapy are essential components of safe regimen selection and switch.

This makes awareness and systematic HBV screening increasingly important. HBV status should be assessed for all patients through a triple panel that includes HBsAg, anti-HBc and anti-HBs, at the moment of HIV diagnosis, in order to inform ART regimen choice. It should also be reassessed on a regular basis, ideally yearly, and particularly before considering switching to a tenofovir-sparing regimen, which would lack adequate HBV activity. Screening identifies active infection, previous exposure and susceptibility to HBV, informing treatment, monitoring and vaccination decisions and reducing the risk that HBV is overlooked during ART optimization.

For people living with HIV and HBV, ART should include agents with activity against both viruses in the nucleos(t)ide backbone, especially tenofovir either as disoproxil fumarate (TDF) or as alafenamide fumarate (TAF). HIV regimens that do not provide adequate HBV activity, including several two-drug regimens and long-acting strategies, should not be used alone in HIV-HBV coinfection. When tenofovir cannot be used as part of the ART regimen, HBV therapy must be maintained separately, with entecavir representing an important alternative when given alongside fully suppressive ART.

Regimen switching requires particular caution and informed rescreening for HBV. Withdrawal of HBV-active therapy in patients with HBV coinfection can lead to HBV DNA rebound and potentially severe hepatitis flares. In patients with previous HBV exposure but no current HBsAg positivity, the risk of reactivation is lower but remains clinically relevant when HBV-active ART is withdrawn, making risk assessment and post-switch monitoring important.

The expanding range of HIV treatment options highlights the importance of considering HBV status in ART decision-making. Simplification considerations and long-acting therapy options can offer substantial benefits, but only when HBV prevention and treatment remain integrated into the decision. In HIV-HBV coinfection, every ART switch is potentially an HBV treatment switch: HBV matters.

Session 8 Host–virus interplay: insights into pathogenesis, immunity and cancer

Roles of Epstein-Barr virus in human diseases

Paul Farrell, United Kingdom

Most people become infected by Epstein-Barr virus (EBV) in early childhood and then carry the virus for life without symptoms or disease. However, infection of primary human B cells in cell culture results in permanently proliferating lymphoblastoid cell lines and several types of cancer involve a contribution from EBV. The incidence of these cancers varies in different parts of the world, to some extent consistent with sequence variation in the EBV (identified by large scale sequencing) and other factors that contribute to the cancer.

Recently the role of EBV in some auto-immune diseases, including Multiple Sclerosis (MS) and Systemic Lupus Erythematosus (SLE) has become better understood and it is now clear that primary infection delayed until teenage years or later is a major factor in Multiple Sclerosis. The mechanisms underlying all these diseases have focussed interest on EBV vaccines that might prevent infection. The value of this approach for prevention of cancers and auto-immune diseases and the merits/risks of various types of EBV vaccine will be considered.



Keynote Lectures

Session 9

The viral combat: antivirals, antibodies, and vaccines

Human intestinal and liver organoids: a window into enteric virus biology & drug discovery

Joana Rocha-Pereira, Belgium

Enteric viruses remain a major cause of global morbidity and mortality. Gastroenteritis viruses such as human norovirus (HuNoV) and rotavirus (HRV) are responsible for a substantial burden of acute diarrheal disease worldwide, while enterically transmitted hepatitis viruses, including hepatitis E virus (HEV), represent an important cause of acute viral hepatitis. Despite their significant clinical impact, therapeutic options remain limited, in part due to challenges in developing physiologically relevant models that support viral replication and antiviral evaluation.

Human organoids have emerged as powerful platforms that recapitulate key features of the intestinal and hepatic epithelium while maintaining translational relevance for drug discovery. This presentation will highlight how the PORTO lab has leveraged intestinal and liver organoids to overcome longstanding barriers in enteric virus research and establish advanced platforms for antiviral development. Building on pioneering work demonstrating the first in vitro cultivation of HuNoV in human intestinal organoids, the laboratory developed and optimized a three-dimensional infection model supporting enhanced viral replication. This platform has enabled antiviral testing and mechanistic studies across a range of enteric viruses in both intestinal and hepatic tissues. Using these systems, the antiviral activity of repurposed drugs and novel compound classes has been evaluated against HuNoV and HRV.

More recently, human intestinal organoids were used to demonstrate productive HEV genotype 3 infection of the gut epithelium, revealing direct viral targeting of proliferative cells and highlighting the importance of epithelial turnover in sustaining infection. The efficacy of approved antivirals against HEV-3 and rat HEV has subsequently been compared in intestinal and liver organoids. Finally, ongoing studies integrating gut-on-chip and liver-on-chip technologies aim to evaluate antiviral responses in increasingly physiologically relevant tissue environments.

European Diagnostic Virology Award

Immune response against infection and vaccination in fragile patients

Irene Cassaniti, Italy

Immunosuppression is a critical condition that can increase the risk of viral infection and delay or reduce immune response against vaccination. Specific clinical conditions, including transplantation, are associated to increased risk of herpesviruses reactivation, including Human Cytomegalovirus (HCMV), Varicella-Zoster (VZV). Even if the standardization of molecular assays for HCMV DNA quantification has significantly improved its management in transplant recipients, the role of virus-specific T-cell response and the introduction of assays for monitoring of immune response has not reached yet. In our research experience virus-specific T-cell response have been optimized and assessed for evaluating T-cell competence and ability to control viral replication. In our recent research, pp65-based assay for T-cell response quantification has been assessed for predicting hematopoietic stem cell transplant recipients at higher risk to develop HCMV severe reactivation after Letermovir prophylaxis, allowing a personalized management of HCMV in these patients. Looking at vaccination preventable diseases, VZV-specific T-cell response wanes with age and immunosuppression, leading to an increased risk of Herpes Zoster events. Recently, the introduction of a gE-based recombinant vaccine (RZV) has improved the quality of life of elderly and immunosuppressed patients. However, better knowledge in terms of long-term immunogenicity in high-risk patients should be obtained. Our research group evaluated immunogenicity and the safety of RZV specifically in the patients with cancer undergoing immunotherapy, demonstrating that strong immune response is elicited by vaccination in these patients, both in terms of humoral and T-cell response. A reduction of T-cell and humoral response, measured as total antibodies against VZV and antibody-dependent cellular cytotoxicity (ADCC) has been observed at 12 months after vaccination.



Keynote Lectures

Session 10

Genomic Strategies in Viral Diagnostics: Innovations and Challenges

Implementing Viral Metagenomic Sequencing in Clinical Diagnostics: Validation and Experience

Michael Huber, Switzerland

Unexplained infections remain a major challenge in clinical diagnostics, with many cases of central nervous system disease, respiratory infection, and gastroenteritis unresolved after conventional testing. This presentation describes the implementation of viral metagenomic sequencing as an unbiased diagnostic approach at the Institute of Medical Virology, University of Zurich, and discusses its validation, clinical experience, and readiness for routine use.

The presentation covers the full diagnostic workflow, from sample preparation for RNA and DNA viruses to Illumina MiSeq sequencing, internal controls, and analysis with the in-house VirMet bioinformatics pipeline. Clinical case studies illustrate the diagnostic value of metagenomics, including the identification of Toscana virus in cerebrospinal fluid from a traveler with CNS infection and the detection of a novel human circovirus in a liver biopsy from an immunocompromised patient. Data from routine clinical cases, outpatient respiratory infections, and suspected CNS infections further demonstrate the capacity of this approach to detect a broad spectrum of known, rare, and unexpected viral pathogens.

Key challenges for routine diagnostics are also addressed, including lower sensitivity compared with PCR, turnaround time, database contamination, reporting criteria, reimbursement, and the optimal positioning of metagenomics alongside established diagnostic methods. The presentation concludes that viral metagenomic sequencing is now a reliable, validated, and accredited test for selected clinical scenarios, with future potential as part of a comprehensive genomic pathogen detection covering viruses, bacteria, genotyping, and resistance markers.

Session 11

Viral diagnostics: innovations and challenges

Sustainable laboratory Practices: Practical Strategies, Challenges, Innovations and Future Directions

Padraig Williams, Ireland

Clinical and diagnostic laboratories are highly resource-intensive environments, with significant demands for energy, ventilation, refrigeration, water and single-use consumables. Reducing the environmental impact of laboratory operations while maintaining quality, patient safety and regulatory compliance is an increasing priority for healthcare organisations.

The Irish Blood Transfusion Service (IBTS) has established sustainability as a strategic priority, with initiatives focused on reducing energy consumption, emissions and waste while improving resource efficiency. The IBTS National Donor Screening Laboratory has been My Green Lab (MGL) certified since 2021, with MGL certification expanded to other IBTS laboratories in 2023/2024. This provides a structured framework for embedding sustainable practices and driving continuous improvement within the laboratory environment.

Key opportunities for sustainable laboratory practice include the optimisation of energy-intensive equipment and cold storage, sustainable procurement, digital transformation, lean laboratory processes, water conservation, and reduction of plastic and hazardous waste. Practical initiatives include optimising ultra-low-temperature freezers by increasing storage temperatures where appropriate (e.g., from -80°C to -70°C), improved maintenance, defrosting and inventory management; the introduction of energy-efficient laboratory equipment and a traffic-light system to identify opportunities for energy reduction, including automated standby and sleep modes; replacement of inefficient lighting with LED and smart management systems; and the use of sustainability criteria when selecting equipment, consumables and services. These initiatives demonstrate how changes to laboratory processes and infrastructure can deliver measurable environmental benefits.

Waste reduction initiatives include right-sizing consumable orders, reducing packaging, supplier take-back schemes and the introduction of reusable hazardous waste containers. Sustainable transport initiatives, including the transition of the IBTS vehicle fleet to hydrotreated vegetable oil (HVO), further contribute to emissions reduction.

Sustainable innovation extends beyond the adoption of new technologies and encompasses process redesign, behavioural change, collaboration and more efficient ways of working. Integrating sustainability into laboratory operations and decision-making can deliver environmental benefits alongside improved efficiency, cost-effectiveness and organisational resilience. Ultimately, sustainable laboratory practice requires a balance between environmental, economic and social outcomes while maintaining the high standards expected of modern clinical laboratories.



Oral Presentations



Oral Presentations

Wednesday | 16th September

Session 1

Viral challenges of our century: evolution, emergence, and re-emergence

OP1

Post-COVID-19 measles reemergence in France despite high vaccine coverage: insights from molecular surveillance, elimination challenges and vaccine failure investigations

Dr. Julia Dina¹; Miss Louise Houelle¹; Dr. Justine Adnet¹;

¹University Hospital of Caen

Aim: Following an unprecedented decline in measles transmission during the COVID-19 pandemic, Europe is experiencing renewed outbreaks threatening WHO elimination targets. In France, measles re-emerged despite sustained vaccine coverage, raising concerns about immunity gaps, population mobility and vaccine failure. We aimed to characterize post-pandemic measles resurgence in France using epidemiological, molecular and surveillance data generated by the French National Reference Centre (NRC) from 2023 to 2025.

Material and Methods: Samples from suspected measles cases referred nationwide were analysed by RT-PCR and serology. Molecular surveillance included N-450 genotyping and whole genome sequencing (WGS). Targeted investigations were conducted in clusters involving vaccinated individuals, including measles IgG avidity testing and molecular characterization.

Results: NRC activity increased markedly after the pandemic, from 516 analysed samples in 2023 to >1,000 in 2024 and 1,217 in 2025. Measles transmission resumed in late 2023, initially involving clusters among adolescents vaccinated with two MMR doses. In 2024–2025, 399 RT-PCR-confirmed cases were identified, with co-circulation of genotypes B3 and D8, indicating repeated introductions and active transmission. Adults aged 21–45 years emerged as an unexpectedly vulnerable group. WGS supported investigation of transmission dynamics, cluster linkage and mass gathering-associated spread. Combined molecular and serological approaches, including IgG avidity testing, helped distinguish recent infection from post-vaccination immune responses.

Conclusions: Post-pandemic measles resurgence in France highlights the fragility of elimination status, even in highly vaccinated settings. Integrated molecular surveillance, advanced sequencing and targeted investigation of vaccine failure remain essential to rapidly identify transmission chains, detect immunity gaps and support measles elimination in Europe.

OP2

Genomic Insights into the Origin and Transmission Dynamics of a cVDPV3 Outbreak in Israel

Dr. Neta Zuckerman¹; Dr. Merav Weil¹; Dr. Itay Bar-Or¹;

¹Central Virology Laboratory, Ministry of Health

Circulating vaccine-derived polioviruses (cVDPVs) remain a major challenge to global polio eradication efforts, with cVDPV3 outbreaks being exceptionally rare. Here, we describe a cVDPV3 outbreak detected in Israel during 2021–2022 through the country's integrated environmental surveillance and acute flaccid paralysis surveillance systems. Genomic analyses linked the environmental and clinical detections to a single outbreak, which involved widespread environmental circulation across multiple regions and included a paralytic case in an unvaccinated child. Whole-genome sequencing and genomic epidemiology analyses were applied to investigate the outbreak's emergence and transmission dynamics using both clinical and wastewater-derived poliovirus isolates.

Recombination analysis identified a consistent Sabin 3/Sabin 1 recombinant structure across outbreak-associated isolates. Phylogenetic inference identified close genetic links between environmental and clinical isolates, helping resolve previously unrecognized transmission chains. Mutation analysis revealed substantial divergence from the Sabin 3 vaccine strain already at the time of first wastewater detection, suggesting prolonged viral replication prior to identification. Furthermore, the observed pattern of synonymous and non-synonymous mutations supported a possible origin from an immunodeficient individual (iVDPV). Our findings highlight the value of high-resolution whole-genome sequencing for resolving poliovirus transmission dynamics and investigating outbreak origins and their application on clinical and environmental surveillance samples for the early detection of poliovirus outbreaks.



Oral Presentations

Wednesday | 16th September

OP3

IVIG-Derived Antibody Profiles Reflect Regional Differences in Arbovirus Circulation

Dr. Tatjana Schwarz¹; Marie Luisa Schmidt¹; Fiona Lawrenz¹; Hazal Kalnins-Köse²; Dr. Florian Zirkel³; Dr. Victor Max Corman⁴;

¹Institute of Virology, Charité Berlin, Germany; ²Institute of Virology, Charité Berlin, Germany. Biotest GmbH & Co. KGaA, Germany; ³Biotest GmbH & Co. KGaA, Germany; ⁴Institute of Virology, Charité Berlin, Germany. Labor Berlin GmbH, Germany;

Introduction/Aim: Intravenous immunoglobulins (IVIG) are plasma-derived products reflecting population-level exposure to circulating pathogens.

Arboviruses exhibit distinct epidemiological patterns in the United States (US) and Europe (EU). West Nile virus (WNV) is endemic in the US and emerging in EU. Chikungunya virus (CHIKV) occurs mainly as travel-associated infection in both regions. Tick-borne encephalitis virus (TBEV) is endemic in parts of EU but absent in the US.

To assess population-level exposure, antibody responses to WNV, CHIKV, and TBEV were analyzed in three IVIG products.

Methods: Antibody responses to TBEV, WNV, and CHIKV were quantified in 71 IVIG batches by ELISA. Products P1 (n=26) and P2 (n=15) were derived from EU plasma (different manufacturing processes), while P3 (n=30) used US plasma (same process as P1).

Results: Comparison of anti-WNV IgG levels showed a significantly higher response in P3(US) (median 8.3 OD ratio) than in P1(EU) (6.9) and P2(EU) (4.6, $p<0.0001$), with a significant difference also observed between the EU products ($p<0.0001$).

Similarly, anti-CHIKV IgG levels were higher in P3(US) (median 85.1 RU/mL) compared to P1(EU) (18.2, $p<0.0001$) and P2(EU) (10.8, $p<0.0001$), with no difference between P1 and P2.

In contrast, anti-TBEV IgG levels were significantly lower in P3(US) (median 597 U/mL) than in P1(EU) (3182, $p<0.0001$) and P2(EU) (3207, $p=0.0052$).

Conclusions: Anti-TBEV and anti-WNV IgG patterns align with their known epidemiology. In contrast, the increased anti-CHIKV IgG levels in US IVIG are unexpected, given the minimal documented local transmission. This may indicate underestimated CHIKV exposure through travel or undetected local infections, or potentially reflect higher vaccine-induced immunity. These findings support evaluating IVIG as a potential intervention for these viruses.

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Introduction/Aim: On 2 May 2026, the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC) were notified of a cluster of severe respiratory illness of unknown aetiology among passengers on board the Dutch-flagged expedition cruise ship M/V Hondius. The ship departed Argentina on 1 April, made several stops, and arrived in the Netherlands on 18 May. Testing of a patient hospitalised in South Africa ultimately confirmed hantavirus on 3 May as the causative agent, further specified as member of the Orthohantavirus andesense complex (ANDV) on 5 May. The outbreak resulted in 13 cases, including three deaths. We summarise the public health and laboratory response in the Netherlands.

Material and Methods: ECDC/WHO classified all people on board the M/V Hondius as well as risk contacts from air travel, healthcare, and laboratory settings precautionarily as high-risk contacts. They were advised to quarantine for six weeks after their last potential ANDV exposure with active monitoring with weekly qPCR and serology testing. Low-risk contacts were passively monitored and offered laboratory testing if developing symptoms consistent with early ANDV infection.

Results: On May 6, two symptomatic individuals were evacuated to the Netherlands and hospitalised. In total, 92 persons were quarantined in the Netherlands. Mandatory notification of ANDV was immediately raised to group A2 of notifiable diseases, enabling real-time monitoring, notification of suspected cases, and medical diagnostics. One high-risk contact tested positive for ANDV during weekly monitoring and was hospitalised before symptoms developed. The quarantine period has ended for contacts on 18 June, except for one completing quarantine on 2 July. All cases had been on board the M/V Hondius.

Conclusions: The value and necessity of national preparedness protocols for expedition and cruise ships and points of entry were highlighted by the public health actions during evacuee management in the Netherlands. Quarantining and testing were important measures for outbreak containment, allowing early recognition of cases with subsequent hospital admission ensuring best chance of survival.

OP4

Andes virus outbreak linked to the Dutch-flagged expedition cruise ship M/V Hondius; an overview of the public health response in the Netherlands

Dr. Olivia Luise Gern¹; Oda Everdina van den Berg²; Dr. Bettie Voordouw³; Dr. Luca M. Zaack⁴; Dr. Annemiek van der Eijk⁵; Dr. Corine GeurtsvanKessel⁶; Dr. Chantal Reusken⁶; Dr. Helma Ruijs⁷; Dr. Margreet J.M. te Wierik⁸; Dr. Annelies Brilman⁴;



Oral Presentations

Wednesday | 16th September

Session 2

Global Warming and the expanding frontiers of arboviruses

OP5

Increased incidence of human West Nile and Usutu Infections in Austria, 2024

David Florian¹; Jeremy V. Camp¹; Christof Jungbauer²; Dirk Werber³; Andreas Reich³; Karin Stiasny¹; Stephan W. Aberle¹; Judith H. Aberle¹;

¹Center for Virology, Medical University of Vienna, Austria; ²Austrian Red Cross, Blood Service for Vienna, Lower Austria and Burgenland, Austria; ³Austrian Agency for Health and Food Safety, Austria;

Introduction: West Nile virus (WNV) and Usutu virus (USUV) outbreaks have been documented across Europe, posing a growing public health concern. In this study, we examined the incidence of human WNV and USUV over 15 years (2009-2024) in Austria to detect changes in epidemiological trends over time.

Methods: We analysed all laboratory-confirmed WNV and USUV cases recorded by the national surveillance system, including data from the blood donation screening. We assessed virus sequences and examined changes in regional infection dynamics in affected areas.

Results: From 2009 to 2024, a total 109 WNV infections were diagnosed. Case numbers peaked in 2024 (WNV, n=34; USUV, n=22), exceeding the total number of annual cases reported between 2009-2023, also including the highest number of severe neurologic WNV patients. We observed a marked increase of local WNV and USUV infections in a previously low-incidence region in eastern Austria. Simultaneously, the Southeast European subclade of WNV lineage 2 was detected in Austria for the first time.

To investigate whether additional WNV infections may have remained undetected, a retrospective screening of biobanked patient samples, submitted by a neurology ward in the most severely affected area of eastern Austria in 2024 season was performed. We identified four (4.5%) WNV infections among 88 received samples, indicating that the true extent of infections in newly affected regions is likely underestimated.

Conclusion: Our data indicate that human infections with WNV and USUV in eastern Austria are underdiagnosed and that case numbers have been increasing over time, emphasizing the importance of surveillance to ensure blood donor safety. Retrospective detection of additional WNV cases in a highly affected region highlights the need for increased clinical vigilance in an outbreak setting.

OP6

Genetic characterization of two novel Aigai virus strains in Greece

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Introduction: Aigai virus (AIGV, Orthonairovirus parahaemorrhagiae) is a novel species in the Orthonairovirus genus. Up to 2021 it was assigned to Europe 2 genotype (genogroup VI) of Crimean-Congo haemorrhagic fever virus (CCHFV). AIGV has been detected in ticks in the Balkans, Turkey and Algeria, while in Turkey and Iran it has been detected also in humans. Here, we present the genetic characterization of two novel Greek AIGV strains.

Methods. In the frame of the EU OH-SURVector project, 12 of 545 (2.2%) *R. bursa* ticks collected in 2024 from livestock and environment in various regions of Greece were found positive for AIGV. RNA from two selected individual ticks collected in two sites in western Greece, close to Achelous River, were subjected to metagenomic next generation sequencing. Various bioinformatic software packages were applied for the genetic characterization of the two strains.

Results: Whole genome sequences were taken from the two strains, provisionally named Achelous-1 and Achelous-2. The respective genetic differences in the S, M, and L segments are 0.3%, 3.0%, and 0.6% at nucleotide level, and 0.0%, 1.8% and 0.3% at amino acid level. The highest aa difference (4.0%) was seen in the heavily glycosylated mucin-like domain (MLD) of the M segment. The MLD of Achelous-1 and -2 differs greatly not only from all CCHFV genotypes, but also from other AIGV strains. Given that the MDL and GP38 are major targets of non-neutralizing, protective antibodies in CCHFV and related nairoviruses, the observed MLD aa changes may modulate antibody binding, immune evasion and potential pathogenicity

Conclusions: A novel AIGV lineage was detected in *R. bursa* ticks collected from livestock in Greece, suggesting that integrated CCHFV/AIGV surveillance is highly recommended. Since novel variants with potential pathogenic determinants may emerge, genetic analysis has to be based on whole genomes.



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OP7

Genomic Surveillance of Chikungunya Virus in Returning Travellers to Germany Reveals Repeated Viral introductions and Potential Sources for Autochthonous Transmission in Europe

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Introduction/Aim: Chikungunya virus (CHIKV), transmitted by *Aedes aegypti* and *Aedes albopictus*, has expanded globally with autochthonous European outbreaks (Italy: 384, France: 788 cases in 2025). We investigated CHIKV infections among returning travellers in Berlin, Germany to assess diagnostic strategies, transmission risk, and viral diversity.

Materials and Methods: Returning travellers (Jan 2025–Feb 2026) with suspected CHIKV underwent ELISA (study threshold: IgM>2.5 RU) and/or RT-PCR. Cases were defined as compatible symptoms & positive RT-PCR and/or IgM>2.5 RU. PCR-positive samples were sequenced by high-throughput sequencing and cultured for virus isolation.

Results: Among 388 travellers who were screened for CHIKV infections at the Institute of International Health, 33 met the case definition (19 by serology only, 14 by PCR). 36% (5/14) of PCR-positive cases were antibody-negative at sampling. 67% (22/33) cases presented in winter when *Aedes albopictus* activity is absent in Germany.

The travel history of patients includes Cuba (10), Seychelles (8), Mauritius (5), Sri Lanka (5), Indonesia (3), India (1), and La Réunion (1).

Viral loads ranged from 1.8×10^3 – 2.4×10^8 cop/mL. Where successful (n=5), sequencing and cell culture isolation revealed multiple distinct CHIKV variants imported into Germany that clustered within the Asian/Caribbean and the East/Central/South African lineages.

Conclusion: Viraemic travellers pose a low current risk due to seasonal *Aedes* inactivity but expanding vector distribution and repeated introduction of diverse CHIKV variants highlight the potential for local establishment in Germany.

OP8

Spatiotemporal Expansion of Japanese Encephalitis Virus Genotype V in the Republic of Korea and Experimental Evaluation of *Aedes albopictus* as a Potential Bridge Vector

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus classified into five genotypes (GI–GV). In the Republic of Korea (ROK), genotype V (GV) has rapidly expanded since its first detection in 2010, yet its ecological characteristics and transmission potential remain insufficiently understood. This study investigated the nationwide distribution of JEV genotypes and evaluated the vector competence of *Aedes albopictus* for JEV GV under laboratory conditions. Nationwide mosquito surveillance was conducted from 2017 to 2022 at 32 sites across the ROK. Collected mosquitoes were identified morphologically and screened for JEV by real-time RT-PCR. Positive samples were subjected to envelope gene-based genotyping. To assess vector competence, laboratory colonies of *Ae. albopictus* were orally infected with JEV GI, GIII, and GV strains. Infection rate (IR), dissemination rate (DR), and transmission rate (TR) were evaluated at 7 and 14 days post-infection (dpi) using RT-qPCR analysis of mosquito bodies, legs/wings, and saliva. A total of 1,102,031 mosquitoes representing 30 species were collected during the surveillance period. JEV GI, GIII, and GV were detected, with GV becoming the predominant genotype after 2020. GV was identified not only in *Culex tritaeniorhynchus* but also in multiple mosquito species, suggesting expanded vector associations. In laboratory experiments, *Ae. albopictus* demonstrated high competence for GV, with a DR of 100.0% and TR of 36.7% at 14 dpi. In contrast, GIII showed limited transmission efficiency (TR 6.6%), while GI exhibited minimal dissemination and no detectable transmission. The increasing dominance of JEV GV and its efficient transmission by *Ae. albopictus* suggest a potential epidemiological shift in JEV circulation in the ROK. These findings indicate that *Ae. albopictus* may function as a bridge vector linking sylvatic and urban transmission cycles. Continuous molecular surveillance and expanded vector monitoring are essential to better understand the public health risk associated with emerging JEV genotypes.



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Session 3

Viral respiratory infections: the ever-present challenge

OP9

From crisis to preparedness: Lessons for Europe's public health laboratories

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Public health and clinical laboratories are central to detecting, monitoring and controlling respiratory infectious threats. To strengthen future preparedness, the European Centre for Disease Prevention and Control (ECDC) commissioned a study to assess the readiness of public health laboratories across EU/EEA countries, drawing on lessons from the COVID-19 pandemic. The study explored key challenges faced by laboratories, how these were addressed, and opportunities to improve long-term preparedness for respiratory pathogens.

A multi-step approach was used, including a rapid literature review, interviews and a survey with national public health authorities, followed by a technical meeting with EU institutions and EU/EEA Member States to discuss expert experiences in more depth.

Laboratories experienced considerable strain during the pandemic. Major challenges included surging testing demand, workforce shortages, supply chain disruptions, and constraints related to infrastructure and procurement. Difficulties in data sharing and coordination affected the timeliness and completeness of surveillance outputs. In response, laboratories recruited and trained additional staff, introduced new workflows, updated guidance and standard operating procedures, and leveraged capacity from academic, commercial and other sectors. Supply chains were diversified and new collaborations established to sustain testing activities.

Strengthening laboratory preparedness requires evidence-based approaches, including defining appropriate levels of genomic surveillance, effectively integrating alternative surveillance methods, and clarifying the laboratory evidence needed for decision-making across different emergency phases. At EU level, establishment of EU reference laboratories for public health will further support laboratory preparedness.

OP10

Expanded Glycan Receptor Recognition and Tissue Tropism of Clade 2.3.4.4b H5Ny Viruses Reveal Zoonotic Risk

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Highly pathogenic avian influenza H5Ny viruses pose a continuing threat to global public health due to their capacity for cross-species transmission and rapid evolutionary adaptation. The recent spread of clade 2.3.4.4b H5N1 viruses in dairy cattle highlights the need to define molecular and tissue-level determinants of zoonotic risk. Here, we investigated the glycan receptor landscape, structural basis, and tissue tropism of contemporary clade 2.3.4.4b H5Ny viruses, focusing on cattle-associated H5N1 and human-infecting H5N8 strains. We show that these viruses do not simply switch from avian-type to human-type receptor preference, but instead expand their glycan recognition repertoire. Cattle-associated H5N1 hemagglutinin retained strong binding to avian-type α 3-linked sialylated glycans while acquiring slight but detectable binding to human-type α 6-linked receptors. Tissue-binding analyses revealed strong HA binding to bovine mammary tissue and selected human mucosal tissues, including conjunctival and respiratory epithelia, supporting a potential route for mammalian adaptation and zoonotic exposure. Human-infecting H5N8 hemagglutinins further showed enhanced binding to complex fucosylated glycans, including sialyl Lewis X, with two naturally occurring substitutions, K222Q and S227R, contributing to broadened receptor recognition. Structural analyses revealed that expanded receptor usage is associated with increased plasticity of the HA receptor-binding site, enabling accommodation of distinct glycan conformations. Together, our findings suggest that clade 2.3.4.4b H5Ny viruses had already evolved features of broadened receptor specificity in the human-infecting H5N8 strain identified in Russia in 2020, and that similar receptor expansion is evident in cattle-associated H5N1 viruses. These results provide a receptor-, structure-, and tissue-based framework for assessing the zoonotic risk of emerging H5Ny viruses in clinical virology and public health surveillance.



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OP11

Host immune suppression associated with SARS-CoV-2 variant diversity and airborne emission heterogeneity

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Introduction/Aim: Human challenge experiments provide a unique opportunity to study viral-host interactions occurring within the first few days of infection. Here, we show differences in viral replication dynamics and shedding in healthy naïve individuals infected with SARS-CoV-2 Wuhan strain. Why some individuals shed far more virus and generate greater viral diversity remains unresolved. We leveraged controlled human challenge in naive adults to investigate the heterogeneity in variant generation and airborne emission and identify its immunological basis.

Materials and Methods: Eighteen healthy, naive adults received controlled inoculation with SARS-CoV-2 Wuhan strain, with dense longitudinal nasal and throat sampling. Participants were stratified by peak within-host mutation count in throat. Analyses included viral sequencing, bulk RNA-seq (nasal), viral load kinetics (qPCR, plaque assay), single-cell RNA-seq, and environmental viral capture from exhaled air.

Results: Within-host variants were predominantly newly generated rather than present in inoculum, arising stochastically via viral polymerase errors. High variant producers exhibited earlier nasal viral peaks (day 5 vs day 7) with sustained subgenomic RNA and mRNA elevation, indicating unconstrained early replication. Throat-to-nose seeding with ~1.5 day delay occurred in all individuals, but high producers amplified diversity further in nasal tissue. Critically, high variant generators were the highest airborne emitters; variants were detected in exhaled air. Mechanistically, high producers showed suppression of antimicrobial genes during days 3–5, despite elevated interferon responses—this pattern was also seen in epithelial cells and monocytes by single-cell analysis.

Conclusions: In controlled primary infection, individuals with suppressed mucosal antimicrobial defences sustain earlier, unconstrained viral replication generating both higher within-host diversity and elevated airborne emission. These findings mechanistically link innate immune heterogeneity to variant-rich phenotypes, with implications for pandemic spread and emergence of variants of concern.

OP12

Pediatric immunity influences seasonal dominance of RSV-A and RSV-B

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Background: Respiratory syncytial virus (RSV) is the leading cause of severe respiratory infections in children under five years of age, with nearly all children infected by the age of two. In Europe, RSV activity peaks during winter, with alternating strong and weak seasons. These epidemics are driven by two co-circulating subgroups, RSV-A and RSV-B, which often alternate in dominance between seasons. The mechanisms underlying this pattern, particularly the role of subtype-specific immunity, remain unclear.

Methods: RSV circulation and seasonality were monitored through a sentinel surveillance network at the Center for Virology, Medical University of Vienna. Subgroup-specific immunity was assessed using RSV-A and RSV-B neutralization assays in serum samples (n=375) from different age groups and consecutive seasons between 2021-2025.

Results: The 2021/22 RSV season was weak due to COVID-19 mitigation measures, followed by an exceptionally strong season in 2022/23, several moderate seasons, and increased activity again in 2025/26. After an unusually early start in 2021/22, seasonality gradually returned to typical winter timing. RSV-B dominated the 2022/23 season (91%), while RSV-A predominated in the following two seasons (84% and 54%). The proportion of circulating RSV subgroups was associated with the distribution of subgroup-specific neutralizing antibodies in sera from children who had experienced only the preceding RSV season. No such association was observed in children exposed to two or three seasons or in adults.

Conclusion: The alternating dominance of RSV-A and RSV-B may partly be explained by immunity acquired during the first RSV infection in early childhood. These findings suggest that young children play a key role in shaping RSV circulation. Future studies should assess how passive immunization in this group may influence overall RSV epidemiology.



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Session 4

Infants at risk: congenital, neonatal and pediatric viral infections

OP13

National Overview of Neonatal Herpes Simplex Virus Infections: Retrospective 2014–2025

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Introduction: Neonatal infection by Herpes simplex viruses (HSV1/2) is rare but have a very poor prognosis. In the absence of mandatory reporting, the National Reference Center for Herpesviruses assumed the national survey of these infections since 2014.

Materials and Methods: A yearly questionnaire was sent to biologists, obstetricians, and pediatricians from public or private hospitals with maternity wards to collect number of births, HSV-positive newborns, type of infection (cutaneous, neurological, disseminated), with detailed case description since 2017 (clinical presentation, HSV type, context of discovery, maternal history of genital herpes, child treatment and outcome).

Results: Based on the 186 reports received, overall incidence over 12 years is 22.3/100,000 births (2.1/100,000 considering the total number of births in France during the period). Cutaneous-mucosal infection (CMI) was the most frequent (59%), followed by disseminated infection (DI) (29%) and isolated encephalitis (E) (12%). HSV1 caused 82% of infections regardless of clinical presentation, with a broadly stable rate over the period.

The context was mainly maternal infection (47.1%), neonatal clinical signs (40.1%), less frequently systematic screening (2.7%) or unexplained death (0.5%). Only 59.4% of cases were associated with maternal herpes history. Mean age at diagnosis was 9.6 days for CMI, 21.6 days for E, 7.3 days for DI; 163/186 newborns (87.6%) were treated, 98.8% with acyclovir (mean duration 14 ± 8.31 days). Outcome was favorable in 94% of CMI, 70% of E and 61% of DI cases.

Conclusion: Epidemiological data over this decade are relatively stable. As no maternal history of Herpes was reported in many cases, increased information on postnatal transmission risk among family and close contacts should improve prevention and early treatment of neonatal Herpes.

OP14

Retrospective Genomic Surveillance of Respiratory Syncytial Virus (RSV) Over 12 Seasons in Hospitalized Children

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This study provides a comprehensive genomic characterization of Respiratory Syncytial Virus (RSV) from a well-defined cohort of hospitalized children enrolled in the Clarity-EU asthma project. Understanding RSV genetic evolution and identifying virulence markers associated with severe disease and long-term respiratory outcomes are therefore of major clinical relevance.

We performed a retrospective genomic analysis of 469 RSV-positive samples collected from hospitalized children with acute respiratory infection over 12 epidemic seasons (2005–2018). Whole-genome sequencing was successfully obtained for 76 isolates (30 RSV-A and 46 RSV-B) using a laboratory-developed protocol. Phylogenetic reconstruction included 7,067 global RSV-A and 7,282 RSV-B sequences. Mutation analysis was performed against reference genomes NC_038235.1 (RSV-A) and NC_001781.1 (RSV-B).

RSV-A accounted for 266 samples (56.7%) and predominated in 9 of the 12 seasons, whereas RSV-B (161 samples, 34.3%) predominated only during the 2006–07, 2010–11, and 2013–14 seasons. Phylogenetic analysis identified seven circulating clades: A.3.1.1, A.D, A.D.2.2, B.D, B.D.4, B.D.4.1, and B.D.4.1.1. Two distinct groups were particularly relevant when the genomic results were linked to the clinical study. Group A1 (RSV A) harbored mutations NS2-D100N, P-N95S, G-I236T, F-D385N, and L-V740A. According to the clinical records, 4 sequences out of 10 were from a recruited patient but three of four patients had no asthma or other respiratory complications. In contrast, Group B1 (RSV B) contained 6 mutations in the G protein (R98G, K103N, A131E, P231I, T289L, and S304L) and one mutation in M2-2 (S26N); notably, all recruited patients in this group presented asthma or other respiratory complications.

This study delineates the genomic epidemiology of RSV in a severe pediatric cohort over 12 epidemic RSV seasons. The findings highlight candidate genomic markers potentially associated with long-term respiratory outcomes.



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OP15

Integrative -omics profiling of congenital cytomegalovirus infection in twins

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Introduction/Aim: Congenital cytomegalovirus (cCMV) infection is the most common congenital viral infection and a leading cause of neurodevelopmental impairment, yet clinical outcomes vary widely even among fetuses exposed to the same maternal infection. Twin pregnancies represent a unique model to investigate fetal-intrinsic and placental factors contributing to this heterogeneity. We hypothesized that host genetic background and cell-specific environments influence viral replication and disease expression in cCMV infection.

Material: Three monozygotic diamniotic twin pairs with suspected or confirmed cCMV infection were enrolled.

Methods: Human cytomegalovirus (HCMV) clinical isolates were recovered from urine samples and phenotypically characterized in human foreskin fibroblasts (HFF), retinal pigment epithelial (ARPE-19) cells, and ex vivo placental explants. In parallel, 1H NMR-based metabolomic profiling of neonatal urine samples and virus genetic analyses were performed. Results were integrated with clinical findings.

Results: HCMV clinical isolates exhibited overall growth properties with limited phenotypic and genotypic variability. However, metabolomic analyses highlighted distinct molecular signatures between the twin pairs. Betaine, creatinine, and gluconate were identified as metabolites discriminating against individuals based on infection status, recovery phase, and nutritional or pharmacological management.

Conclusions: This integrative multi-omics study in twins provides evidence that host background and cellular context contribute to variability in viral replication and biological outcomes in cCMV infection. These findings support a role for fetal-intrinsic factors in disease heterogeneity and may inform future strategies for early risk stratification and personalized management.

OP16

gN-gM complex II genotype co-occurrence as a candidate marker of congenital HCMV virulence

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Introduction/Aim: Congenital human cytomegalovirus (cHCMV) causes sensorineural hearing loss and neurodevelopmental impairment, but viral determinants remain unclear. HCMV gN (UL73) and gM (UL100) form complex II, involved in viral assembly and spread. We investigated whether gN-gM genotype combinations show non-random pairing and congenital-associated enrichment.

Materials and Methods: A dataset of 375 gN-gM paired sequences was retrieved and analysed for genotype co-occurrence patterns. Of these, 31 isolates were from congenital cases and had complete gN-gM paired assignments. Associations were tested using contingency analysis (²), Cramer's V (effect size for categorical association strength), and disease-category comparisons.

Results: gN and gM showed non-random pairing in the overall cohort ($\chi^2=147.55$, $df=14$, $p=2.22 \times 10^{-2}$; Cramer's $V=0.44$). The most frequent pairs were gM1-gN4d (60/375; 16.0%), gM3-gN4a (45/375; 12.0%), and gM3-gN2 and gM1-gN3a (each 33/375; 8.8%). Among paired congenital-associated isolates, gN4 variants predominated collectively (20/31; 64.5%), with gN4d the most frequent individual genotype (7/31; 22.6%), followed by gN4a and gN4b (each 5/31; 16.1%). gM1 and gM3 were equally represented (each 12/31; 38.7%), followed by gM2 (7/31; 22.6%). The most frequent gN-gM combinations were gM1-gN4d (5/31; 16.1%), gM3-gN4a (4/31; 12.9%), and gM2-gN4b (3/31; 9.7%).

Conclusions: Congenital HCMV isolates demonstrate structured gN-gM complex II organisation rather than random glycoprotein pairing. Congenital HCMV-associated combinations, particularly of gN4 and gM3 variants, support further investigation of gN-gM signatures as candidate genotypic biomarkers of congenital HCMV virulence.



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Session 5

Viral infections in special populations and vulnerable hosts

OP17

Successful treatment of a UK case of Lymphocytic choriomeningitis virus meningoencephalitis associated with bispecific antibody teclistamab therapy for myeloma

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Introduction: Lymphocytic choriomeningitis virus (LCMV) is a rare cause of meningoencephalitis in humans. Although LCMV is a rodent zoonosis with a global distribution, no cases have been reported in the UK since the 1950s, and testing is rarely performed. LCMV meningoencephalitis is self-limiting in immunocompetent hosts, but infection in the immunocompromised causes more severe disease. We report a severe presentation of LCMV meningoencephalitis in a 60-year-old man with relapsed multiple myeloma receiving teclistamab, and describe his treatment with a novel combination of antivirals.

Results: The patient presented with a five-month history of progressive neurological symptoms, including hearing loss, ataxia, headache, and weight loss. Cerebrospinal fluid (CSF) analysis showed lymphocytic pleocytosis, markedly elevated protein, and hypoglycorrhachia. Extensive microbiological and autoimmune investigations were negative, and empirical antimicrobial and antituberculous therapies did not prevent clinical deterioration. Metagenomic next-generation sequencing (mNGS) of CSF identified LCMV, and a history of rodent exposure was subsequently identified.

Combination antiviral therapy was initiated. Favipiravir dosing was guided by serum and CSF therapeutic drug monitoring. The patient showed neurological improvement, with clearance of LCMV from CSF and blood within two months. Antiviral therapy continued for six months. The patient regained functional independence and returned to work.

Conclusions: In addition to describing a new association of severe

LCMV disease with teclistamab, this case highlights the presence of LCMV in the UK, and the utility of mNGS for pathogen identification in undiagnosed encephalitis. Finally, the case provides evidence supporting combination antiviral therapy in persistent LCMV infection.

OP18

Pigeon Paramyxovirus Type-1 (PPMV-1) Detection by Metagenomics Next Generation (mNGS) Sequencing in a Case of Fatal Pneumonia in a Patient with Relapsed Acute Lymphoblastic Leukaemia (ALL)

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Avian paramyxovirus type 1 (APMV-1) is an important pathogen in poultry, and an emerging cause of severe infection, including pneumonia, in immunosuppressed individuals¹. We report an APMV-1 infection in a patient with relapsed acute lymphoblastic leukaemia (ALL) five months post allogeneic stem cell transplant. He received profoundly immunosuppressive chemotherapy, developing a fever and respiratory failure on day 6 of admission. He was transferred to critical care for intubation and ventilation and on day seventeen died of multi-organ failure. Using Oxford Nanopore mNGS, we detected



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pigeon Paramyxovirus type 1 (PPMV-1), an antigenic variant of APMV-11, in two respiratory secretion samples. This sequence was identified and highlighted through mSCAPE. Retrospective testing using a generic APMV-1 PCR detected viral RNA in stool on admission indicating community acquisition and confirming metagenomic detections. Consensus genome showed greatest similarity to known genotype VI.2.1.1.2.2 PPMV-1 sequences and clustered with UK wild pigeon isolates on phylogenetic analysis. Although the patient had no known pigeon contact, they resided in central London where pigeons are ubiquitous. Detection of unexpected or emerging pathogens, particularly in immunosuppressed individuals, is likely to increase as mNGS becomes more prevalent in clinical practice. Genomic data from emerging organisms can inform pandemic preparedness, with surveillance supporting public health services. Differentiating pathogenic and bystander detections is a significant challenge for infection specialists implementing this new diagnostic technology.

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OP19

Deciphering the Impact of Epstein-Barr Virus Serostatus on Early DNAemia after Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Epstein-Barr virus (EBV) reactivation is frequent after allogeneic hematopoietic stem cell transplantation (HSCT), and can sometimes progress to post-transplant lymphoproliferative disorder, a serious condition. While donor EBV seropositivity (sero+) is recognized as a risk factor, the specific impact of serological marker profiles (anti-VCA and anti-EBNA IgG) on EBV DNAemia has not been studied.

Aim: To investigate factors associated with EBV DNAemia during the first 100 days post-HSCT, with a focus on the impact of EBV serostatus patterns.

Methods: We conducted a monocentric study on a large cohort of HSCT. Inclusion required pre-transplant EBV serology for both donor and recipient, and EBV PCR follow-up with at least 3 samples within the first 100 days post-HSCT. Logistic regression was used to identify factors associated with EBV DNAemia and clinically significant (CS) viremia ($\geq 4 \log \text{ IU/mL}$).

Results: A total of 926 donor-recipient couples were included. EBV DNAemia was detected in 491 recipients (53%), of whom 39.5% reached the clinically significant threshold. On multivariable analysis, donor EBV sero+ (aOR: 5.57) and use of anti-lymphocyte globulins (aOR: 2.82) were the strongest independent predictors of DNAemia. Among seropositive donors, high donor anti-EBNA IgG levels (≥ 3 rd quartile) were independently associated with an increased risk of EBV DNAemia (aOR: 1.56). Donor sero+ increased the risk of CS viremia by 60–69% across all major transplant risk factors (stem cell source, donor origin and drug regimen).

Conclusions: Donor EBV sero+ is the dominant driver of early EBV DNAemia after HSCT, with high donor anti-EBNA titers identifying a higher-risk subgroup. Transplant-related factors independently modulate the overall risk of CS viremia. These findings support a multifactorial approach to risk stratification.

OP20

The human DNA virome and HLA variation as predictors of transplantation outcomes in HLA-matched HSCT donor–recipient pairs

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Aim: To investigate the role of the human DNA virome recipient-donor matching in allogeneic hematopoietic stem cell transplantation (HSCT) outcomes, with focus on the development of graft-versus-host disease (GVHD).

Materials and methods: We analyzed peripheral blood DNA samples from 46 HLA-matched donor–recipient pairs pre-HSCT. The primary indication for HSCT was acute myeloid leukemia. We performed custom viral hybrid-capture next-generation sequencing, targeting 41 DNA viruses. In addition, selected viruses were quantified by qPCR. Clinical data was analyzed to evaluate associations between viral prevalence, viral recipient-donor matching and viral load, and HSCT outcomes.

Results: Across the 92 sequenced samples, at least one virus was detected in 58.7% of samples, and among these virus-positive samples, 40.7% had two or more detected viruses. The most frequently identified viruses were human herpesvirus 7 (HHV-7), Epstein–Barr virus (EBV), and torque teno virus (TTV). Before HSCT, EBV was more prevalent in recipients, whereas TTV was more frequently detected in donors. Discordant virus profiles between donors and recipients were associated with the development of chronic GVHD. Preliminary, EBV negativity was associated with HLA-B*08:01, and HHV-7 with HLA-B*35:01, suggestive of HLA-associated determinants of viral persistence.

Conclusions: These findings suggest that recipient HLA variation and donor–recipient differences in the DNA virome may influence HSCT outcomes, including chronic GVHD, and warrant further investigation for risk stratification.



Oral Presentations

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Session 6

Viral infections of the nervous and gastrointestinal systems

OP21

Etiology of Viral Encephalitis in Bangladesh: Defining the Role of Metagenomic Next-Generation Sequencing in an Observational Cohort

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Introduction/Aim: In most patients with encephalitis no cause is identified despite extensive diagnostics. We aimed to characterize the etiologies of encephalitis in Bangladesh, develop practical diagnostic algorithms, and evaluate the added value of metagenomic next-generation sequencing (NGS).

Material and methods

Patients hospitalized at the Dhaka Medical College Hospital in Dhaka, Bangladesh fulfilling established clinical criteria of acute viral encephalitis were included. Clinical data, CSF, blood, throat and rectal samples were collected. A broad diagnostic panel of viruses was tested in CSF using RT-qPCR and targeted-capture Illumina sequencing. Throat- and rectal swabs were tested for Influenza virus (A and B) and enterovirus using RT-qPCR. Serological testing included ELISA IgM and IgG for hepatitis E, Zika, and dengue virus (DENV). Virological etiology was defined using the Granerod laboratory classification.

Results: A viral etiology was identified in 69/210 patients (33%). Detection methods included CSF RT-qPCR (35/69, 51%), RT-qPCR of throat, rectal, or blood samples with or without serology (20/69, 29%), and serology alone (14/69, 20%). The most commonly confirmed etiologies were DENV (n=9, 4%), JEV (n=8, 4%), and HSV-1 (n=4, 2%).

Among 31/114 tested cases (27%), viral genomes were detected by RT-qPCR but not by NGS. Conversely, in 16 cases (14%), NGS detected viral genomes with potential clinical relevance not identified by RT-qPCR. These included parvovirus-4 (n=10), HSV-1 (n=2), and single cases of hepatitis B virus, polyomavirus, rotavirus A, and JEV. Among the Parvovirus-4 cases, four patients had a possible alternative viral etiology and two had a confirmed alternative viral etiology.

Conclusions: DENV is a major cause of encephalitis in Bangladesh. Although NGS contributed to pathogen detection, expanding sampling to extra-neural specimens may be more important to increase diagnostic yield. Further studies are needed to clarify the pathogenic role of parvovirus-4 in encephalitis.

OP22

Surveillance of Enterovirus and Parechovirus-A Shedding in Children With and Without Acute Gastroenteritis in the United States, 2012-2024

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Background: Diarrheal disease remains a major cause of childhood morbidity worldwide. While several enteric viruses are well characterized, data describing intestinal shedding of enterovirus (EV) and parechovirus A (PeV-A) among children with and without acute gastroenteritis (AGE) in the United States are limited. We evaluated EV and PeV-A detection and genotype diversity over 12 years among children with medically attended AGE.

Methods: From 2012-2024, stool samples were collected from children <18 years enrolled through the CDC New Vaccine Surveillance Network at Children's Mercy Kansas City, including children with AGE (n=4,366) and healthy controls (HC; n=1,544). All samples were tested for PeV-A; EV testing was performed during 2012-2021. Detection used RT-PCR targeting the 5' untranslated region. PeV-A positive samples underwent VP1 sequencing. Detection rates were compared using Fisher's exact tests.

Results: Among 5,910 samples, PeV-A detection was similar among AGE children (449/4,366, 10.3%) and HC (175/1,544, 11.3%; p=0.27). EV was detected more frequently among children with AGE (413/3,547, 11.6%) than HC (105/1,372, 7.7%; p<0.001). Among children with AGE, EV and PeV-A detection rates were similar (11.6% vs 10.3%), whereas among HC, PeV-A was detected more frequently than EV (11.3% vs 7.7%; p=0.001). EV- and PeV-A-positive children were predominantly <2 years. Co-detection occurred among children with AGE (59/413, 14.2%) and HC (21/105, 20%). Among genotyped PeV-A positive samples from AGE, seven genotypes were identified, predominantly PeV-A1 (68%), followed by PeV-A4 (20%) and PeV-A3 (5.4%). Among HC, three genotypes were identified, with PeV-A1 most common (65%). Most PeV-A3 and PeV-A5 detections coincided with years of PeV-A-associated CNS infections.

Conclusion: EV was more frequently detected among AGE children, whereas PeV-A was detected at similar proportions in both groups and more frequently than EV among HC, consistent with asymptomatic shedding. Genotype diversity highlights dynamic circulation and supports continued molecular surveillance.



Oral Presentations

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OP23

Identifying intra-hospital Norovirus GII transmission using whole-genome sequencing

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Background: Norovirus genogroup II (GII) is a common gastrointestinal pathogen that can cause spatially and temporally linked outbreaks due to its resistance to disinfectants and desiccation. Despite this threat, routine whole-genome sequencing has rarely been systematically applied to Norovirus.

Methods: We performed whole-genome amplification of Norovirus GII from 665 clinical stool specimens collected between 2019 and 2025 at Children's Hospital of Philadelphia (CHOP), and 47 samples from Children's National Hospital, using a modified PCR protocol and Oxford Nanopore Technologies (ONT) sequencing. Viral genomes were assembled using long-read sequencing and paired with clinical metadata from the electronic medical record to identify suspected transmission events.

Results: Our assay improves on previous methods and achieved an 84% success rate in assembling CHOP samples into near-complete genomes. Genotyping analysis showed dominance of GII.4 Sydney [P16] from 2019 to 2024, with recent emergence of GII.17 [P17]. We identified multiple cliques (n=44) of genetically indistinguishable or near-identical (≤ 5 SNP) strains. 32% (14/44) of these cliques were from patients on the same units and are considered "Likely" Norovirus GII transmission events. 20% (9/44) of the cliques are from different units or inpatient and emergency department and are "Possible" transmission events. All "Likely" Norovirus GII transmission events have a confirmed epidemiological link or occurred entirely within unit and 56% (5/9) of the "Possible" transmission events have an epidemiological link - either a shared clinician or shared off unit location or both.

Conclusions: This study demonstrates that whole-genome sequencing of Norovirus GII is a powerful tool for high-resolution viral surveillance. Prospective genomic monitoring combined with informatics enables detection of nosocomial outbreaks and reveals hidden transmission chains. Routine implementation of this platform could facilitate earlier implementation of containment measures that may ultimately reduce healthcare-associated Norovirus transmission.

OP24

Emergence and spread of multirecombinant enterovirus A71 lineages C1.3/RF5 and C1.3/RF6 associated with neurological diseases in Europe, 2016–2022, ENPEN multicentre study

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Oral Presentations

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Introduction/Aim: Since its re-emergence in Europe in 2015, enterovirus A71 (EV-A71) has been associated with sporadic cases of severe infections and outbreaks. The aim of our study was to characterise the EV-A71 cases in Europe and track the evolution of the virus between 2016 and 2022.

Material: We compiled the data of confirmed EV-A71 infections collected by 19 institutes participating to the European Non-Polio Enterovirus Network (ENPEN) between 2016 and 2022.

Methods: We also sequenced whole EV-A71 genomes to report on the viral evolutionary history of the virus and its molecular changes.

Results: Between 1 January 2016 and 31 December 2022, 627 cases of EV-A71 were reported in 14 European countries. Most cases (59.3%) occurred in children under two years of age. Of the 537 cases for which clinical information was available, 52.7% were diagnosed with neurological signs, and 11% presented with acute flaccid paralysis or acute flaccid myelitis. EV-A71 infections were linked to two subclades, C1.2 and C1.3, within the C1 subgenogroup. Two marked epidemic waves, in 2016 and 2019, were characterised by the predominance of C1.3 viruses. Comparisons of 153 whole genomes revealed nine recombinant forms (RFs). Only C1.3/RF5 and C1.3/RF6 lineages achieved widespread geographic dissemination initially in Europe and subsequently in Asia and the Americas, and long-term circulation. Phylodynamic analyses suggest that C1.3 emerged around 2013–2014, displacing earlier C1.1 viruses. Molecular analyses of the capsid revealed several amino acid changes, but few of which occurred within known antigenic sites.

Conclusions: Our findings document the role of two EV-A71 multi-recombinant lineages (C1.3/RF5 and C1.3/RF6) as frequent causes of neurological disease in young children in Europe in 2016–2022.

Oral Presentations

Friday | 18th September

Session 7

Advances in HIV and viral hepatitis

OP25

Rat hepatitis E virus infection in cultured human and rat hepatocytes confirms a partial species barrier and distinct host responses

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Introduction/Aim: Rat hepatitis E virus (rHEV) is a recently identified cause of human hepatitis. rHEV is genetically divergent from other human-infecting hepatitis E virus (HEV) variants; however, the impact of this divergence on rHEV's tropism for human hepatocytes remains unknown. We compared rHEV and HEV in terms of binding, entry, replication efficiency, and host responses in human and rat hepatocytes. Through this, we gained insights into mechanisms underlying the clinic-epidemiological features of human rHEV infection.

Material: Differentiated HepaRG cells (representing human hepatocytes) and primary rat hepatocytes (PRHs) were infected with human and rodent-derived rHEV strains (representing subtype Ia and IIb), a HEV genotype 4 strain, and the HEV-Kernow C1 p6 infectious clone.

Methods: Viral replication was assessed using RT-qPCR and viral protein immunoassays. Hepatocyte binding and entry were quantified using virus-like particles (VLPs). Host transcriptomic responses including interferon (IFN) expression and interferon-stimulated gene (ISG) expression were evaluated.

Results: rHEV strains replicated more efficiently in PRHs than HepaRG cells with higher RNA loads and robust viral protein expression in PRH supernatants and cells. rHEV VLPs preferentially bound to and entered PRHs than HepaRG cells, whereas the reverse was observed for HEV VLPs. Entry was blocked by endocytosis inhibitors. Despite robust type I/III IFN induction by rHEV in both HepaRG cells and PRHs, ISG induction was significantly more attenuated in PRHs supporting viral persistence.

Conclusions: rHEV preferentially infects PRHs and innate host responses within this reservoir support viral persistence. There is a partial species barrier at the human hepatocyte level, which may explain rHEV's low incidence and milder clinical phenotype.

OP26

Adenovirus and AAV2-Associated Severe Hepatitis in Children Before 2022

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Introduction/Aim: In 2022, an outbreak of severe hepatitis of unknown aetiology was reported across multiple countries. Adenovirus and Adeno-Associated Virus 2(AAV2) were implicated as potential co-factors. Data from before the COVID-19 era on this association remain limited. This study aims to investigate the presence and role of AAV2 in children with acute severe hepatitis(ASH) and/or paediatric acute liver failure(PALF) before 2018.

Material and Methods: A retrospective, single-centre study of children aged 0-16 years admitted with ASH and/or PALF and a positive whole-blood PCR for adenovirus, conducted between 2004 and 2018. Archived historical liver tissue was examined by PCR for AAV2, adenovirus, CMV, EBV and HHV-6. The histological and clinical data were also reviewed.

Results: During the study period approximately 225 children presented with PALF. Twenty-seven patients were included (15 PALF, 12 ASH), therefore adenovirus was detected in at least 6.7% of the PALF cases. Among the PALF patients, 8 underwent liver transplantation, with no deaths reported. Liver tissue obtained during the acute disease phase was available from 14 patients (11 PALF, 3 ASH); AAV2 was detected in 10 of 14 (71%), with low cycle threshold (Ct) values indicating a high viral burden (median Ct 26.12), including 7/11 PALF cases tested. Adenovirus, CMV and EBV were not detected in any liver tissue by PCR. Low-level HHV-6 positivity was detected in 9/14 cases. Histological findings showed non-specific hepatitis, with a predominantly lymphocytic infiltrate and no evidence of direct adenoviral toxicity.

Conclusions: Our findings confirm that adenovirus-associated ASH and PALF in children occurred well before the 2022 outbreak and provide tissue-level evidence of a longstanding association with AAV2. The detection of AAV2 in historical liver samples at high levels (low Ct values) supports the hypothesis that AAV2 is implicated in the pathophysiology of severe paediatric hepatitis. AAV2 testing should be considered in selected cases of indeterminate hepatitis.



Oral Presentations

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OP27

Double reflex testing reveals a still significant HDV burden in Europe and represents an effective strategy for earlier disease-stage diagnosis

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Background / Aim: Hepatitis D virus (HDV) causes the most severe viral hepatitis, however often remains underdiagnosed. Here, we investigate HDV screening and seroprevalence in a large set of HBsAg+ individuals in Europe and Middle East before (2021-2023) and after (2024-2025) the implementation of double reflex testing (DRT: antiHDV-testing in all HBsAg+ with unknown HDV serology and HDV-RNA if antiHDV+ as per EASL guidelines).

Methods: This multicenter study included 25527 HBsAg+ subjects (15483 collected in 2021-2023 and 10044 in 2024-2025) from 22 hospitals in Europe and the Middle East.

Results: Subjects were 57% males with median age of 49 years and only 28% under NUC-treatment. The rate of HDV screening strongly increased after DRT implementation from 56% to 92% ($p < 0.001$).

HDV seroprevalence decreased after DRT implementation from 6% to 4% ($p < 0.001$). Notably, HDV-RNA testing raised from 81% to 94% ($p < 0.001$) while HDV-RNA positivity remained stable (47% and 39%, $p = 0.04$) supporting a relevant circulation of active HDV infection.

Compared to 2021-2023, anti-HDV+ subjects diagnosed by DRT more frequently had ALT < 40 U/l (66% vs 33%, $p < 0.001$) and a less advanced liver fibrosis (F0/F1 in 94% vs 33.8%, $p < 0.001$), supporting HDV detection in milder liver disease. In the 343 subjects with active infection, median HDV-RNA was 5.2 logIU/ml. Notably, compared to 2021-2023, subjects with active HDV infection detected by DRT had significantly lower HBsAg (2313 vs 5158 IU/ml), HBV-DNA (30 vs 131 IU/ml), and ALT (45 vs 79 U/L) ($p < 0.001$ for all), supporting DRT as a valuable approach for an early HDV diagnosis, with lower cytolytic activity.

Conclusions: This study reveals a still relevant burden of HDV infection with an intense HDV replication across Europe. Implementing double reflex testing increases HDV case identification and enables diagnosis at earlier stages of liver disease.

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OP28

The Effect of Sample Transport and Storage Conditions in EDTA-Containing Gel Tubes on the Performance of Hepatitis B (HBV) and Human Immunodeficiency Virus (HIV) Nucleic Acid Tests During the Pre-analytical Process: A Preliminary Study

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Introduction/Aim: The performance of viral load tests is important for diagnosing and monitoring viral infections. Preanalytical sample handling may affect the test performance(1). This study aimed to evaluate the effects of different storage temperatures, durations, and freeze-thaw procedures on viral load measurements obtained from Vacusera K2EDTA-Gel (Disera, Izmir, Turkey) tubes and compare the results with Vacutainer K2-EDTA tubes (Becton Dickinson Franklin Lakes, USA).

Materials and Methods: Blood samples were collected from 125 donors at the Ege University Faculty of Medicine Blood Center between August 2025 and May 2026. Positive samples were prepared using external quality control materials (American Proficiency Institute and Qnostics Molecular Controls) and plasma samples with known viral loads. After appropriate storage, HBV DNA and HIV RNA levels were measured using Alinity m assays (Abbott Molecular, USA). Coefficient of variation (CV), repeatability, and correlation analyses were performed.

Results: Repeatability analysis after storage at room temperature and +4°C for 1, 3, and 7 days demonstrated CV below 10% for intra-assay and below 20% for inter-assay measurements in Vacusera K2E EDTA-gel tubes. After freeze-thaw procedures, HBV-spiked samples showed a CV below 20%, while one low-level sample showed a CV of 63% with results below the dynamic range.

Linearity analysis of Vacusera tubes showed r^2 values above 0.97 for both HBV and HIV under different preanalytical conditions. In the accuracy analysis, all average bias results were below 0.5 Log₁₀. A high correlation was found between the measurement values of the two EDTA gel tubes (HIV: $r=0.991$, HBV: $r=0.992$, $p<0.001$).

Conclusions: HIV RNA and HBV DNA quantification in EDTA gel tubes showed stable, reliable, and reproducible performance with low variability under different storage conditions. Viral load results remained consistent during both short- and long-term storage at +4°C; however, freeze-thaw cycles in samples near the lower limit of detection may affect the measurement reliability.



Oral Presentations

Friday | 18th September

Session 8

Host-virus interplay: insights into pathogenesis, immunity and cancer

OP29

Cutavirus Across Borders: A Large-Scale Study in Malignant and Non-Malignant Tissues

Ms. Irini M. Assimakopoulou¹; Ms. Sally Chesnut¹; Ms. Zhenying Li¹; Dr. Alexander Salava²; Dr. Olga Bugaeva²; Dr. Liisa Väkevä¹; Prof. Mahmoud Kamel³; Prof. Ahmed S. Abdel-Moneim⁴; Prof. Julia Scarisbrick⁵; Dr. Hanne Vinter⁶; Prof. Francesco A. d'Amore⁶; Dr. Susanne Buder⁷; Prof. Vasiliki Nikolaou⁸; Prof. Leonidas Marinou⁹; Dr. Patrick Obermeier¹⁰; Dr. Maria Söderlund-Venermo¹;

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Cutavirus (CuV), the newest human parvovirus, is statistically associated with cutaneous T-cell lymphoma/mycosis fungoides (CTCL/MF), a group of rare skin malignancies of unknown etiology. CuV skin persistence is also linked to parapsoriasis (PP), a long-term inflammatory condition and precursor of CTCL/MF. In contrast, it has been observed only sporadically in other skin diseases or healthy skin. Our aim was to confirm this association, in a larger, multinational cohort. We searched for CuV DNA by qPCR in 942 biopsied formalin-fixed paraffin-embedded (FFPE) tissues (CTCL of different subtypes, PP, cutaneous B-cell lymphoma, malignant or benign breast tissues, other lymphomas or cancers, and non-malignant T-cell-rich dermatoses) from a total of 679 patients (Finland, Germany, Greece, Denmark, the UK, and Egypt). In total, CuV DNA was detected in 49 of 942 FFPE specimens (5.2%) corresponding to 41 of 679 patients (6.0%). Of the 41 CuV DNA-positive patients, 35 had CTCL/MF or PP, 4 had other lymphomas, and one each had melanoma in situ and a benign mole. Altogether, 35/365 (9.6%) patients with CTCL/MF or PP harbored CuV DNA in skin in contrast to 6/314 (1.9%) patients with other malignant or benign disease manifestations ($p < 0.001$), which verifies the association of CuV with CTCL/MF and PP. RNAscope in-situ hybridization of MF skin showed CuV-specific signals both in the epidermis and dermis. This ongoing study aims to elucidate the role of CuV in CTCL carcinogenesis, helping to determine whether CuV merely thrives in cancer or T cells or actively contributes to the development of CTCL/MF.

OP30

Expression of Human Endogenous Retroviruses in colorectal cancer patients

Maria Dolci¹; Fabio Frosio²; Federico Ambrogio³; Saleha Irfan Arodi⁴; Carolina Cason⁴; Lucia Signorini¹; Francesca Camurri¹; Estefanía Calvo Alvarez⁵; Manola Comar⁶; Serena Delbue¹;

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Introduction: Human Endogenous Retroviruses (HERV), which constitute 8% of human genome, are generally transcriptionally silent but can be reactivated in cancer. In colorectal cancer (CRC), their role in tumor pathogenesis remains unclear, while gut microbiota alterations have been linked to disease progression. This study evaluates clinical relevance of HERV expression in CRC and its association with clinical features and gut microbiota composition. Methods: 104 CRC patients undergoing surgical resection at Papa Giovanni XXIII Hospital (Bergamo-Italy) were enrolled. Tumor (TT), adjacent healthy (HT) tissues, serum samples were collected. Clinical data, including tumor stage, histological grade (G), lymph-node status, mismatch repair (MMR) protein expression, and CEA levels were obtained. HERV gene expression (HERV-H, -K, -P, -R env, and -K gag, pol, rec, np9 genes) was quantified by RT-qPCR in tissues and serum extracellular vesicles (EV). Gut microbiota was characterized by 16S rRNA gene sequencing. Correlations between HERV expression, microbiota profiles, and clinical data were evaluated using non-parametric and multivariable analyses.

Results: HERV expression differed significantly between TT and HT, with HERV-Kgag, -Knp9 and -Renv showing higher expression among TT. HERV-Knp9 showed a significant decrease of expression in TT between G1 and G2, while -Kenv expression was associated with loss of function of MMR protein. HERV transcripts, particularly HERV-Knp9, were frequently detected in EV. Reduced microbiota diversity correlated in HT with lymphovascular invasion and in TT with metastases, but no association between microbiota profiles and HERV expression was observed.

Conclusions: HERV expression may reflect relevant molecular features of colorectal cancer, with HERV-Knp9 and -Kenv as potential biomarkers of tumor progression and mismatch repair deficiency. HERV sequences, detected in EV, may be transferred between cells, promoting cellular transformation. Gut microbiota alterations reflect disease severity but do not seem to be involved in HERV expression.



Oral Presentations

Friday | 18th September

OP31

Functional Roles of tRNA-Derived Small RNAs in Dengue Virus Infection

Dr. Bhupendra Kumar Verma

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Introduction: Dengue is an arboviral disease that presents a wide range of therapeutic outcomes, from mild to life-threatening conditions. The absence of effective treatments underscores the need for a deeper understanding of DENV pathogenesis. tRNA-derived small RNAs (tsRNAs), is an emerging class of sncRNAs which play an important role in regulating transcription and translation machinery thus holding the immense diagnostic and therapeutic potential during DENV infection.

Aim: To investigate the modulation of ribonucleases and tRNA fragmentation during DENV pathogenesis.

Methodology: During DENV infection, we characterized the modulation of RNases primarily involved in tRNA fragmentation. To further investigate tsRNA generation, small RNA sequencing was performed on DENV-infected Huh7 cell lines. The RNA-seq data were validated using an in-house optimized Biotin-based Northern Blotting (BiNoB) technique. Antisense oligonucleotides (ASOs) were then used to target the candidate tsRNAs, to assess their impact on DENV replication and to observe the activation of PI3K-Akt pathway.

Results: During DENV infection, RNase levels are modulated, with angiogenin markedly upregulated, leading to increased tRNA fragmentation and disease progression. RNA-seq revealed subtype-specific changes, with 3YRFs upregulated and i-tRFs downregulated. Targeting i-tRF-40 (tRNA-Tyr-GTA) using ASOs reduced infection by over 70%. Pathway analysis highlighted PI3K-Akt signaling, where specific i-tRFs showed distinct roles: i-tRF-40 TyrGTA and i-tRF-25 LysCTT promoted viral infection via Akt activation, while i-tRF-28 LysCTT inhibited Akt phosphorylation and exhibited antiviral effects.

Conclusion: This study gives insights about the potential regulatory roles of tRFs in DENV pathophysiology, contributing to the better understanding about host-virus interaction.

OP32

How pandemic viruses Activate, Evade, and Exploit host immunity

Dr. Rayhane Nchioua

Institute of Molecular Virology, Ulm University Medical Center, Ulm University

Pandemic viruses continuously evolve to escape the antiviral defenses of their hosts. My research focuses on the molecular interactions between viruses and innate immune restriction factors that act as early barriers to infection.

In particular, I study the antiviral protein ZAP, which recognizes CpG dinucleotides in viral RNA and targets these viruses for degradation. Many successful viruses, including Human immunodeficiency virus 1 and SARS-CoV-2, have evolutionarily suppressed CpG dinucleotides in their genomes to evade ZAP-mediated restriction. In our work, we demonstrated that SARS-CoV-2 and its closest bat coronavirus relatives show exceptionally strong CpG suppression compared to other coronaviruses, suggesting evolutionary preadaptation to evade ZAP-mediated antiviral activity. Nonetheless, endogenous ZAP still restricts SARS-CoV-2 replication in lung cells, particularly upon IFN- γ stimulation. I investigate how these virus-host interactions shape viral evolution, species transmission, and pathogenicity.

In parallel, I investigate how interferon-induced restriction factors such as IFITM proteins regulate viral entry and how viruses adapt to overcome or exploit these barriers. We discovered that SARS-CoV-2 hijacks endogenous IFITM proteins, particularly IFITM2, as entry cofactors for efficient infection, despite their classical antiviral roles. In addition, I study how emerging viral variants evolve resistance to innate immune responses, including reduced sensitivity to type I, II, and III interferons observed for the Omicron BA.1 variant.

Finally, I investigate innate immune sensing pathways, including inflammasome activation by viral proteins, to understand how infected cells detect viral invasion and how viruses evade these cellular alarm systems.

Together, my work aims to define how pandemic viruses evade, suppress, or exploit intrinsic immune defenses and how these interactions can be leveraged to develop new antiviral strategies.



Oral Presentations

Friday | 18th September

Session 9

The viral combat: antivirals, antibodies, and vaccines

OP33

Effectiveness of HPV vaccination in a population-based cervical screening programme: implications for cervical cancer elimination in Portugal

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Introduction/Aim: Monitoring the real-world impact of HPV vaccination in organised screening programmes is essential to assess population-level effectiveness. We aimed to evaluate the long-term effectiveness and population-level impact of quadrivalent HPV vaccination in Portugal on HPV16/18 prevalence and genotype-specific distribution.

Material and Methods: We performed a population-based retrospective cross-sectional study including women aged 25–32 years participating in the Northern Portugal cervical cancer screening programme (2016–2021). HPV genotyping and cytology data were linked to the Portuguese National Vaccination Registry.

Results: A total of 87,180 women were included, including 47,987 unvaccinated and 39,193 (44.8%) vaccinated women, of whom 97.2% received the quadrivalent vaccine. Overall hr-HPV prevalence was similar in unvaccinated and vaccinated women (~20%). HPV16/18 prevalence was markedly lower in vaccinated women (0.4% vs 3.1%), corresponding to ~88% vaccine effectiveness and ~90% among women vaccinated before age 18. Among hr-HPV-positive women, HPV16/18 accounted for 16.6% of infections in unvaccinated versus 1.8% in vaccinated women. Non-vaccine hr-HPV genotypes accounted for a higher proportion of infections in vaccinated women, particularly HPV51, HPV52 and HPV39, although their absolute prevalence remained stable. Across successive birth cohorts, HPV16/18 prevalence declined by 73–93% ($p < 0.001$), confirming a strong population-level impact.

Conclusions: HPV vaccination has led to a marked reduction in HPV16/18 prevalence in a screening population, demonstrating high real-world effectiveness. Genotype redistribution reflects proportional shifts rather than true type replacement, supporting continued surveillance and the role of vaccination in cervical cancer elimination.

OP34

Characterization of Potential Drug-resistance Mutations in the HSV-1 Thymidine Kinase

Dr. Hanna Schalkwijk¹; Ms. Vera Jonges¹; Prof. Graciela Andrei¹;

¹KU Leuven

Introduction/Aim: Herpes simplex virus 1 (HSV-1) is a ubiquitous pathogen that establishes life-long latency in neurons. Though infections often cause mild and self-limiting mucosal lesions, severe, potentially life-threatening disease can occur in immunocompromised hosts. The extensive use of antivirals in these patients resulted in the frequent isolation of drug-resistant strains, which cause difficulties in infection management. Novel mutations are often found in the clinic and complicate the interpretation of genotypic resistance testing. In this study, we evaluated the effect of novel mutations in the HSV-1 thymidine kinase (TK) on drug susceptibility.

Methods: 14 novel HSV-1 TK mutations detected in clinical samples by RegaVir (the Belgian National Reference Center for antiviral resistant Herpesviridae) were included. Pure viral clones were isolated by serial dilution from clinical samples that propagated in cell culture. If not, recombinant viruses were constructed by en passant mutagenesis of an HSV-1 bacterial artificial chromosome. Susceptibility of the 14 mutant viruses to TK dependent (acyclovir, penciclovir, ganciclovir, brivudine, trifluridine) and TK independent (foscarnet, cidofovir) drugs was evaluated by cytopathic effect reduction assays.

Results: Viral clones harboring the E83Q, A93V or Q104H mutation were isolated and could be linked to drug resistance. Recombinant virus strains harboring 11 distinct TK mutations (E36D, S74A, E83G, E95K, P155Q, E210K A234E, F289C, L327P, S332W, or L343P) were constructed and are currently being evaluated for their drug susceptibility.

Conclusions: By linking novel HSV-1 TK mutations that were detected in the clinic to drug-resistant or polymorphism phenotypes, this study facilitates the interpretation of genotypic resistance testing, ultimately improving patient care.



Oral Presentations

Friday | 18th September

OP35

Improving Immune Responses In Avian IAV H5 Immunization By Sustained Antigen Release Technology

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The recent outbreaks of highly pathogenic avian influenza A(H5N1) virus among domestic and wild birds worldwide underscore the need for a vaccine to prevent human transmission. In 2024, Seqirus's zoonotic influenza A(H5N8) vaccine was approved to protect against highly pathogenic clade 2.3.4.4b H5N1 avian influenza viruses. However, the regimen requires a booster dose after primary immunization to improve efficacy, which can reduce compliance. Research suggests that prolonged antigen exposure during the primary immunization strengthens humoral immunity, motivating improved vaccination strategies. Sustained antigen release via DelSiTech Silica Matrix™ Technology, coupled with adjuvant incorporation, may enhance immunogenicity and overall vaccine effectiveness.

This study aimed to compare humoral responses after extended (silica-formulated), escalating (seven doses; 0.2%–63.3%; positive control), or traditional bolus subunit immunization in mice. C57BL/6 mice were immunized subcutaneously with recombinant H5 hemagglutinin (~3.75 µg) and MF95-like (Addavax) adjuvant. Encapsulated H5 microparticles were embedded in silica hydrogel (Silica Matrix™ Technology) for sustained antigen release. Serum H5-specific IgG, IgM, IgA and cross-reactivity to H3/H1 were evaluated at weeks 3 and 6 by an in-house ELISA method.

While the escalating antigen-dosing regimen elicited robust IgG responses, these remained lower than those observed in the silica groups, which generated strong IgG at week 3 with only minor attenuation by week 6 post-priming. An adjuvant was necessary for good IgG responses in all groups except those receiving silica. Weak H5-specific IgM responses appeared only in escalating dose groups, and no IgA was detected. The escalating dose + adjuvant group showed limited cross-reactivity against H1 and H3 subtype virus proteins.

The findings suggest that a single dose immunization using Silica Matrix™ Technology yields superior humoral IgG responses, outperforming traditional bolus injections.

OP36

Development of VHH anti-ACE2 to broadly inhibit SARS-CoV-2 infection

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¹Institut Pasteur

Introduction/Aim: Six years after the COVID-19 pandemic, the continuous emergence of antibody-escape variants of SARS-CoV-2 requires the identification of alternative methods of protection against infection. Indeed, currently approved antibodies target the viral spike protein, which accumulates mutations over time, thereby rendering new variants resistant to these antibodies. Here, we targeted the human angiotensin-converting enzyme 2 (hACE2), the major entry receptor for SARS-CoV-2, instead of the viral spike protein. This host-targeting strategy requires preserving the physiological function ACE2, given its essential role in blood pressure regulation.

Materials and methods: We generated heavy-chain-only antibodies (VHHs) from an alpaca immunized with a soluble form of hACE2. They were tested for their ability to bind ACE2, disrupt or not ACE2 physiological function, and inhibit SARS-CoV-2 infection both in vitro and in vivo in hACE2-expressing mice.

Results: These VHHs bind hACE2 without affecting the enzymatic activity of the protein and two of them inhibit all SARS-CoV-2 isolates tested. Their X-ray structure in complex with hACE2 show that their epitope overlaps with the footprint of the receptor binding domain (RBD) of the SARS-CoV-2 spike on hACE2. A dimeric fusion construct, named B07-Fc, avidly binds hACE2 (KD = 0.1 nM) and inhibits in vitro infection of ten tested variants with an IC50 ~ 1 nM. In vivo, experiments using K18-hACE2 mice show that intranasal prophylactic administration of B07-Fc confers dose-dependent protection against SARS-CoV-2.

Conclusions: These VHHs targeting hACE2 represent potential broad-spectrum therapeutic candidates against potential new emerging coronaviruses using hACE2 as a receptor.



Oral Presentations

Friday | 18th September

Session 10

Genomic strategies in viral diagnostics: innovations and challenges

OP37

Respiratory Metagenomics in the UK National Health Service: A Prospective Multisite Observational Study

Dr. Luke Blagdon Snell¹; Dr. Rahul Batra²; Dr. Jonathan Edgeworth²; Dr. Gaia Nebbia²; Dr. Ronan Doyle³; Dr. Jasmin Islam⁴; Dr. Alberto San Francisco⁴; Dr. Jonnie Cohen²; Dr. Julian Muller²; Dr. Manon Jaboyedoff²; Dr. Nicholas Price²; Dr. Adela Alcolea-Medina²;

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Introduction: The UK National Health Service (NHS) Respiratory Metagenomics Network (<https://metagenomics.nhs.uk>) is deploying rapid metagenomic testing for respiratory infection in several centres. We present the first multisite evaluation of this implementation.

Methods: A prospective observational study across five UK NHS hospital sites. Data were captured on demographics, clinical context and antimicrobial prescribing. Outcomes included detection of extra pathogens and impact on antimicrobial therapy. Univariable analysis used binary logistic regression.

Results: 179 cases underwent metagenomic testing. Overall, 58/179 (32%) were female, with a median age of 55 years (IQR 31-64). Three quarters (100/179, 74%) were intubated with 33/179 (18%) on ECMO. Bronchoalveolar lavage accounted for half (98/179, 55%) of samples, with other lower respiratory tract samples comprising 61/179 (34%). Ten percent (18/179) were non-respiratory samples. Additional pathogens beyond standard-of-care testing were detected in 50/179 (28%) cases. Antimicrobial impact occurred in 56/179 (31%), comprising de-escalation in 32/179 (18%) and escalation in 24/179 (13%).

Extra pathogen detection was more common in paediatric cases (16/32, 50% vs 34/147, 23%; OR 3.3, 95% CI 1.51-7.34, $p=0.003$). Antimicrobial impact was more common in paediatric cases (15/32, 47%) than adult cases (41/147, 28%) (OR 2.3, 95% CI 1.04-4.99, $p=0.04$).

There was no difference in extra pathogen detection in immunocompetent cases (40/133, 30%) compared to immunocompromised cases (10/46, 22%) (OR 0.65, 95% CI 0.29-1.43, $p=0.28$). There was also no difference in antimicrobial impact between immunocompetent cases (39/133, 29%) and immunocompromised cases (17/46, 37%) (OR 1.4, 95% CI 0.70-2.86, $p=0.34$).

Conclusions: Respiratory metagenomic testing identified additional pathogens in over a quarter of cases and influenced antimicrobial prescribing in nearly one third. Further evaluation will assess impacts across populations, clinical indications and utilise multivariable analysis to control for confounders.

OP38

A three-year assessment of clinical metagenomics applied to complex diagnostic support in the Portuguese National Reference Laboratory

Dr. Rita Ferreira¹; Dr. Luís Coelho¹; Dr. João Dourado Santos¹; Dr. Daniel Sobral¹; Dr. Nuno Lopes¹; Dr. Joana Isidro¹; Dr. Verónica Mixão¹; Dr. Miguel Pinto¹; Dr. Alexandra Nunes¹; Dr. Sílvia Duarte²; Dr. Daniela Santos²; Dr. Sara Rangel²; Dr. Mariana Antunes²; Dr. Luís Vieira²; National Hospital Network³; Dr. Vítor Borges¹; Dr. João Paulo Gomes¹;

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Introduction: The Portuguese National Institute of Health (INSA) is the reference laboratory which, among its multiple roles, supports the national hospital network in diagnosis. In this context, INSA implemented a metagenomics pathogen detection methodology to aid clinicians in complex diagnosis. Here, we provide a three-year overview of this experience, addressing the types of samples received, experimental issues, microorganisms detected, and the evaluation of the method's impact based on clinicians' feedback.

Material and Methods: Over these three years, INSA processed 255 samples from multiple matrices, including CSF (47%), biopsies (18%), among others (blood, BAL, etc), most of them collected from hospitalized patients with severe conditions and no established diagnosis, using an established probe-based metagenomics pathogen detection methodology [Ferreira et al, 2025]. An harmonized survey was provided to clinicians to assess metagenomics impact.

Results: Genetic sequences of microorganisms were detected in 171 (67%) of samples, ranging from a median of 1.25 microorganisms detected in CSF to 3.6 in naso/oropharyngeal swabs. Detections enrolled 46% viruses, 50% bacteria, 4% fungi, and only one parasite. The 5 most frequently identified microorganisms were Epstein-Barr virus (HHV-4), Citomegalovirus (HHV-5), Torque teno virus, HSV-1 and HSV-6.

According to clinicians' feedback, clinical metagenomics had a positive benefit in approximately 50% of cases, including identification of microorganisms consistent with clinical presentation, exclusion of an infectious etiology (e.g., suggesting autoimmune or neoplastic disease), changes in therapeutic management, among others.

Conclusions: This study consolidated the added-value of metagenomics towards a more informed clinical decision for the management of complex clinical cases.



Oral Presentations

Friday | 18th September

OP39

Feasibility of Monkeypox Virus Genome Sequencing from Antigen Rapid Diagnostic Tests to Enhance Decentralized Genomic Surveillance

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Genomic surveillance of monkeypox virus (MPXV) is critical to monitor viral evolution and inform public health responses, but remains limited in remote resource-constrained settings. MPXV antigen rapid diagnostic tests (Ag-RDTs) are increasingly considered for decentralized diagnosis and may represent an alternative source of viral DNA for sequencing. We evaluated the feasibility of recovering and sequencing MPXV DNA from used Ag-RDT devices.

Three commercially available MPXV Ag-RDTs were evaluated using serial dilutions of a cultured MPXV subclade Ib isolate. Following testing, positive Ag-RDTs with different test band intensities were stored for 19 days at room temperature or +4°C. Viral DNA was extracted from test pads and tested by PCR. Whole-genome sequencing was performed using metagenomic and tiled amplicon-based Oxford Nanopore approaches. Sequencing performance was assessed by genome coverage, depth, and subclade assignment. The same protocol is being applied to a subclade Ib clinical sample.

MPXV DNA was successfully recovered from all Ag-RDTs, with an average of 3 log₁₀ reduction in viral copies versus input. Both sequencing approaches enabled subclade identification in nearly all testing conditions (across three Ag-RDTs, three band intensities, and two storage temperatures). The tiled amplicon-based method showed superior and consistent performance, achieving >85% genome coverage in all samples and >90% in 77.8% of cases. In contrast, metagenomic sequencing demonstrated more variable coverage, but better representation of genome extremities and potential to detect new mutations and variants. Sequencing results from the clinical sample are pending.

This proof-of-concept study demonstrates that MPXV genomes can be reliably sequenced from used Ag-RDTs, even after prolonged storage without cold chain. Ag-RDTs could therefore serve not only as diagnostic tools but also as practical sampling devices for genomic surveillance, potentially expanding sequencing capacity and enhancing outbreak monitoring in remote and resource-limited settings.

OP40

Detection of Human Pegivirus by Clinical Metagenomics: Association with Neurological Presentations and Clinical Suspicion

Dr. Sonia Lacourt¹; Dr. Nicolas Veyrenche¹; Dr. Vichita Ok¹; Dr. Hervé Lecuyer¹; Dr. Emilie Sitterlé¹; Dr. Agnès Ferroni¹; Dr. Anne Jamet¹; Dr. Marianne Leruez-Ville¹; Dr. Jacques Fourgeaud¹;

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Introduction and Objectives: Human pegivirus (HPgV) is a common blood-borne RNA virus historically considered non-pathogenic. However, cases of pegivirus-associated encephalomyelitis (PAEM) characterized by optospinal involvement (optic neuropathy and myelitis) were described in immunocompromised patients. HPgV was detected in plasma, brain tissue and cerebrospinal fluid (CSF), with compartmentalization in the central nervous system (CNS) suggesting neurotropism. We aimed to evaluate the association between HPgV detection by clinical metagenomics (mNGS) and this neurological phenotype, as well as the clinicians' ability to suspect it.

Materials and Methods: A retrospective analysis was conducted on samples analyzed by mNGS between 2020 and 2026. HPgV detections were cross-referenced with clinico-biological data (sample type, indication, immune status), and proportions were compared using Fisher's exact test. A PAEM phenotype (optic neuropathy/atrophy and/or myelitis) was clinically defined and compared to other neurological presentations. Clinical suspicion was classified as de novo versus follow-up of a known case.

Results: HPgV was detected in 2.8% (63/2,241) of samples (52 patients), including 18 CSF samples. Positivity was significantly higher in immunocompromised patients (4.8%; 45/945) than in immunocompetent individuals (1.1%; 7/642) (OR = 4.5; p < 0.001). It was more frequent in neurological indications (4.2% vs. 2.3%; OR = 1.9; p = 0.02). Among these, HPgV was strongly associated with the PAEM phenotype (11.5% [9/78] vs. 2.3% [9/388]; OR = 5.5; p < 0.001). For target cases (PAEM with positive HPgV), a priori suspicion had a positive predictive value of 71% (5/7) and a sensitivity of 56% (5/9); about one-fifth of these cases were incidental findings.

Conclusions: These data show a strong and specific association between HPgV and the optospinal PAEM phenotype in immunocompromised patients. While not proving causality, they encourage HPgV screening in unexplained optospinal encephalomyelitis. Virological confirmation (CSF/serum viral load, CNS compartmentalization) and prospective multicenter studies remain necessary.



Oral Presentations

Saturday | 19th September

Session 11

Viral diagnostics: innovations and challenges

OP41

Improved Methods to Diagnose iciHHV-6

Sr. Alexander Lennon¹; Dr. Sarah Buddle¹; Dr. Leticia Scalioni²; Dr. Oscar Torres²; Prof. Judith Breuer²; Dr. Julianne Brown¹;

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Introduction/Aim: Human herpesvirus 6 (HHV-6) integrates into the telomeric regions of host chromosomes. If this occurs in sperm or ovum cells the viral genome is passed down by Mendelian genetics, known as inherited chromosomally integrated HHV-6 (iciHHV-6), where one copy of the virus exists within every nucleated cell in the host's body. When HHV-6 is detected by PCR, it is essential to differentiate between iciHHV-6 and active infection to enable appropriate clinical management. Patients with iciHHV-6 are expected to have one copy of HHV-6 per copy of the human genome.

We aimed to develop digital PCR (dPCR) to accurately assess the ratio of HHV-6-to-human genomic DNA and explore sequencing to identify integration sites.

Material and Methods: DNA was extracted from 46 residual whole blood samples known to be positive by PCR for HHV-6 and analysed using a triplex dPCR assay targeting HHV-6A, HHV-6B and the human RPP-30 gene to determine viral-to-host genomic ratios.

A subset of samples, including iciHHV-6 and non-iciHHV-6, underwent target enriched whole genome Illumina sequencing (n=10) or a modified target enrichment protocol for nanopore sequencing optimized for ~2kb fragments (n=8), followed by viral integration site analysis using CLC Genomics Workbench.

Results: In patients with suspected iciHHV-6 (n=13 whole blood, n=2 CSF), HHV-6 genomic ratios ranged from 0.88 - 1.12 copies/genome (mean 0.96 copies/genome). When whole blood that had been frozen was excluded, the ratio was 0.94 - 1.02.

Illumina sequencing achieved high genome coverage (>99.6%), but failed to identify integration sites. Nanopore read length and bioinformatics tools require optimisation for integration site detection.

Conclusions: dPCR from fresh whole blood provides an accurate and reproducible method to detect 1:1 ratio of viral to human genomic DNA, suggestive of iciHHV-6. Short read sequencing is insufficient to span the telomeric repeat regions therefore cannot identify integration sites, however this may be overcome by long-read sequencing.

OP42

Next-Generation Sequencing of Human Cytomegalovirus for the Identification of Resistance Mutations

Ms. Hope Johnston¹; Dr. Olivia Turner¹; Sr. Max Djemal¹; Dr. Maria-Teresa Cutino-Moguel¹; Dr. Kathryn Harria¹;

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Human cytomegalovirus (CMV) is a significant cause of morbidity in immunocompromised patients, particularly solid organ and haematopoietic stem cell transplant recipients. Antiviral resistance, often emerging after prolonged exposure to ganciclovir or valganciclovir, limits treatment options and complicates management. In the UK resistance testing relies on referral laboratories using Sanger sequencing, with turnaround times of up to two weeks. A next-generation sequencing (NGS) workflow for CMV resistance testing was developed and evaluated for implementation into existing virus sequencing service.

An NGS assay targeting CMV resistance-associated genes was developed using multiplex PCR, Nextera XT library preparation and sequencing on Illumina MiSeq/MiniSeq platforms. Validation was performed using the WHO International Standard for HCMV and twelve clinical samples (viral loads 1.05×10^3 – 1.9×10^7 IU/mL), including 3 with known resistance mutations. Bioinformatic analysis included sequence assembly, quality control, variant detection, and resistance interpretation using the University of Ulm, CHARMD and UCL databases. Results were compared with reference reports.

The estimated limit of detection was ~2 log IU/mL. Complete sequence concordance was achieved in 8/12 (66.7%) samples, with partial concordance in 1/12 (8.3%), UL97 failed to amplify. 3 samples failed to amplify, primarily at low viral loads. UL97 mutations (H411L) associated with maribavir resistance were correctly identified in concordant samples. No ganciclovir resistance mutations were detected by either laboratory.

This study demonstrates the feasibility of in-house CMV resistance testing using NGS. Ongoing work will include further limit-of-detection studies, analysis of additional clinical samples during a 12-month period of testing in parallel with the reference laboratory. Implementation into the existing service would be at minimal additional cost due to shared sequencing runs and has the potential to improve access to testing and enable faster clinical decision-making in high-risk populations.



Oral Presentations

Saturday | 19th September

OP43

When fever misleads atypical measles in a patient with high-risk Lassa virus exposure

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¹Geneva University Hospitals

Aim: We describe the case of a 38-year-old woman evaluated for fever developing 15 days after high-risk contact with Lassa virus, who had received a 10-day post exposure prophylaxis course of ribavirin started on day 2.

Methods: Diagnostic work-up included daily plasma and repeated naso-pharyngeal (NP) testing by RT-PCR for Lassa virus. Respiratory viruses, arboviruses, HIV, CMV, EBV and malaria infections were assessed by RT-PCR, serology, rapid tests and blood smears. Lassa virus serology (IgG, IgM) was conducted at the Bernhard-Nocht-Institute, Germany. Final diagnosis was retrieved by unbiased metagenomic next-generation sequencing (mNGS) after hospital discharge.

Results: Fever up to 40°C degrees was the only relevant clinical sign during hospitalization (no pharyngitis, no rash). Laboratory testing revealed microcytic anemia, lymphocytopenia and elevated CRP level. All RT-PCR tests for Lassa virus were negative, as was testing for Dengue, Chikungunya, Zika, HIV, respiratory viruses and malaria. Serological results indicated past EBV, CMV and dengue infections. A 5-day course of levofloxacin was empirically started on day 16 after contact. Fever subsided and the patient was discharged on day 18. Blood cultures and Lassa virus serology assessed on day 15 and 21 after contact remained negative. Unbiased mNGS on two pooled plasma samples retrieved respectively one and two read pairs of measles virus (genotype B3). The diagnostic was later confirmed by RT-PCR on plasma samples and on the second NP swab and displayed high Ct-values. The patient had received 1 documented dose of MMR vaccine 4 years prior and 2 self-reported doses during childhood. Measles IgG were > 1000 UI/l, with no detectable IgM.

Conclusion: Breakthrough measles infection may occur without rash in vaccinated individuals thus representing a diagnostic pitfall. This may have significant logistical impact in the context of evaluation of a high-consequence infectious disease.

OP44

Masking Recurrent Contaminants in Reference Sequences Improves Specificity of Clinical Metagenomic Sequencing

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Viral metagenomic next-generation sequencing (mNGS) is a powerful approach for pathogen detection in clinical diagnostics; however, accurate virus identification depends critically on the quality of reference databases used for taxonomic classification. A recurrent challenge is the erroneous assignment of mNGS reads to viral taxa, frequently arising from kitome-derived sequences aligning to contaminant regions embedded within viral reference sequences. Such systematic misclassifications occur across biologically unrelated samples and compromise diagnostic specificity, leading to false-positive viral detections.

VirMask is a novel computational strategy to systematically identify and mask recurrent contaminant regions within reference sequences. In a first step, VirMask aligns simulated human reads against a viral database and masks all human-derived regions identified within reference sequences. In a second step, VirMask identifies and masks persistent contaminant regions based on their high prevalence across independent metagenomic datasets. In our analysis, such contaminant regions commonly originated from non-viral elements, including ribosomal RNA, vector contamination (e.g., CMV enhancers), and sequencing adapters.

Finally, VirMask employs alignment-based similarity searches to detect and mask homologous regions from multiple reference sequences, thereby reducing noise in mNGS outputs and improving diagnostic specificity.

Using data from clinical metagenomic sequencing runs, we demonstrate that our masking approach usefully reduces the incidence of recurrent artefactual viral hits without impacting true pathogen detection. Our results underscore the necessity of rigorous viral database curation and offer a reproducible framework for enhancing diagnostic confidence in mNGS-based clinical virology.



Posters



Posters

1 Concurrent Surveillance for Chikungunya Virus in Dengue-Suspected and Confirmed Cases in Jazan, Saudi Arabia

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dengue share similar clinical symptoms, CHIKV infections are frequently misdiagnosed, complicating surveillance and control efforts. In Saudi Arabia, dengue is endemic in regions such as Jazan, where climatic conditions support mosquito breeding. Although CHIKV mortality is low, it can cause severe and long-lasting joint disease. This study aimed to detect CHIKV in dengue-suspected and confirmed cases from 17 provinces in Jazan.

A total of 138 blood samples were included in this study. These samples were obtained from patients who were clinically suspected of dengue infection but tested negative for DENV using both enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR). Detection of CHIKV RNA was performed using a commercial VIASURE RT-PCR kit, employing a set of CHIKV-specific primers according to the manufacturer's instructions.

All 138 blood samples tested negative for CHIKV RNA by RT-PCR, indicating no molecular evidence of CHIKV infection among the studied dengue-negative cases during the study period.

The absence of detectable CHIKV in the examined samples suggests limited or no circulation of CHIKV in the studied population in Jazan region at the time of investigation. However, continuous arboviral surveillance remains essential to promptly identify potential introductions or emerging outbreaks and to support effective public health preparedness strategies in dengue-endemic regions.

Chikungunya virus (CHIKV) is an emerging mosquito-borne virus of global public health importance, mainly transmitted by *Aedes* mosquitoes. It has caused outbreaks across Africa, Asia, Europe, and the Americas, often in areas where dengue virus (DENV) is also present. Because CHIKV and

Posters

3

A One Health, pan-European network strengthening surveillance, clinical management and control of zoonotic hepeviruses

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Background: Zoonotic hepeviruses, particularly hepatitis E virus (HEV, species *Paslahepevirus balayani*), represent an underestimated public-health threat in Europe. HEV is the leading cause of acute viral hepatitis, yet surveillance remains fragmented, blood donation screening for HEV is not always implemented and diagnostic algorithms differ widely between countries, leading to substantial under recognition. Chronic infections are increasingly reported in immunocompromised individuals, especially solid-organ transplant recipients, and therapeutic options remain limited in the absence of authorised antivirals or vaccines. Along the animal and food chain, HEV is classified by EFSA as a priority food-borne pathogen, but surveillance in livestock, wildlife and food products lacks harmonised standards. The identification of ratHEV (species *Rocahepevirus rattii*) as a cause of acute and chronic hepatitis in European countries further broadens the spectrum of zoonotic hepevirus infections

Methods: ONWARD (One Health Zoonotic Hepevirus Network; COST Action CA24140) was established to bridge scientific, clinical and policy gaps that limit Europe's preparedness and response. The network connects experts from 27 countries across virology, hepatology, veterinary medicine, food safety, epidemiology, ecology and public health. Its activities are organised into five Working Groups and cross-cutting Task Forces to promote harmonised diagnostics, integrated One Health surveillance and coordinated research agendas

Results & Conclusion: ONWARD is standardising molecular and serological methods for zoonotic HEV and ratHEV, defining common surveillance indicators and mapping transmission pathways at the human-animal-environment interface. The network builds a sustainable pan-European One Health framework through collaboration and shared resources.

4

Comparison of two IGRA assays exploring cell-mediated immunity against CMV, BKV and EBV in kidney transplant patients

Dr. Aurélie TRUFFOT

CHUGA

Monitoring cellular immune responses (CMI) has become essential in managing immunocompromised patients, especially kidney transplant recipients. This study compared the performance of two interferon-gamma (IFN- γ)-based assays — ELISpot and VIDAS®-IGRA — for detecting T-cell responses against cytomegalovirus (CMV), Epstein-Barr virus (EBV), and polyomavirus BK (BKV). Twenty-eight blood samples from kidney transplant recipients were analyzed using both assays. CMV-specific CMI was assessed using overlapping peptide pools from pp65 and IE-1 proteins (JPT Peptide Technologies GmbH) and a CMV lysate (bioMérieux). All patients were monitored for opportunistic viral replication through qPCR for up to six months after blood sampling.

For CMV-CMI, the results showed that both assays gave comparable proportions of positive responses following antigen stimulation (> 100 spots/250,000 PBMC in ELISpot or level of IFN- $\gamma > 1$ UI/mL in VIDAS®), with no significant differences observed between peptide pools. Positive responses were consistent with CMV serology. For EBV and BKV specific responses, assay agreement was acceptable, though responses were generally weaker than those for CMV.

This study highlights the importance of standardizing IGRA assays. While VIDAS®-IGRA RUO offers an open, automated and standardized solution, improvements in BKV peptide formulation and refinement of predictive thresholds are needed for optimal clinical use.

Posters

5

Torque teno virus load and antiviral T-cell immune response after belatacept initiation in kidney transplant recipients

Dr. Aurélie TRUFFOT

CHUGA

Emerging biomarkers are being investigated in renal transplant recipients to predict the level of immunosuppression, including Torque teno virus (TTV) DNA load and T-cell-mediated immunity (CMI) assays (e.g., ELISpot-T IFN- γ). This study assessed the impact of conversion from tacrolimus to belatacept on TTV load and CMI in kidney transplant recipients and explored their relationship with opportunistic viral infections as a proxy for immunological balance.

A total of 124 patients converted from tacrolimus to belatacept were included. TTV load and CMI were evaluated at baseline and 12 months. CMI was measured by ELISpot-T IFN- γ using anti-CD3 stimulation. Cytomegalovirus, Epstein-Barr virus and BK polyomavirus DNAemia were also assessed.

After 12 months, polyclonal CMI responses significantly decreased (1740 vs 1099 spots/250,000 PBMCs, $p = 0.04$), while the reduction in virus-specific responses was not significant. This decrease was independent of belatacept concentration, age and time from transplantation to conversion. Belatacept conversion had no effect on TTV load, although higher loads were observed in patients converted earlier (<4 years post-transplant). TTV load was not associated with age, belatacept exposure or time since transplantation. No increase in viral reactivation was observed.

Belatacept conversion did not significantly modify TTV load compared with tacrolimus but was associated with reduced polyclonal and antiviral CMI, supporting further investigation of its effect on T-cell IFN- γ secretion.

6

Donor-Derived West Nile Virus Infection in Kidney Transplant Recipients, France, 2025

Dr. Aurélie TRUFFOT

CHUGA

We report 2 cases of donor-derived West Nile virus infection in kidney transplant recipients in France. Both recipients had mild disease develop and recovered without sequelae. A more proactive screening strategy in France, particularly during periods of highest risk for West Nile virus circulation, would help reduce risk for donor-derived infections.



Posters

7

BK Virus Infection After Kidney Transplantation: A 7-Year Retrospective Study at a University Hospital Laboratory

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Introduction: BK virus is a small double-stranded DNA virus. Most commonly persist in healthy individuals after primary infection and may re-activate when immune system suppressed such as solid organ transplantation, bone marrow transplantation and cancer. The aim of this retrospective study was to investigate the presence of BKV in the clinical samples of adult kidney transplantation patients

Material and methods: A total of 2373 samples obtained from 228 kidney transplantation patients between 2018 to 2025, were included to the study. Viral nucleic acids were extracted from the samples with EZ1 Virus Mini Kit v2.0 in EZ1 Advanced device. BKV DNA were detected by using polymerase chain reaction PCR Artus®BK virus RG-PCR Kit in Rotor-Gene Q5plex instrument.

Results: Among the 228 patients included in the study, 154(67.4%) male and 74(32.6%) female, with a mean age of 40.47 ± 12.28 (range: 18–72 years). Total BKV positivity rate was found as 46%(105/228). The most frequently observed viral load was between 10^2 and 10^4 copies/mL. Our study shows that the distribution of the sample decreased over the years during the pandemic period (2020–2023). However, the number of tests increased again after the pandemic ended.

Conclusions: BKV infection is particularly important in immunosuppressed patients, especially kidney transplant recipients. Regulations in healthcare systems and infection control measures during the COVID-19 pandemic have led to disruptions in routine follow-up programmes for kidney transplant patients. Our study also showed a significant decrease in the number of samples analysed during the pandemic period. Routine quantitative monitoring of BKV viral load using PCR is particularly important for the effective clinical management of this patient group.

8

A challenging diagnosis in a premature infant: HHV-6 infection presenting with seizures and hydrocephalus

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Introduction/Aim: Chromosomally integrated HHV-6 (ciHHV-6) may re-activate through excision from the host chromosome in cases of immunodeficiency. The aim of this study was to determine whether HHV-6 detected in the cerebrospinal fluid (CSF) of a neonate by a central nervous system panel represented active infection or ciHHV-6.

Materials and Methods: A 28-week, 1080gr preterm female newborn delivered via emergency cesarean section due to chorioamnionitis was intubated and admitted to the NICU. Due to the development of hydrocephalus and worsening neurological status, a CSF sample was obtained and HHV-6 was found positive by Meningitis/Encephalitis panel and a quantitative real-time PCR. Nail, hair follicle and plasma samples from the parents of the newborn were collected to determine the ciHHV-6.

Results: Infant's CSF and plasma viral loads were 16920 copies/mL and 1210 copies/mL, respectively. Following deterioration of clinical status and increase in seizures, PCR analysis was repeated in both CSF and plasma samples. The viral loads in CSF and plasma were 289.000 copies/mL and 240 copies/mL, respectively. Based on these findings, ganciclovir treatment was initiated. During treatment CSF HHV-6 DNA levels were 71620/90610 copies/mL at one weeks intervals, respectively. HHV6-DNA viral loads were <185 copies/mL, 1350 copies/mL, 23000 copies/mL in the mother's plasma, nails and hair follicles, respectively, whereas all samples from the father were tested negative.

Discussion: A clinical response to ganciclovir treatment was observed with a decline in viral load. In preterm infants, HHV-6 DNA positivity cannot be assumed as ciHHV-6 without further evaluation. Serial measurements of CSF and plasma viral loads, interpreted with clinical findings, are necessary to distinguish active CNS infection and to guide appropriate treatment.



Posters

9

Molecular Surveillance of Enterovirus D68 in Portugal, 2024–2025

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Enterovirus D68 (EV-D68) has reemerged as a pathogen of public health concern, associated with respiratory illness and, in some cases, neurological complications. Despite its global spread, EV-D68 circulation in Portugal remains poorly characterized. This study aimed to investigate the presence, clinical spectrum, and molecular diversity of EV-D68 in the Algarve region between October 2024 and February 2025. A total of 150 respiratory samples previously testing positive for rhinovirus/enterovirus were screened for EV-D68. EV-D68 detection was performed by RT-qPCR. Positive samples underwent genomic characterization through Oxford Nanopore long-read sequencing. Bioinformatic analysis included quality filtering, host decontamination, and taxonomic classification. Phylogenetic analysis was performed using multiple sequence alignment and maximum-likelihood inference. Eight samples were confirmed EV-D68 positive, yielding a detection rate of 5.44%. Cases spanned a wide age range, from infants to older adults. Clinical presentations ranged from mild respiratory symptoms to severe disease, including dyspnea and seronegative autoimmune encephalomyelitis/encephalitis. Co-detections with other respiratory pathogens were observed in some cases. Genomic analysis identified one partial VP1 sequence within clade B3 and a complete genome belonging to clade A2, with phylogenetic clustering alongside contemporaneous European strains. These findings demonstrate the active circulation of multiple EV-D68 clades in Portugal and highlight the broad clinical spectrum associated with infection. Notably, this study represents the first genomic documentation of clade A2 in Portugal. Continued molecular surveillance is essential to monitor viral evolution and assess the public health implications of EV-D68 in the country.

10

Occurrence of Rat Hepatitis E Virus in Small Mammals of Portugal

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Rocahepevirus rattii (RHEV), classified within the family Hepeviridae, is an emerging pathogen of zoonotic concern, with human infections documented across Europe, Asia, and the Americas. Although rodents are widely regarded as the main reservoir hosts, information on RHEV circulation among wild small mammals in Portugal is currently limited. The present study sought to determine the occurrence and molecular features of RHEV in wild rodents throughout mainland Portugal. Liver samples (n = 145) were obtained from wild small mammals of Portugal. Initial HEV screening was performed using a pangenotypic RT-qPCR targeting ORF3, while RHEV detection employed a dual-target RT-qPCR assay with two primer/probe sets targeting distinct ORF1 fragments, improving sensitivity and minimizing false negatives. RHEV-positive samples underwent Sequence-Independent Single-Primer Amplification followed by Oxford Nanopore long-read sequencing. Bioinformatic analysis included quality filtering, host decontamination, and taxonomic classification and phylogenetic analysis was performed. No classical HEV RNA was detected. However, RHEV RNA was found in one *R. norvegicus* specimen. Phylogenetic analysis placed the found RHEV isolate within European clade I, genotype 1, sub-genotype a, in close relationship with strains linked to human cases. These results represent the first documentation of RHEV in wild rodents from Portugal, complementing earlier environmental detections in wastewater and suggesting a potential role of rodents in sustaining viral presence in urban environments. Despite the low detection rate, ongoing surveillance of rodent populations and environmental samples, alongside clinical monitoring, remains essential to better characterize the zoonotic risk posed by RHEV in Portugal.



Posters

12

Leveraging the Host Cell for Antiviral Therapy Through Drug Repurposing

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Introduction/Aim: Viral diseases pose a major global health burden, and the emergence of pathogenic viruses underscores the need for new strategies to identify antiviral therapeutics. Host-directed antivirals remain largely underexplored and may overcome limitations of direct-acting antivirals, including viral resistance and restricted broad-spectrum activity. Here, we present a host-centric drug repurposing strategy using SARS-CoV-2 as a model to identify antiviral candidates relevant to current and future viral threats.

Materials/Methods: We screened over 5,200 repurposable compounds using an integrated phenotypic framework combining Cell Painting-based morphological profiling, antibody detection of viral proteins, and a cytopathicity assay.

Results: We show that Cell Painting detects infection-induced phenotypic changes that are reversed by antiviral treatment. The screen identified 324 primary hit compounds, which were validated in human A549ACE2 lung epithelial cells and counter-screened for drug-induced phospholipidosis, a confounding mechanism associated with apparent antiviral activity. We present 74 phenotypic hit compounds, including 44 novel or previously reported broad-spectrum antiviral candidates. Pathway enrichment analysis of the host targets highlighted cellular processes commonly exploited by viruses, including protein kinase signaling and innate immune pathways. To assess broader relevance, we show that morphological profiling can detect virus-induced phenotypic changes across multiple RNA viruses and cell systems, including Zika virus and HCoV-229E.

Conclusions: Together, these findings demonstrate a host-centric framework for identifying antiviral candidates with relevance to emerging and re-emerging RNA viruses, contributing to global antiviral preparedness.

13

The Role and Significance of Gastrointestinal Multiplex PCR Panel in Inflammatory Bowel Disease Flares: Evaluation of Viral Agents and Clostridioides difficile Positivity

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Introduction/Aim: Inflammatory bowel disease (IBD) is a chronic disorder characterized by remission and flares. Infections should be ruled out both at diagnosis and during flares; this study evaluated the role of gastrointestinal multiplex PCR panels in differentiating infectious gastroenteritis from IBD flares.

Materials and Methods: Stool samples from 1450 patients were analyzed using the QIAstat-Dx® Gastrointestinal Panel test in 2024–2025. Positive panel results from IBD patients were evaluated according to demographic characteristics, pathogen distribution, and microbiological findings

Results: Among all samples, 539 (37.2%) were positive. A confirmed IBD diagnosis was present in 220 patients (15.2%) with a mean age of 37 years; 97 (44%) were female, 123 (56%) male, 79 (36%) children, and 141 (64%) adults. Panel positivity was found in 61/220 (27.72%) IBD patients. Among panel-positive IBD patients, 8.8% of pathogens were viral (Norovirus, Rotavirus, Sapovirus), 13.8% were Clostridioides difficile, 76.25% bacterial agents other than C. difficile, and 1.25% a parasitic agent. No statistically significant differences were found between IBD and non-IBD patients regarding viral or C. difficile positivity ($p > 0.05$). Clostridioides difficile toxin A/B testing was negative in both groups.

Conclusions: Panel positivity was lower in IBD patients than in other groups tested for diarrhea etiology. This suggests every diarrheal episode in IBD is not infection related and flares and infections must be carefully differentiated. Also, high bacterial detection in panels may indicate colonization. Overall, gastrointestinal multiplex PCR panels can be useful for identifying infectious agents in IBD patients; however, results, particularly for C. difficile and viral pathogens, should always be interpreted with clinical and laboratory data.



Posters

14

Viral Load Evaluation in EBV-Associated Malignancies and the Prognostic Impact of Recurrent Positivity

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¹Gazi University Faculty of Medicine, Department of Medical Microbiology; ²Gazi University Faculty of Medicine, Department of Medical Microbiology, Division of Virology; ³Gazi University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology;

Introduction/Aim: Epstein-Barr virus is the first virus to be identified as being associated with malignancies. The aim of this study was to determine the rate of EBV positivity in EBV-associated malignancies and to evaluate the prognostic impact of recurrent viral positivity.

Material and Methods: In this study, a total of 6444 samples from 2062 patients, collected for the detection of EBV DNA between 2022 and 2025. Viral nucleic acid extraction was performed on the EZ1 Advance-dXL instrument using the EZ1&2 Virus Mini Kitv2.0 and viral nucleic acid amplification determined with Artus® EBV RG-PCR Kit. The test results were analyzed according to age, sex and the malignancies associated with EBV.

Results: According to the test results, 379/2062 (18%) patients were positive for EBV. Among the EBV-positive patients, 209 (55%) were male, 170 (45%) were female; 157 (41%) were children and 222 (59%) were adults. Among the EBV-positive patients, 51 (13%) were diagnosed as malignancies strongly associated with EBV and we found the most frequent EBV-associated malignancy was non-Hodgkin lymphoma (39%), followed by nasopharyngeal carcinoma (33%), T-cell lymphoma (10%), Burkitt lymphoma (8%), Hodgkin lymphoma (6%) and both B-cell lymphoma and gastric cancer (2% each).

Conclusions: Previous reviews have reported that, among EBV-associated malignancies, nasopharyngeal carcinoma shows the strongest association with EBV. According to our results, non-Hodgkin lymphoma was the most common EBV-associated malignancy. It is also among the ten most common cancers in both sexes worldwide, according to GLOBOCAN 2022 data. Particularly in immunosuppressed patients, reactivation of latent infection may lead to the development of EBV-associated malignancies; therefore, quantitative viral load monitoring is important for predicting disease progression and directing therapeutic strategies.

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Longitudinal viral kinetics in healthcare workers infected with SARS-CoV-2: implications for infection prevention and return to work strategies for future threats

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Introduction: The SARS-CoV-2 pandemic highlighted the need for harmonized surveillance and evidence-based infection prevention strategies in health care personnel. Despite extensive experience, optimal duration of isolation and the utility of test-based return-to-work approaches for healthcare workers (HCWs) remain uncertain. Insights into within-host viral dynamics across variants are essential to refine infection prevention measures and improve preparedness for future outbreaks. We aim to analyse longitudinal viral kinetics in HCWs infected with different SARS-CoV-2 variants, evaluate the implications for infection prevention and work-ban strategies, and inform evidence-based policies for future pandemic preparedness.

Material and Methods: We conducted a longitudinal observational study among HCWs with 4975 confirmed SARS-CoV-2 cases across successive variant periods. Sequential oro- and naso-pharyngeal swabs were collected for RT-PCR testing to monitor Ct-value trajectories over time. Viral kinetics were compared across variants and viral loads at set time point were analysed.

Results: Delta and Omicron show high peak loads compared to Alpha and Non-VOC. All variants display high loads at day 5 and 7 after initial test. Median time to reach cyclic threshold values of 30 was approximately 10 days for all variants.

Conclusion: This study offers detailed insight into the variant-specific viral kinetics of SARS-CoV-2 among HCWs, with implications for infection prevention policies and return-to-work decisions. By applying longitudinal Ct-value monitoring, we demonstrate the limitations of uniform time-based isolation strategies. Test-based return-to-work approaches may improve safety and workforce continuity in future outbreaks. Moreover, we opt for comparable data for cross-country comparability aiding in rapid insight in novel threats.

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Seasonal Distribution and Respiratory Viral Coinfections in Influenza Cases

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Introduction: Influenza viruses are responsible for significant morbidity and mortality every year in the world. Early diagnosis is important in preventing the spread of the disease, as well as in providing effective treatment. This study aimed to analyze the seasonal distribution of influenza infection and coinfections with other respiratory pathogens.

Material and method: Our study includes a total of 11.358 nasopharyngeal swab samples sent to our laboratory between 2024 and 2025. Of the samples included in the study, 6.128(54.0%) male and 5.230(46.0%) female. Patients were between 0 and 89 years old. Influenza positivity was detected using three commercial assays (FTD Respiratory 21, Neumodex and QIAstat Respiratory Panel) applied according to routine laboratory practice. Patients were classified according to season and coinfections with other respiratory pathogens.

Results: Among the analyzed samples, 934(8.22%) were positive for influenza A and 114(1.00%) for influenza B, with a total of 1.048(9.22%) influenza positive cases. Influenza positive cases were most frequently reported in January, February and March. The highest positivity rates were observed in the 18-49 and ≥65 age groups. Coinfections were detected in 139(13.3%) influenza positive cases, most common Rhino and RSV A/B with the highest proportion in the ≥65 age.

Conclusion: Influenza A was detected more frequently than influenza B, consistent with previous epidemiological studies. The increase in cases during January-March corresponds to the typical winter circulation of influenza viruses in temperate regions. In addition, the higher positivity rates observed in the 18-49 and ≥65 age groups and the presence of coinfections with other respiratory viruses emphasize the importance of continuous surveillance and early diagnostic testing during peak influenza seasons.

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Age Distribution and Seasonal Variation of Human Respiratory Syncytial Virus (RSV) Cases Between 2018 and 2025

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Introduction: Respiratory syncytial virus (RSV) is one of the leading causes of lower respiratory tract infections (LRTIs) in children, particularly during the winter and spring seasons. This study aimed to evaluate the age and seasonal distribution of RSV positivity rates detected in our laboratory between 2018 and 2025.

Material and method: Our study included a total of 16860 nasopharyngeal swab samples collected from patients aged 0-102 years between 2018 and 2025. Nucleic acid extraction was performed using the EZ1 & 2 Virus Mini Kit on the EZ1 Advanced XL instrument, followed by amplification using the FTD Respiratory 21, FilmArray Respiratory Panel 2 Plus (bioMérieux, France), QIAstat-Dx Respiratory SARS-CoV-2 Panel (Qiagen, Germany), and NeuMoDx Flu A-B/RSV/SARS-CoV-2 (Qiagen, Germany) kits. Positive cases were analyzed according to gender, age, and season.

Results: In our study, the RSV positivity rate was 5.9%. Of the positive patients 52.6% were male and 47.4% were female. The highest positivity rates were observed in the 1-5 months age group (31.3%). RSV-positive cases showed an increase in November and peaked in December. The positivity rates were 13.6% in December, 10.6% in November, and 10.2% in January.

Conclusion: The highest positivity was observed in the 1-5 age group, indicating that the epidemiological and clinical burden of RSV is predominant in early childhood and remains a significant health problem. RSV maintains its seasonal characteristics. RSV has been shown to cause LRTIs particularly in early childhood; therefore, the implementation of preventive strategies and strengthening of infection control measures are essential.



Posters

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External Quality Assessment in the Portuguese Screening Network for HIV, HBV, HCV and Syphilis: Participation, Performance and Team Characterization (2018–2025)

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Introduction: Rapid diagnostic tests (RDTs) are used in screening programs for the early detection of infections of public health relevance: HIV, HBV, HCV and syphilis. External Quality Assessment (EQA) programs enable monitoring of analytical performance and promote improvement of testing practices. This study describes the participation and performance of entities within the Portuguese Screening Network (PSN) in EQA programs between 2018 and 2025 and characterizes team organization through a questionnaire applied in early 2026.

Material and Methods: A retrospective observational study was conducted using data from the PSN EQA programs between 2018 and 2025. The number of registered entities, participation rates and proportion of correct results were analyzed by infection and year, as well as performance according to RDT brand. In February 2026, a questionnaire was applied to characterize organizational structure, training needs, and practices related to reagent use and lot traceability. Data were analyzed descriptively.

Results: Between 2018 and 2025, the number of registered entities increased overall, reaching the highest value in 2025 (18). Participation rates and analytical performance were high, particularly for HBV (99% and 93%), HIV (93% and 95%) and HCV (94% and 99%). Syphilis showed slightly lower participation rate (83%) and analytical performance (90%). The questionnaire revealed that most teams consisted of 3–5 members (56%), with moderate staff turnover (56%) and predominantly mixed operation (fixed and mobile) (67%). More than half reported the need for additional training (56%). The use of different reagent brands varied by infection, and 59% of teams reported systematic recording of reagent lot numbers.

Conclusions: The PSN showed high participation and consistent analytical performance in these EQA programs.

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Results of a Proficiency Testing Scheme for molecular testing of West Nile Virus

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Introduction/Aim: Molecular techniques in WNV RNA testing are standard in Europe for blood and organ donation screening from subjects from endemic WNV areas, and in the identification of viral genomes in sample matrices from patients presenting a clinical picture typical of WNV infection. As an increasing number of commercial assays have come to market, an understanding of the limitations of some assays is important to assess when implementing into clinical practice. To allow laboratories to assess their proficiency in the detection of WNV and differentiation from other flaviviruses, proficiency testing (PT) schemes are an invaluable tool.

Material/Methods: WNV RNA PT samples over 5 consecutive years were analysed. Non-WNV Flaviviruses like Dengue Virus Type 1 (DENV-1), Japanese Encephalitis Virus (JEV), Yellow fever Virus (YFV), Zika Virus (ZIKV), and/or Usutu Virus (USUV) samples were included in the panels. Out of 50 samples, 38 samples were defined WNV samples with different viral loads, 7 were specificity samples (non-WNV Flavivirus) and 5 samples were true negatives.

Results: Specificity non-WNV Flavivirus samples were correctly tested as negative for WNV by 90% of the in-house PCR assays and commercial assays. The USUV samples were correctly tested negative with the WNV specific in-house PCR assays, whereas some of the commercially available assays gave false positive results for WNV RNA.

Conclusions: Cross-reactivity was observed for some WNV molecular tests. The clinical relevance of non-detected or as WNV-positive detected USUV infection in humans and the risk of USUV to blood safety are unknown however needs to be carefully considered.



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Interpreting Anti-HEV Total Positivity: The Role of Clinical Context in Routine Viral Serology

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Background: Hepatitis E virus (HEV) is an underrecognized cause of acute hepatitis and is often overlooked in routine diagnostic algorithms. Patients with elevated transaminases are typically evaluated for hepatitis A, B, C, and toxic causes, whereas HEV testing is not systematically performed, potentially leading to underdiagnosis.

Methods: This retrospective study included consecutive patients with anti-HEV total positivity identified at a tertiary care center between January 2019 and December 2025. Clinical indications were categorized based on diagnostic codes. Biochemical severity was classified into three tiers using ALT, AST, and INR levels. Liver injury patterns were assessed using the R ratio. Concurrent viral markers (HAV IgM, HBsAg, anti-HCV) were analyzed, and triple-negative status was defined.

Results: Among 1,245 patients tested, 160 (12.9%) were anti-HEV total positive. Median age was 68.5 years, with most patients older than 50 years. Biochemical findings were heterogeneous, with 39.6% classified as severe (Tier 3). R ratio analysis showed predominantly cholestatic (59.0%) and mixed (21.2%) patterns. Among patients with complete viral serology, 84.3% were negative for HAV, HBV, and HCV markers. Patients with hepatic presentation and triple-negative serology showed a higher proportion of severe biochemical injury, although not statistically significant.

Conclusions: HEV may be an overlooked cause of elevated transaminases in patients negative for conventional viral hepatitis markers. The high rate of anti-HEV positivity suggests that HEV infection may be more common than currently recognized. Incorporating HEV testing into the evaluation of unexplained liver enzyme elevation may improve diagnostic accuracy.

Keywords: Hepatitis E, Anti-HEV total, Acute hepatitis, Serological markers, Liver injury patterns

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Characterization of a Sabin-like type 2/3 recombinant poliovirus with a triple nucleotide deletion in the VP1 region isolated from sewage

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Introduction/Aim: Nucleotide deletions within the VP1 capsid region of poliovirus are exceptionally rare. This study characterized a natural Sabin-like type 2/3 recombinant poliovirus strain (CHN-E12-018) isolated from environmental sewage in 2012, which possessed a unique triple nucleotide deletion in its VP1 region.

Material: The study material was the poliovirus strain CHN-E12-018. It was obtained from sewage samples collected in Guangdong Province in 2012.

Methods: Virus isolation was performed using Hep-2, RD, and L20B cell lines. Viral RNA was extracted, and the full-length genome was sequenced via RT-PCR and Sanger sequencing. Phylogenetic trees for P1, P2, and P3 genomic regions were constructed. Recombination analysis was conducted using RDP4 and SimPlot. A comparative protein structure simulation of the VP1 capsid between the variant and the Sabin 2 reference strain was performed using AlphaFold3 and PyMOL.

Results: It featured a triple-nucleotide deletion (positions 2,497-2,499) in the VP1 region, leading to the loss of leucine at position six of the VP1 protein. This deletion was not located at known attenuation or major neutralization sites. Phylogenetic and recombination analyses identified the strain as a type 2/3 recombinant, with a crossover site between nucleotides 6,981 and 7,439 (spanning 3Dpol and 3'UTR). Protein structure simulation indicated the VP1 capsid of the variant was highly similar to that of Sabin 2 (RMSD: 0.201 Å).

Conclusions: The triple nucleotide deletion in the VP1 region of this poliovirus variant likely occurred during an early evolutionary stage. The findings highlight the critical importance of continuous environmental surveillance to monitor for such unusual poliovirus variants, which supports ongoing global polio eradication efforts.

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Development of a field-deployable RT-LAMP assay for rapid detection of Nipah virus

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Nipah virus (NiV) is a World Health Organization priority zoonotic pathogen causing severe encephalitis and respiratory disease with high mortality rates. Outbreaks frequently occur in rural areas where access to laboratory infrastructure is limited. While RT-PCR remains the diagnostic gold standard, its deployment in field conditions is challenging. This study aimed to develop and evaluate a rapid field-deployable RT-LAMP assay capable of detecting both NiV Bangladesh (NiV-B) and Malaysian (NiV-M) lineages.

Primers targeting conserved regions of the NiV nucleocapsid were designed for dual lineage detection. Analytical sensitivity was assessed using serial dilutions of quantified in vitro transcribed RNA, with limit of detection (LOD) determined by Probit analysis (95% detection probability). Analytical specificity was evaluated against a panel of 21 related pathogens. RT-LAMP reactions were performed at 65°C for 30 minutes using both fluorescence-based (Mic PCR, Genie III) and colourimetric detection formats. No cross-reactivity was observed with non-target pathogens. Fluorescence-based assay demonstrated improved sensitivity and reduced time to detection compared to colourimetric assay. For NiV-B, LODs were 36-63 copies/μL (fluorescence) within 10 minutes, and 400 copies/μL (colourimetric) within 25 minutes. For NiV-M, LODs were 23-38 copies/μL (fluorescence) within 15 minutes and 138 copies/μL (colourimetric) within 26 minutes.

This study demonstrates a sensitive and specific RT-LAMP assay for NiV detection. Fluorescence-based detection offers enhanced sensitivity and real-time monitoring while colourimetric readout provides a simpler, minimal equipment alternative suitable for field deployment. This platform has potential utility for rapid outbreak detection and surveillance in resource-limited settings.

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Evaluation of Epstein–Barr Virus Seroprevalence and Atypical Serological Patterns: A Retrospective Study of 11,503 Cases

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Introduction/Aim: Epstein–Barr virus (EBV) infection is highly prevalent worldwide, and its seroepidemiology varies according to age, sex, and sociodemographic factors. Recent observations have raised concerns about possible changes in viral exposure dynamics following the COVID-19 pandemic, as well as ongoing challenges in interpreting atypical EBV serological profiles.

Material/Methods: This retrospective cross-sectional study evaluated EBV serological patterns in 11,503 patients who underwent simultaneous testing for VCA IgM, VCA IgG, EA IgG, and EBNA IgG between January 2023 and August 2025 at a tertiary-care hospital in Türkiye. Serological profiles were classified as seronegative, primary infection, past infection, chronic infection, or atypical. Age- and sex-related distributions were analyzed, and logistic regression models were used to assess factors associated with EBV seropositivity and atypical patterns.

Results: Past EBV infection was the most frequent profile (64.0%), followed by seronegativity (19.8%) and atypical patterns (14.3%). EBV VCA IgG seropositivity exceeded 50% for the first time in the 5–9 age group and increased steadily with age, suggesting a shift toward later EBV exposure in the post-pandemic period. Age was strongly associated with EBV seropositivity, and female sex independently increased the likelihood of VCA IgG positivity. Atypical serological patterns were more common in younger individuals and showed an inverse association with age, while sex had no independent effect.

Conclusion: These findings provide updated post-pandemic data on EBV seroepidemiology and highlight significant age- and sex-related differences in serological patterns. Interpretation of atypical EBV profiles should incorporate patient age, immune status, and test performance to ensure accurate clinical assessment.



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Age-Specific Seroprevalence of Six Viral Infections Over 10 Years in a University Hospital in South Korea

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Introduction: Age-specific seroprevalence reflects patterns of prior exposure and immunization. This study evaluated the age-stratified seroprevalence of herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), measles, mumps, and rubella using data from a university hospital in South Korea.

Materials and Methods: We conducted a retrospective cross-sectional study using IgG serologic data collected between 2016 and 2025 at a single university hospital. Individuals were categorized into ten age groups (0, 1–4, 5–9, 10–19, 20–29, 30–39, 40–49, 50–59, 60–69, and ≥70 years).

Results: A total of 4,277 HSV, 2,912 VZV, 6,035 CMV, 2,545 measles, 1,767 mumps, and 2,054 rubella IgG tests were analyzed. HSV seroprevalence increased with age, from 17.9% (1–4 years) to approaching 100% (≥70 years), with an overall seropositivity of 73.8%. VZV also increased with age, from 53.4% (1–4 years) to ≥98% (≥50 years) (overall 87.9%). CMV showed a similar pattern, from 43.6% (1–4 years) to approaching 100% (≥60 years) (overall 86.9%). Measles seroprevalence was 86.5% (1–4 years), 94.1% (5–9 years), 84.3% (10–19 years), and 79.5% (20–29 years), with ≥96% in those aged ≥50 years (overall 88.1%). Mumps seroprevalence remained high across age groups, with 92.7% (1–4 years), 94.7% (5–9 years), 81.1% (10–19 years), 85.1% (20–29 years), and higher seropositivity in those aged 60–69 years (94.0%) (overall 85.2%). Rubella seroprevalence varied by age, with 63.1% (10–19 years), 69.2% (20–29 years), and 51.8% (60–69 years) (overall 69.1%).

Conclusions: HSV, VZV, and CMV showed increasing seroprevalence with age, whereas measles, mumps, and rubella showed variable patterns. These findings provide baseline data on age-specific immunity in a university hospital setting in South Korea.

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Research Progress on Immunodeficiency ? Associated Vaccine ? Derived Poliovirus (iVDPV): from Viral Evolution to Public Health Responses

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Introduction/Aim: The large scale vaccination with oral poliovirus vaccine (OPV) has led to a dramatic decline in the number of global polio cases. However, due to the genetic instability of OPV, the accumulation of errors in the replication process and the lack of proofreading function of RNA polymerase, abnormal gene expression will occur in the genome. Some mutations can lead to neurovirulence reversion, resulting in the emergence of vaccine-derived polioviruses (VDPVs).

Material: This review is based on published scientific literature, reports from authoritative bodies like the World Health Organization, and documented iVDPV case studies.

Methods: A systematic search and analysis of peer-reviewed studies, surveillance reports, and expert reviews were conducted to collate information on the definition, virological characteristics, evolution mechanisms, global epidemiology, and public health strategies related to iVDPV.

Results: Since the global switch from trivalent to bivalent OPV in 2016, the proportion of type 2 iVDPVs has decreased, with types 1 and 3 becoming relatively more common. Key control strategies include: (a) strengthening AFP and environmental surveillance, as well as active screening of PID patients; (b) upgrading vaccination strategies, such as sequential IPV/OPV schedules and deploying novel, more genetically stable oral polio vaccines (nOPVs); and (c) developing and applying antiviral drugs, with the combination therapy of POCAPAVIR and IMOCITRELVIR showing the most promise.

Conclusions: Future research should focus on clarifying the mechanisms of viral evolution, coordinating global surveillance networks and improving the accessibility of prevention and control technologies to help achieve the ultimate goal of global polio eradication.

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Genetic variation of E6, E7 and LCR genes of human papillomavirus type 16 in a Swedish population

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Introduction/Aim: Cervical cancer is one of the most common cancers affecting women worldwide. Persistent infection with high-risk human papillomavirus (HR-HPV) is a key factor in the development of cervical cancer. Human papillomavirus type 16 (HPV-16) is the most prevalent HR-HPV genotype globally, and variations in its genetic sequence have been associated with differences in clinical outcomes. The aim of this study is to examine the distribution of HPV-16 variants in a population in Sweden through sequence analysis of HPV-16 DNA obtained from cervical samples. The study also aims to establish the phylogenetic relationships among the identified variants, determine the most common variants, and predict B-cell epitopes within E6 and E7 proteins.

Methods: DNA was extracted from a total of 100 cervical liquid-based cytology samples obtained from women tested positive for HPV-16 with different lesion categories. LCR, E6, and E7 genes were then amplified by PCR. The amplified products were then sequenced and subjected to genetic variation analysis, phylogenetic analysis, and B-cell epitope prediction.

Results: The findings showed that HPV-16 sequences from this Swedish cohort predominantly belonged to the European A lineage, particularly sublineage A1. Several mutations were identified, mainly within the E6 and LCR genes, whereas the E7 gene was more conserved. No statistically significant association was observed between genetic variation and abnormal cytology. Some substitutions in E6 and E7 genes impacted B-cell epitopes.

Conclusions: Overall, a predominance of European variants was observed, with a degree of genetic variability among the studied HPV-16 strains. This study contributes to a better understanding of the molecular epidemiology of HPV-16 variants and may be useful in informing the design of therapeutic vaccines.

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Investigation into the profiling of SARS-CoV-2-specific IgG responses elicited by natural infection and vaccination.

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The spike (S) glycoprotein of SARS-CoV-2 facilitates viral host cell entry via binding with the angiotensin converting enzyme 2 (ACE2) receptor. The S protein is composed of two subunits: S1, responsible for receptor binding, S2, mediating fusion of viral and host cell membranes. Emergence of variants of concern raised concerns regarding increased transmissibility and immune evasion. The Omicron variant carries over thirty mutations in the receptor binding domain (RBD) of the S protein, enhancing ACE2 affinity and spike cleavage.

This study investigates SARS-CoV-2-specific IgG and IgG avidity targeting ancestral and Omicron spike proteins following vaccination or natural infection, along with antibody-mediated inhibition of ACE2-spike binding. Samples were obtained from 14 wild-type infected patients, 14 Pfizer-vaccinated patients, and 10 pre-pandemic controls. IC50 values were calculated using non-linear regression with a sigmoidal dose-response model based on binding inhibition data from Spike-ACE2 and variant (E484K) assays.

Vaccinated individuals demonstrated higher S1 RBD-specific IgG levels (convalescent 67.73±7.08; vaccinated 141.26±134.44, p<0.05). Avidity to S1 RBD IgG was >40% in 3 convalescent individuals, while vaccinated individuals remained <40% (p=0.498). Detectable Omicron-specific IgG was observed in vaccinated individuals but absent in all convalescent samples (vaccinated 37.1±43.02, p<0.001). IC50 values for SARS-CoV-2 binding to ACE2, measured against wild-type S1 RBD domain were not significantly different (convalescent: 61.29±67.73; vaccinated: 109.25±138.31, p=0.097).

This study suggests vaccination induces stronger IgG response and broader activity against wild-type and Omicron variants, whereas infection-derived IgG provides limited protection against Omicron and other variants.

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Immune Evasion and Clinical Outcomes in Vaccinated Patients Hospitalized with COVID-19 (2021–2022)

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In Brazil, COVID-19 vaccination commenced in January 2021. Although vaccination was widely implemented, severe cases continued to be reported throughout 2021-22 and cause significant hospitalisations and fatalities until a sufficient level of immunity was established within the population. Patients who developed moderate to severe disease and required hospitalisation may have served as reservoirs for the emergence of viral immune evasion mechanisms. Accumulated favourable mutations in the viral genome that result in immune escape constitute a well-described evolutionary strategy. This immune escape permits reinfection of both the original and new hosts, sustaining transmission as if the population were immunologically naive. We aim to evaluate the neutralising capacity of antibodies in hospitalised patients with moderate to critical COVID-19 during 2021-22, and determine whether a loss of neutralisation capacity could be associated with the emergence of SARS-CoV-2. Methods of this study comprises a retrospective analysis of a patient population hospitalised at Hospital São Paulo in 2021-22. Three groups were analysed with PCR. Group 1 Hospitalised patients admitted due to COVID-19 severity. Group 2 Non-hospitalised individuals treated as outpatients. Group 3 Control group negative by PCR. Serum/plasma samples collected from participants will be tested using the VNT to assess the neutralising capacity against five variants and subvariants of SARS-CoV-2. A commercial ELISA will be performed to detect IgA, IgG, and IgM levels. The results will be comparison of serum neutralisation profiles across the three groups to determine whether immune escape mechanisms occurred in hospitalised patients and investigation into whether IgA levels are consistently lower than IgG and IgM in hospitalised indicating compromised mucosal immunity.

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Hepatitis E virus in shrews – a novel zoonotic risk?

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Introduction: *Parvovirus balayani* (hepatitis E virus, HEV) is the most relevant human-pathogenic species of the *Hepeviridae* family. Since 2018, additional zoonotic infections with ratHEV (species *Rocaepevirus rattii*) have been reported. Recently, a novel hepevirus, provisionally termed shrewHEV, was identified in *Crocodyrus russula* (greater white-toothed shrews, GWTS) that is phylogenetically more closely related to HEV than ratHEV. We investigated the prevalence of shrewHEV in Germany and its infectivity in various cell lines.

Materials and Methods: 222 GWTS collected in Germany between 2002 and 2022 were screened for shrewHEV RNA by RT-qPCR in liver, intestine and kidney tissue. ShrewHEV RNA sequences were determined and phylogenetically analysed. Liver homogenates of ten positive shrews were inoculated onto shrew-derived SUN-3-R cells and HEV- and ratHEV-permissive human liver cell lines PLC/PRF/5, HuH-7 and HuH-7-Lunet BLR. Cells and supernatants were further characterized by immunofluorescence assay, sequencing and density gradient centrifugation. Finally, we assessed the antiviral activity of ribavirin (RBV) and sofosbuvir (SOF) against shrewHEV.

Results: ShrewHEV RNA was detected in 16/222 GWTS. Five of 10 liver-derived strains replicated in human liver cell lines, but none in SUN-3-R shrew cells. Replication was most efficient in HuH-7 followed by HuH-7-Lunet BLR cells, while PLC/PRF/5 cells were substantially lower permissive. Density gradient centrifugation demonstrated quasi-enveloped particles. In addition, both RBV and SOF showed antiviral activity against shrewHEV in vitro.

Conclusions: ShrewHEV was detected in 7.2% of GWTS samples across its distribution range. The virus replicated efficiently in human-derived liver cells indicating the necessity of further studies on its zoonotic potential.

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Molecular diagnosis of Epstein-Barr virus infection: clinical performance of a high-throughput molecular platform on whole blood samples.

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Background: Epstein-Barr virus (EBV) occurred as asymptomatic infection or mononucleosis syndrome in immunocompetent hosts, while it could represent a life-threatening pathogen in immunocompromised subjects. Quantitative molecular assays on whole blood or plasma represent the gold standard for the diagnosis and the monitoring of EBV infection. Due to its clinical impact, an accurate standardization among different centers and diagnostic kits represents an effort to improve clinical management of EBV-positive patients. This study aims to evaluate the clinical performance of a novel qPCR kit on a high-throughput molecular platform starting from whole blood samples.

Materials: 220 clinical whole blood specimens were tested simultaneously with Panther Fusion® EBV Quant Assay (Hologic, USA) on the Panther Fusion Plus high-throughput automated platform (Hologic, USA) and the routine laboratory method, based on the platforms Elite InGenius® and BeGenius® (ELITechGroup, Italy) with the EBV ELITe MGB® qPCR kit (ELITechGroup, Italy). The EBV detection rate and correlation analysis between two tested methods were evaluated.

Results: Data showed an agreement of 92.81% for detected/quantified positive samples and 86.79% for negative samples between the two tested methods. The evaluation of viraemia in positive samples performed with a Bland-Altman analysis highlighted a good correlation between the tested platforms in terms of International Units ($\Delta \log_{10}$ IU/ml: $+0.32 \pm 0.41$), while linear regression resulted in an $R = 0.79$.

Conclusions: The novel high-throughput qPCR assay performed on whole blood samples showed good agreement with our Standard of Care. Furthermore, this approach provides advantages such as an automated workflow with minimal hands-on time and random access, thus enabling rapid and reliable diagnostics in clinical routine.

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Metabolic reprogramming across COVID-19 severity reveals stage-specific signatures of host response

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Introduction/Aim: Coronavirus disease (COVID-19) is associated with heterogeneous clinical outcomes, reflecting complex host metabolic responses to SARS-CoV-2 infection. This study aimed to characterize stage-specific metabolic alterations across disease severity.

Material and Methods: Untargeted metabolomic profiling was performed using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) on plasma samples from 25 individuals, including healthy controls (n=5) and COVID-19 patients (n=20) stratified into four severity groups based on pulmonary involvement and respiratory support. Multivariate statistical and pathway enrichment analyses were applied.

Results: A total of 541 metabolites were identified, revealing progressive metabolic divergence across disease stages. Early and moderate COVID-19 were characterized by dysregulation of monocarboxylic acids and significant perturbations in phenylalanine metabolism, indicating early immunometabolic stress. In contrast, severe disease demonstrated a shift toward pyrimidine metabolism, consistent with increased nucleotide turnover associated with viral replication and immune activation. Principal component analysis confirmed a continuum of metabolic changes correlating with disease severity rather than discrete transitions.

Conclusions: COVID-19 progression is associated with dynamic metabolic reprogramming reflecting both host immune responses and viral demands. Identified stage-specific metabolic signatures may serve as potential biomarkers for disease stratification and provide a basis for metabolomics-guided precision approaches in viral infections.

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Transcriptomic and epitranscriptomic remodeling of host RNA reveals regulatory mechanisms in COVID-19 pathogenesis

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Introduction: Host response to SARS-CoV-2 involves complex transcriptional and post-transcriptional regulation, yet the contribution of RNA processing and modifications remains incompletely understood. This study aimed to characterize transcriptomic and epitranscriptomic alterations in COVID-19.

Material and Methods: Whole-blood samples from COVID-19 patients and controls were analyzed using RNA sequencing and direct RNA sequencing. The analysis included gene expression profiling, alternative splicing, allele-specific expression, circular RNAs, RNA modifications (m5C, m6A, pseudouridine), and poly(A) tail dynamics. Functional enrichment and pathway analyses were performed.

Results: SARS-CoV-2 infection induced extensive transcriptomic remodeling, including altered expression of ribosomal protein genes, widespread alternative splicing events, and allele-specific expression changes. Epitranscriptomic analysis identified hundreds of m5C, m6A, and pseudouridine sites enriched in infected individuals, predominantly localized in 3' untranslated regions. These modifications frequently co-occurred in immune-related genes such as CSF3R and PIK3CD. Poly(A) tail alterations and non-adenine incorporation further indicated disruption of RNA stability and translational control. Functional analysis revealed enrichment in pathways related to endocytosis, Fc receptor-mediated phagocytosis, and antiviral immune responses.

Conclusions: COVID-19 is associated with multi-layered RNA regulatory remodeling, spanning transcriptional, post-transcriptional, and epitranscriptomic mechanisms. These findings highlight RNA processing as a key component of host-virus interaction and identify potential molecular targets for antiviral strategies.

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Comparison of two PCR point-of-care instruments, Deaou-ART04 and FlashDx-1000-E, for detection of Respiratory Syncytial Virus, Influenza A/B virus and SARS-CoV-2

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Introduction: Influenza A/B virus (FluA/B), SARS-CoV-2, and respiratory syncytial virus (RSV) are major respiratory pathogens with important implications for both clinical management and infection control. Rapid and accurate diagnostics are therefore essential. We compared two point-of-care PCR-platforms, Deaou-ART04 (Deaou Gene) and FlashDx-1000-E (FlashDX), with our in-house routine respiratory panel (TaqMan array card).

Material and Methods: A total of 83 clinical samples were analyzed, including SARS-CoV-2 (n=12), FluA (H1/H3/H5) (n=21), FluB (n=10), RSV A (n=15), RSV B (n=10), RSV untyped (n=2), and negative samples for FluA/B, RSV and SARS-CoV-2 (n=22). Nine co-infections were included: RSV B + SARS-CoV-2 (n=3), RSV A + FluA (n=5), and RSV A + RSV B (n=1). All samples were tested on the same day using both platforms. A broad range of Cq-values was represented (16.84–36.21). To assess specificity, 'negative' samples included other respiratory viruses (rhinovirus, adenovirus, ...).

Results: Among the first 9 samples tested on Deaou, 4/9 yielded false-positive RSV results; which was resolved after a software update. FlashDx showed no false positives. Invalid results occurred in 2/83 samples for Deaou and 1/83 for FlashDx. Deaou and FlashDx identified 44/61 and 50/61 true positives, respectively. False negatives were observed in 13/83 samples with Deaou (incl. 4 co-infections) and 10/83 with FlashDx (incl. 1 co-infection), mainly at Cq-values above 33.

Conclusions: Both platforms demonstrated comparable performance for mono-infections, with a turnaround time of +/- 1 hour and providing Cq-values. Deaou allows up to 4 simultaneous tests per run, whereas FlashDx processes 1 cassette per run. FlashDx requires a smaller sample volume (120 µL vs. 250 µL), differentiates RSV subtypes A/B and showed superior detection of co-infections.



Posters

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Low-positive CMV IgG serostatus before allogeneic hematopoietic cell transplantation is not associated with increased CMV reactivation risk

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Pretransplant CMV IgG serostatus guides CMV risk stratification and letermovir prophylaxis after allogeneic hematopoietic cell transplantation (HCT), but low-positive results near the assay cutoff remain difficult to interpret.

In this single-center study, pretransplant sera from 335 adult allogeneic HCT recipients were tested with Elecsys CMV IgG (Roche) and classified as negative (<0.5 U/mL), low-positive (0.5-9 U/mL), or high-positive (>9 U/mL). To assess whether low-positive Elecsys results were reproducible, 95 Elecsys-reactive sera were retested with VIDAS (bioMérieux), SERION (Virion/Serion), and recomLine (Mikrogen). Plasma CMV DNAemia was monitored weekly to day+100 after HCT.

At transplantation, 202 allogeneic HCT recipients were high-positive, 71 low-positive, and 62 negative. Of 38 low-positive Elecsys sera retested, only 2 (5.3%) were confirmed by any alternative assay, whereas agreement increased at higher IgG levels. In recipients with serial pre-HCT sera, CMV IgG classification changed between initial testing and transplantation in none of initially high-positive recipients, 15.8% of initially low-positive recipients, and 47.1% of initially seronegative recipients. Day+100 cumulative incidence of CMV DNAemia (>34.5 IU/mL) was 40.5% in high-positive recipients receiving letermovir, 16.7% in low-positive recipients without prophylaxis, 3.7% in low-positive recipients receiving letermovir, and 5.6% in seronegative recipients. Compared with high-positive recipients, low-positive and seronegative recipients had markedly lower sub-distribution hazards of CMV DNAemia.

Low-positive Elecsys CMV IgG results were rarely reproducible on orthogonal immunoassays and were not associated with the CMV reactivation risk seen in truly seropositive HCT recipients, suggesting they are less likely to reflect prior CMV exposure.

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Target-capture metagenomic sequencing of cerebrospinal fluid as a reflex test for suspected viral CNS infection

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Background: Metagenomic next-generation sequencing (mNGS) has expanded diagnostic options for central nervous system (CNS) infections, but conventional shotgun approaches often lack sensitivity for low-titer viral pathogens in cerebrospinal fluid (CSF). Target-capture (TC)-mNGS may address these limitations and improve diagnostic yield.

Methods: In this retrospective single-center study, TC-mNGS was performed on CSF from 87 patients in three adjudicated cohorts: BioFire®-ME panel-confirmed viral CNS infection (n=25), suspected viral CNS infection despite negative BioFire®-ME testing (n=30), and non-infectious neurological disease controls (n=32). Shotgun mNGS and Nanopore TC-mNGS were evaluated in predefined subcohorts.

Results: TC-mNGS confirmed all virus-positive cases in the BioFire®-ME-positive cohort. In suspected viral CNS infection with negative BioFire® results, TC-mNGS detected viruses in 6/30 patients, yielding 20.0% additional detections versus BioFire® (95% CI 7.7-38.6; NNT 5) and 6.7% beyond the full routine molecular workup (95% CI 0.8-22.1; NNT 15). Compared with shotgun mNGS, TC-mNGS increased viral read recovery by 21- to 210,000-fold and retained detection in low-titer infections missed by shotgun mNGS. Nanopore TC-mNGS generated fewer viral reads than Illumina but missed no viral pathogens. TC-mNGS also enabled near-complete viral genome recovery for JC polyomavirus NCCR rearrangement analysis, HSV and HIV antiviral drug-resistance assessment, and enterovirus phylogenetics.

Conclusion: TC-mNGS improves viral CSF detection over shotgun mNGS while enabling clinically relevant genome-level characterization. It is best positioned as a second-line reflex test after negative syndromic panel testing when suspicion for viral CNS infection remains high or genomic characterization may inform clinical management.

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Towards Improving Cytomegalovirus (CMV) IgM Assays For The Detection of Congenital CMV Infection

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Towards Improving Cytomegalovirus (CMV) IgM Assays For The Detection of Congenital CMV Infection

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Cytomegalovirus is the leading cause of congenital infection worldwide. Total IgM levels in infants have been reported to be lower than those observed in adults. Current CMV specific IgM assays are developed using adult sera and may lack the sensitivity required to detect CMV IgM in infants. This study compares total IgM levels in CMV IgM positive serum samples from infants and adults and compares the reactivity to individual CMV antigens. Ten CMV IgM positive infant serum samples, 10 CMV IgM positive adult serum samples, and 12 CMV IgM negative infant serum samples were studied. Adult samples were also tested after dilution to a total IgM concentration comparable to that in infants. Infant sera when compared to adult sera showed significantly lower levels of total IgM (mean $\pm$ SD: $1.35 \pm 1.6\text{mg/ml}$ vs $7.51 \pm 3.84\text{mg/ml}$, $p < 0.001$), CMV IgM ($150.89 \pm 204.33 \text{ U/ml}$ vs $403.36 \pm 243.72 \text{ U/ml}$, $p = 0.03$), and anti-CMV p52 IgM (ISR: 2.49 ± 2.02 vs 4.84 ± 1.83 , $p = 0.028$). The diluted adult samples showed significantly lower levels of CMV IgM ($p = 0.002$) and anti-CMV p52 IgM ($p = 0.005$) when compared to the neat adult samples. The infant CMV IgM and anti-CMV p52 IgM levels were similar to those in the diluted adult samples. Interestingly, of the 10 diluted adult samples tested on the CMV IgM line immunoassay, one was negative and one was indeterminate. This study shows that current CMV specific IgM assays may not be sensitive enough for the detection of congenital CMV in infants due to the lower levels of total IgM in infant sera and further research is required to develop CMV specific IgM assays using infant sera.

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Evaluation of Adherence to Diagnostic Guidelines in Laboratory Practices at Initial HIV Diagnosis: Strengths and Gaps

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Introduction/Aim: Baseline evaluation in newly diagnosed HIV patients extends beyond immediate disease management and plays a critical role in identifying coinfections, assessing immune status, and guiding vaccination strategies.

Material/Methods: This single-center retrospective study evaluated laboratory test requests at initial HIV diagnosis (January–June 2025) and compared them with national and EACS guideline recommendations.

Results: Fifty-nine patients were included (86.4% male; mean age 39). Adherence was high for core and serological assessments, indicating a comprehensive baseline evaluation. The most frequently requested tests included CD4/CD8 ratio (94.9%), Toxoplasma IgG (93.2%), CMV IgG (91.5%), measles IgG (89.8%), and mumps IgG (89.8%). Notably, serologies not explicitly recommended in guidelines, such as mumps and HSV, were frequently ordered, reflecting an expanded serological profiling approach. However, some guideline-recommended assessments, including Streptococcus pneumoniae, influenza, sexually transmitted infections, and HPV, were not performed. Seropositivity rates revealed high exposure to CMV, HAV, and HSV-1, while anti-HBs positivity was only 44%, indicating insufficient hepatitis B immunity. Seropositivity for Toxoplasma, HSV-2, and syphilis further highlighted coinfection risks.

Conclusion: While baseline evaluation demonstrates strong overall adherence and comprehensive serological assessment, inconsistencies remain. A more structured, guideline-based testing strategy may optimize clinical decision-making and preventive care.

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Performance evaluation of the Panther Fusion® EBV Quant Whole Blood Assay

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Introduction: The Panther Fusion® EBV Quant Whole Blood Assay, currently for investigational use only (IUO), is a fully automated, high-throughput real-time PCR assay designed to monitor EBV DNA in whole blood from solid-organ and hematopoietic stem cell transplant patients. This study evaluated analytical performance, comparability to a commercial assay, and traceability to the 1st WHO International Standard for EBV.

Material and Methods: A total of 317 whole blood samples from transplant patients were tested with the Panther Fusion® EBV Quant Whole Blood assay and compared to the EBV R-Gene assay (BioMérieux). External Quality Assessment (EQA) was performed using QCMD EBVWB24S and EBVWB25S panels. Traceability to the 1st WHO International Standard for EBV (NIBSC 09/260) was also evaluated.

Results: Across 117 samples in the common quantitation range, the Panther Fusion® EBV Quant Whole Blood assay showed a mean difference of 0.13 Log₁₀ IU/mL versus EBV R-Gene® (Bland–Altman) and strong correlation (Deming regression: $R = 0.95$). The assay showed robust traceability to the 1st WHO International Standard, with a maximum overall bias of 0.08 Log₁₀ IU/mL and near-perfect correlation ($R = 0.997$). For the QCMD panels, measured viral loads closely matched consensus values, with all differences ≤ 0.22 Log₁₀ IU/mL.

Conclusions: The WHO calibrated Panther Fusion® EBV Quant Whole Blood Assay provides accurate and comparable quantitation of EBV DNA in transplant patient samples relative to R-Gene EBV assay. EBV viral load results obtained from QCMD panels are consistent with market-leading assays, supporting interchangeability of quantitative results. When used on the fully automated Panther Fusion® system, the EBV Quant Whole Blood Assay supports effective viral load monitoring and extends the infectious disease menu on the platform.

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Mitochondrial Network Dynamics and APOBEC3A-mediated stress during HIV-1 infection

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Mitochondria are key regulators of innate immune signalling and metabolic homeostasis, and their dysfunction influences antiviral responses. This PhD project investigates the interplay between mitochondrial network dynamics and the cytidine deaminase APOBEC3A (A3A) in HIV1 infected macrophages. A3A, strongly induced by type I interferon signalling and resistant to Vif-mediated degradation, primarily is involved in DNA catabolism in myeloid cells. Preliminary proteomics data reveal an association between A3A and the mitochondrial phosphatase PGAM5, a regulator of mitophagy and mitochondrial quality control. We hypothesize that A3A-PGAM5 interaction disrupts mitochondrial network structure and functions, leading to reactive oxygen species accumulation, mitochondrial DNA (mtDNA) leakage, and sustained interferon responses.

To test this, we will characterize A3A-mediated effects on mitochondrial function, metabolic remodelling, and innate immune signalling in infected macrophages. In vivo relevance will be assessed using a Macaca fascicularis cohort covering pre-infection through chronic phases, quantifying plasma cell-free mitochondrial DNA (mt-cfDNA) levels, oxidation, and fragmentation as markers of mitochondrial stress. Parallel human studies will compare people living with HIV on ART and HIV-negative individuals on PrEP to distinguish infection- and drug-driven mitochondrial perturbations. By linking intracellular mitochondrial damage to systemic mt-cfDNA release, this work aims to establish extracellular mitochondrial DNA as a biomarker of immune-metabolic dysregulation and to identify strategies targeting A3A-PGAM5 signalling to restore mitochondrial health and reduce inflammation in chronic HIV infection.

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Clinical and molecular epidemiology of Respiratory Syncytial Virus (RSV) Infections during the 2025/26 Season in Tyrol, Austria

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Background: This study aimed to characterise RSV-positive paediatric patients and the impact of the widespread Nirsevimab use on the genetic stability of the targeted viral region during the 2025/26 RSV season

Methods: The study population comprised children under five years of age presenting with respiratory symptoms and had confirmed RSV-infection. We gathered data on demographic, clinical, and immunisation status. Oropharyngeal swab samples were submitted for RSV-genotyping and whole-genome sequencing using Oxford Nanopore technology. We also analysed anonymised historical data on RSV-related hospital admissions from the regional hospital characterizing hospitalisation patterns in the pre- and post-Nirsevimab periods.

Results: RSV hospitalization was significantly lower in the 2025/26 season than in the 2023/24 season. The median age at admission increased significantly in the post-Nirsevimab era (469 vs. 238 days, $p < 0.0001$), while the mean length of hospital-stay decreased (3.19 vs. 3.4 days, $p = 0.0008$). RSV-A, clade A.D.3 predominated, but RSV-B infections were associated with a higher likelihood of hospitalisation. In our study, we detected 21 Nirsevimab breakthrough infections among eligible infants. No significant differences were observed in RSV subtype, clade distribution or mutation patterns across breakthrough status. No novel mutations were detected in the Nirsevimab-binding site.

Conclusions: The decline in hospitalization, the higher mean age at admission and a shorter length of hospital-stay in the post-Nirsevimab era, underscores the high real-world impact of the immunization. In addition, the lack of emerging mutations, especially at the Nirsevimab-binding site, indicates genetic stability with no molecular evidence of selective pressure despite the widespread use.

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Nanoparticle Vaccines Based on the Truncated VZV gE Elicit a Robust Immune Response in Mice

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Introduction: Herpes zoster (HZ), caused by the reactivation of varicella-zoster virus (VZV), primarily affects elderly populations worldwide. Although current recombinant HZ vaccines show strong immunogenicity, their high cost and potential side effects may limit their widespread use. Therefore, developing a cost-effective HZ vaccine with improved safety profiles would have significant clinical and public health implications.

Methods: Building upon our previously optimized truncated gE (tgE350) from VZV, we developed the tgE350+Fe nanoparticle vaccine using Spy-Tag/SpyCatcher covalent conjugation. The tgE350 protein and the Fe protein were expressed in HEK293F and E. coli BL21, respectively, enabling spontaneous nanoparticle assembly. Protein expression and nanoparticle formation were confirmed through SDS-PAGE and negative-stain electron microscopy. BALB/c mice were inoculated with either tgE350+Fe or tgE350 combined with Al and CpG adjuvants. Immune responses were evaluated using ELISpot and flow cytometry for cellular immunity, along with ELISA, VZV microneutralization, and fluorescent antibody membrane antigen (FAMA) assays for antibody titers.

Results: Compared with the truncated vaccine tgE350, the nanoparticle vaccine tgE350+Fe significantly enhanced VZV neutralizing antibodies and specific antibody responses in mice without causing significant changes in lymphocyte populations. Moreover, the tgE350+Fe group had significantly more lymphocytes secreting IFN- γ IL-2, and IL-4 than the tgE350 group. No apparent pathological damage was observed in the organs of mice in any experimental group.

Conclusions: This experiment successfully developed the HZ nanoparticle vaccine tgE350+Fe. It enhanced VZV-specific neutralizing antibodies, generated better cellular and humoral immune responses, and showed good safety.

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Global epidemiology and pathogen spectrum of enterovirus-associated encephalitis and meningitis outbreaks from 1960 to 2025: a systematic review and meta-analysis

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Introduction: Enteroviruses (EVs) are major causes of viral encephalitis and meningitis. We analyzed outbreaks reported from 1960-2025 to map epidemiology, clinical manifestations, and serotypes, informing prevention and control.

Material: Data were systematically retrieved from four electronic databases: Web of Science, PubMed, CNKI, and Wanfang, covering the period from 1960 to July 1, 2025.

Methods: We included laboratory-confirmed EV meningitis/encephalitis outbreaks. A random-effects model meta-analysis was used to estimate attack rates, hospitalization rates, and serotype distribution.

Results: A total of 56 outbreaks, comprising 28,622 cases across 20 countries, were included in this meta-analysis. Outbreaks were most prevalent in the Western Pacific and Europe, exhibiting seasonal peaks in June–July (Northern Hemisphere) and April–May (Southern Hemisphere), with a median outbreak size of 90 cases. Schools and medical institutions were the major outbreak settings. EV-B was the dominant species (91.1%, 51/56 outbreaks), with echovirus 30 (E30) being the most prevalent serotype. The two decades from 2000–2009 and 2010–2019 saw the highest number of reported outbreaks. The overall attack rate was estimated at 13.2% (95% CI: 6.1–22.3). Notably, the pooled hospitalization rate was exceptionally high at 94.0% (95% CI: 85.9–99.2). The most frequently reported symptoms were fever, headache, and vomiting.

Conclusions: EV meningitis/encephalitis outbreaks persist globally with high hospitalization rates, seasonal peaks, and pediatric predominance. Enhanced surveillance is urgently needed.

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Age Related Patterns in the Clinical and Molecular Epidemiology of Rhinoviruses

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Introduction/Aim: Respiratory viruses exhibit distinct circulation patterns. While the epidemiology of influenza viruses and respiratory syncytial viruses is well characterized, that of other respiratory viruses remains less well documented.

Material and Methods:

We investigated the epidemiology of respiratory viruses in southern France from 2018 to 2023, with a particular focus on rhinovirus circulation according to age. In addition, we analyzed the molecular epidemiology of rhinoviruses circulating during the autumn peaks of 2022 and 2023.

Results: Rhinoviruses were the most frequently detected respiratory viruses during the study period. Except for the year 2020, rhinovirus circulation followed a bimodal annual pattern, characterized by two epidemic waves and a summer nadir. During the SARS-CoV-2 epidemic, the circulation of respiratory viruses markedly declined between weeks 14 and 25 of 2020; notably, rhinoviruses were the first to show substantial recirculation. Rhinovirus peaks in children younger than three years generally preceded those observed in adults. Wave-driving relative risk analyses suggest that young children contributed more than adults to rhinovirus epidemics, except during periods associated with COVID-19-related mitigation measures. The autumn rhinovirus peaks were driven by substantial genotypic diversity, with 27 and 23 distinct genotypes identified in 2022 and 2023, respectively.

Conclusions: Rhinovirus circulation predominates in children throughout the year and is characterized by high genotypic diversity during epidemic peaks. Although the SARS-CoV-2 pandemic affected rhinovirus circulation, its impact was less pronounced than that observed for other respiratory viruses.

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First fully nanobody-based sandwich ELISA for sensitive and specific detection of the hepatitis E virus capsid antigen

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During viral replication of the hepatitis E virus (HEV), ORF2 (the capsid protein) is released into the bloodstream and can be detected in infected individuals. Diagnosis is based on the detection of specific antibodies, RNA, and/or antigen. We aimed to develop a nanobody-based sandwich ELISA to detect ORF2.

Anti-HEV nanobodies (Nbs) were modified by cloning to incorporate plastic-binding (PSW), biotin-binding (BIO), and alkaline phosphatase (PAL) tags, followed by expression and purification. The immunoassay was optimized with respect to nanobody concentrations, blocking agents, streptavidin–peroxidase (S-HRP) dilution, and other parameters. Reactivity against ORF2 proteins from all 4 human-infecting genotypes, limit of detection, and cutoff value were evaluated. Acute and chronic hepatitis E sera and samples from individuals with other viral infections were assessed. 4 ORF2 proteins and 10 modified nanobodies (Nbs) were produced with high yield and purity. Capture (NbPSW96) and detection (NbBIO39 and NbBIO74/NbPAL39 and NbPAL74) Nbs were selected from 16 tested combinations. The detection limit was 10 ng/mL for the BIO-HRP-E system and 2.5 ng/mL using NbPAL. Both assays detected HEV-Ag from zoonotic genotypes in spiked samples. No cross-reactivity was observed.

7 sera from patients with acute and 9 with chronic hepatitis E, all positive for HEV RNA and serology, were analyzed. Acute samples were negative, consistent with reports indicating that HEV-Ag positivity rates and concordance with HEV RNA are inversely correlated with the presence of anti-HEV antibodies in acute patients. In contrast, HEV Ag was detected in 6 of the chronic patient samples, reinforcing HEV-Ag as a marker of chronic infection.

We report on the first fully Nb-based ELISA for HEV-Ag, offering a valuable and low cost diagnostic tool for hepatitis E.

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Use of a composite comparator method to ascertain performance of a rapid molecular syndromic PCR panel for the detection of 26 pathogens

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Introduction: The investigational-use-only Cobas® Eplex respiratory pathogen panel 3 (RP3) detects 26 respiratory viral and bacterial pathogens in ~90 minutes in a single sample to answer test, reducing the time to diagnosis and subsequent management. Its performance was assessed in support of CE-IVD submission.

Methods: Frozen and fresh nasopharyngeal swab specimens (NPS) were collected from 2023- 2025, respectively, and combined with contrived specimens with spiked organisms to support testing on the RP3 panel. Composite comparator method required 2 positive results to define pathogen detection, determined clinical sensitivity/PPA and clinical specificity/NPA alongside the two-sided 95% score confidence intervals (CI) for 20 targets, whereas a single comparator method was used for the remaining 5 targets.

Results: A total of 3,171 samples (2,506 prospective, 255 retrospective, and 410 contrived) were collected from sites in the United States and tested on RP3 to support submission to BSI (British Standards Institute). Human rhinovirus/enterovirus (9.7%), Influenza A (6.3%), and human coronavirus (5.5%) were the most frequently detected pathogens. Of the original samples tested, there were 139/3,171 consumables that were invalid for an initial validity of 95.6% (95% CI: 94.8–96.3%). Retesting returned 132/139 valid results for a final validity of 99.8% (95% CI: 99.5–99.9%). PPAs across targets ranged from 88.2% - 100% and NPAs ranged from 99.0%-100%. After discordant resolution PPAs improved to 88.9%-100%.

Conclusion: RP3 performance was strong and consistent across all targets, including targets added or updated in comparison to the original RP/RP2 panels, which supported CE-IVD approval in December 2025.

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Anti-hepatitis C virus direct-acting drug therapies for chronically-infected children

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Introduction/Aim: Chronic hepatitis C virus (HCV) infection occurs in >50% of children born to viremic mothers and poses a risk of long-term liver complications. Data on the use of direct-acting anti-HCV drugs (DAA) in the pediatric population, including children ≥3 years of age, remain limited. Here we aimed to describe the therapeutic management using DAAs of children chronically-infected with HCV and evaluate the virological efficacy and tolerability of these treatments.

Materials and Methods: This retrospective study included chronically infected children between Jan-2014-Jan-2025 (11 years). Data collected included viral load (VL; NeumoDx Qiagen assay) and genotyping by Sanger or next-generation sequencing and phylogenetic analysis, and tolerability data. Efficacy was defined as sustained virological response (SVR; VL undetectability 12 weeks post-treatment end).

Results: Seven children were included, aged 1.5-15 years at treatment initiation (mean age, 12 years). Mean VL at diagnosis was 5.7 log₁₀ IU/mL (range, 3.5-6.9). Genotypes were 3a, 1a, and 2i in 3, 2, and 1 cases, respectively. Three patients received glecaprevir/pibrentasvir, one sofosbuvir/ledipasvir, and one sofosbuvir/velpatasvir. SVR was observed in 5/7 children; one child was lost to follow-up, and another was still on treatment at time of analysis. Premature treatment discontinuation occurred in a 12-year-old patient due to difficulty swallowing tablets. SVR was obtained post-treatment reintroduction. Clinical and biological tolerance was deemed satisfactory in all patients.

Conclusion: In this pediatric cohort, DAA demonstrated high virological efficacy associated with good tolerability. These results support the value of early therapeutic management of HCV in children to prevent long-term liver complications.

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Genomics of influenza A H1N1 viruses in public and university hospitals of Marseille, southeastern France, over 7 years (2017-2024)

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Introduction/Aim: Influenza A viruses (IAV) encounter significant genetic evolution overtime, including through genome reassortments. This can notably promote immune escape. The SARS-CoV-2 pandemic was associated with a transient, drastic reduction of IAV circulation. Here, we aimed to obtain and characterize H1N1 IAV whole genomes for infections diagnosed over 7 years in southeastern France.

Materials and Methods: IAV genome next-generation sequencing (NGS) used residues of respiratory samples diagnosed as H1N1 IAV RNA-positive between 2017-2024 in public and university hospitals of Marseille, southeastern France. NGS, after PCR with a described non-commercial procedure, used Illumina on MiSeq/NovaSeq 6000 (2017-2022) or Nanopore on GridION (2023-2024). NGS reads and genome analyses used Minimap2, MAFFT, IQ-TREE, and iTOL. Clades and mutations were identified using Nextclade, reassortments using GiRaF.

Results: 934 IAV complete genomes were obtained. They belong to 11 hemagglutinin (HA)-based clades including predominant clade 6B.1A.5a.1 (54.0%), and other clades with prevalences ranging between 0.1% (n=1; 6B.1A.2) and 16.3% (153; 6B.1A.5a.1). In addition, they belong to 10 neuraminidase (NA)-based clades including predominant (50%) clade B.3.1.2. Some HA clades included different NA clades, and vice versa. Genomes and clades that (co)-circulated changed through years. A putative reassortant was identified for 17 6B.1A.5a.1 clade HA genomes that involved segment 2 of clade 6B.1A.5a.2 and segment 3 of clade 6B.11.5a.2a.1.

Conclusion: These analyses revealed a broad diversity of H1N1 IAV clades in our geographical area. A reassortment involving segments 2 and 3 highlights the importance of comprehensive pan-genomic surveillance to better understand influenza evolution and the emergence of new strains, beyond HA and NA segments alone.

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Parvovirus B19 real-time PCR detection in clinical samples from a tertiary care hospital in Sarajevo, Bosnia and Herzegovina (2024–2025)

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Introduction/Aim: Parvovirus B19 (B19V) causes a wide spectrum of manifestations, from erythema infectiosum to severe complications in immunocompromised patients and fetuses. Real-time PCR (rtPCR) remains the gold standard for detecting active viremia. This study evaluated the diagnostic value of B19V rtPCR in different clinical sample types in routine practice in Bosnia and Herzegovina.

Material and Methods: We retrospectively analyzed all B19V rtPCR requests from January 2024 to December 2025 using the Bosphore Parvovirus B19 Detection Kit (Anatolia Genework, Türkiye), including those detected within a multiplex central nervous system (CNS) panel. Data included patient demographics, requesting department, sample type, and result.

Results: Of 34 valid requests (20 blood, 11 nasopharyngeal swabs, and 3 cerebrospinal fluids—CSFs), 12 tested positive (35.3%). Positivity was observed in blood (9/20; 45.0%) and CSF (3/3; 100%); all nasopharyngeal swabs tested negative. Seven positive results (58.3%) originated from a 17-year-old male post-kidney transplant recipient over eight months, indicating persistent viremia. The remaining 5 positives included a single blood detection in an adult outpatient and three CSF detections in pediatric and neurological patients. Most tests were in the 5–17 years age group (18/34; 52.9%), with 44.4% positivity. No seasonal pattern was observed.

Conclusions: B19V positivity was confined to blood and CSF, while nasopharyngeal swabs had no diagnostic value. Persistent viremia in a post-kidney transplant adolescent and detections in CSF highlight the risk of prolonged infection and possible CNS involvement in immunocompromised and pediatric patients. Our data support the selective use of blood and CSF for suspected systemic or neurological B19V infection and emphasize the need for larger multicenter studies.

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Prevalence of HIV-1 Drug Resistance Mutations in Treatment-Naïve Patients

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Introduction: The acquisition of drug resistance mutations (DRMs) may lead to loss of virological suppression, limit future treatment options, and reduce the effectiveness of pre-exposure prophylaxis (PrEP).

In recent years, major advances in HIV/AIDS treatment have been achieved, including long-acting (LA) injectable therapies and more convenient drug formulations.

The prevalence of transmitted drug resistance (TDR) varies geographically and its study supports updated guidelines and identification of transmission chains.

Aims: To evaluate the prevalence of resistance mutations to protease (PIs), reverse transcriptase (NRTIs and NNRTIs), and integrase strand transfer (INSTIs) inhibitors in HIV-1 treatment-naïve patients followed in an hospital center in Lisbon.

Materials and Methods: A population of 401 treatment-naïve patients was studied between January 2024 and April 2026. 76% were male, mean age 38 years, and mean viral load 1088810 copies/mL. Next-generation sequencing (NGS) technology was used, allowing simultaneous sequencing of the HIV-1 RT, protease, and integrase regions. Results were analyzed using the Stanford HIV Drug Resistance Database (version 9.6), considering a resistance score >30.

Results: 12.7% of patients had mutations conferring resistance.

By class: NNRTIs 8.5%; NRTIs 5.2%; PIs 1%; INSTIs 0.5%.

Most frequent mutations: NRTIs M184V, M41L; NNRTIs K103N, G190A; PIs D30N; INSTIs E138K and G118A, were the only two mutations identified.

Predominant subtypes: B (34%), CRF02-AG (17%), C (16%).

Conclusions: There has been an increase in the prevalence of TDR compared to our 2022 study (11.7%) INSTI resistance remained low (0.5%) Low resistance to cabotegravir and rilpivirine (LA injectable) suggests this regimen as a good alternative to oral therapies. Resistance to doravirine was very low and does not compromise its clinical use.

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Native-like GP38–Gn–Gc Glycoprotein Complex Enables Discovery of VHH Antibodies Targeting Surface Antigens of Crimean-Congo Hemorrhagic Fever Virus

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne pathogen causing severe hemorrhagic disease and represents a significant public health threat. The viral M-segment encodes a glycoprotein precursor processed into GP38, Gn, and Gc. While Gn and Gc are established envelope components, the fate of GP38 has remained unclear and it was not considered a virion component. Yet recent evidence indicates that GP38 is surface-accessible and is associated with viral particles, supporting its relevance as a target antigen. Here, we reconstructed a native-like GP38–Gn–Gc complex to enable the discovery of antibodies targeting viral surface glycoproteins, including GP38.

We engineered a recombinant construct containing the ectodomains of GP38, Gn, and Gc, preserving the SKI-1 cleavage site between GP38 and Gn and the downstream Gn/Gc processing site to allow physiological maturation. Complementary heterodimerization domains were fused to the C-termini of Gn and Gc to stabilize the complex, and distinct affinity tags (His-tag on Gn and double Strep-tag on Gc) were incorporated for purification and validation of co-assembly. The resulting heterotrimeric GP38–Gn–Gc complex was produced and used to immunize llama for VHH discovery.

This approach yielded multiple VHH candidates recognizing the glycoprotein complex, including one that was characterized and shown to bind GP38. We further assessed its binding affinity to the heterotrimeric complex and initiated epitope mapping to define its specificity.

In conclusion, preserving native glycoprotein architecture enabled the identification and characterization of antibodies targeting surface-associated GP38 and establishes a robust platform for discovering functionally relevant binders against viral envelope glycoproteins, supporting the development of targeted countermeasures against CCHFV.

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Rapid PCR to differentiate measles wild-type and vaccine genotypes: Clinical Validation and Outbreak Relevance

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Introduction: Measles is a highly contagious viral disease. It usually presents with symptoms such as fever and rash. However, both may also occur after the measles, mumps and rubella (MMR) vaccine. Vulnerable measles contacts may be advised to isolate for up to 21 days. We carried out the first validation of the VIASURE assay on clinical samples. We aimed to assess whether this differentiates between natural measles infection and post-MMR rash.

Materials and Methods: Samples sent by Alder Hey Children's Hospital to Liverpool Clinical Laboratories for measles PCR testing were retrospectively identified. Repeat in-house measles PCR testing was done to confirm persistent RNA positivity after sample storage. All positive samples were then analysed with the VIASURE Measles + Rubella PCR kit (CerTest, Spain) for genotype differentiation. Analysis was done blind to clinical data, which were then confirmed with the Infection Prevention and Control records.

Results: Of 19 samples with detectable measles RNA on in-house testing, all were positive for measles RNA and 10 were positive for genotype A RNA on the VIASURE assay. Of these, eight had a difference in CT value between total measles RNA and genotype A RNA of ≤ 1 , consistent with recent vaccination. Post-MMR rash was listed as a discharge diagnosis in seven of the eight patients. Despite this, all patients were advised to isolate on discharge.

Conclusions: The VIASURE assay results on stored clinical specimens correlated well with gold standard PCR testing, vaccination and clinical history. There is clinical need for this test, as symptoms alone are not sufficient to differentiate natural measles from post-MMR rash. Further work is required for formal clinical validation, assess impact of test availability on clinical decision-making and feasibility of rollout in an outbreak setting.

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Hepatitis D virus screening and active infection in chronic HBV: a cascade-of-care analysis at a Portuguese tertiary centre, 2023-2025

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Introduction/Aim: Chronic hepatitis delta causes the most severe form of viral hepatitis. EASL 2023 and WHO 2024 guidelines recommend anti-HDV testing in all HBsAg-positive patients, yet uptake varies widely. We characterised the HDV cascade of care at a Portuguese tertiary hospital immediately before reflex testing.

Material and Methods: Retrospective single-centre study (ULS Santa Maria, Lisbon, 2023-2025). The primary population comprised adults with chronic HBV, defined as two HBsAg-positive results at least six months apart, or any detectable HBV viral load. The cascade was analysed in four steps: (1) anti-HDV testing in chronic HBV; (2) anti-HDV positivity in those tested; (3) HDV-RNA testing in anti-HDV positives; (4) HDV-RNA detection in those tested. Proportions with Wilson 95% CI.

Results: The primary population comprised 863 chronic HBV patients. Anti-HDV was tested in 517/863 (59.9%, CI 56.6–63.1); 33/517 tested positive (6.4%, CI 4.6–8.8). HDV-RNA was requested in 28/33 seropositives (84.8%) and detected in 13/28 (46.4%). Active HDV infection was identified in 13/863 chronic HBV patients (1.5%, CI 0.9–2.6), representing the minimum diagnosed burden through existing clinical pathways.

Conclusions: Before reflex testing, our centre screened 60% of chronic HBV patients for anti-HDV — above Iberian baselines (~8%, Buti et al., Spain) but ~30 points below centres with systematic surveillance (~90%, Kamal et al., Stockholm). Nearly half of seropositives had active viraemia, and 40% of the cohort remained untested across three years. Identifying viraemic patients is now clinically actionable given bulevirtide availability. Reflex testing was implemented in March 2026; these data constitute the baseline for a planned before/after comparison of this implementation.

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Genetic Diversity and Transmitted Drug Resistance in HIV-1 Protease and Reverse Transcriptase from Benguela, Angola

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Prior to the 2019 WHO recommendation of dolutegravir (DTG), HIV-1 therapy primarily relied on protease inhibitors (PIs), and nucleoside (NRTIs) and non-nucleoside (NNRTIs) reverse transcriptase inhibitors. In Angola, which transitioned to DTG-based regimens in 2021, the prolonged use of these drug classes likely contributed to the emergence of transmitted drug resistance (TDR). The high HIV-1 genetic diversity in Angola, characterised by multiple subtypes and recombinants, may further influence TDR dynamics. This study estimates TDR prevalence and updates the molecular epidemiology of HIV-1 in Benguela, Angola.

A total of 243 dried blood spots from treatment-naïve individuals attending the General Hospital of Benguela were stored on FTA™ cards. Proviral DNA was purified, and the protease (PR)/reverse transcriptase (RT) coding regions were amplified by nested PCR and sequenced. Maximum-likelihood phylogenies were inferred using IQ-TREE 3, with branch support assessed by bootstrap analysis. Drug resistance mutations (DRMs) were identified using the Stanford HIV Drug Resistance Database (HIVdb).

Subtype C was the most prevalent in both RT (36%) and PR (38%), followed by F1 and CRF02_AG. Additional subtypes included A1, B, D, G, H, J, and CRF124_cpx. PI-associated DRMs were rare (5%), compared with NRTI (20%) and NNRTI (32%) resistance. Intermediate/high-level resistance was most frequently observed for emtricitabine (FTC) (16%), lamivudine (3TC) (16%), efavirenz (28%), etravirine (20%), nevirapine (28%), and rilpivirine (22%).

The subtype diversity found suggests that Benguela may represent a transition zone between the diverse HIV-1 epidemics of Central Africa and the subtype C-dominated epidemics of Southern Africa. The high prevalence of FTC and 3TC resistance is concerning given their continued use in current therapeutic regimens.

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Matrix-specific performance of human RNA and DNA internal control targets across clinical matrices

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Reliable endogenous internal controls are essential in viral clinical diagnostics to confirm sample quality and assay validity, particularly when interpreting negative results. The performance of human internal control targets may vary across specimen matrices. This study aimed to evaluate matrix-specific performance of candidate human RNA and DNA internal control targets in whole blood, urine, swabs, and feces.

Candidate targets were selected to represent different expected abundance levels: two RNA targets with high and low expression (ACTB and C12orf45), and three DNA targets with high, medium, and low copy content (CYTB, rDNA, and AHSP). Assays were first evaluated in single-plex and multiplex formats using purified RNA and DNA. Matrix-specific comparison of internal control abundance and consistency was analyzed using Cq values, replicate variability and detection rate. LOD was calculated for every target. Stability/decay of endogenous internal controls was also assessed in volunteer specimens and compared with selected spike-in viruses.

Preliminary analyses indicate matrix-dependent differences in the performance of the evaluated internal control targets. ACTB, C12orf45, CYTB, rDNA, and AHSP displayed distinct patterns of detectability and stability across whole blood, urine, swabs, and feces. Final results will determine the relative performance of each target across specimen types and assay formats.

Internal control performance should not be assumed to be transferable between matrices without validation. This study provides practical evidence to support more informed selection and implementation of internal controls in human RT-qPCR across matrices, thereby improving the reliability of viral clinical diagnostic testing.

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First Outbreak of Imported Enterovirus A71 Sub-genotype B5 in China - An Emerging Lineage driving Shift in Older Children

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¹National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention; ²National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention; As one of the primary pathogens responsible for severe hand, foot and mouth disease (HFMD), sub-genotype C4a of Enterovirus A71 (EV-A71) has circulated in China for many years. However, an outbreak of hand foot and mouth disease cases that were caused by sub-genotype B5 have been identified in Tianjin municipality of China in 2024. Epidemiological data were collected and phylogenetic characteristics were analyzed based on their VP1/ whole-genome sequences. This outbreak caused 12 related cases and triggered a three-generation transmission chain in a month. These strains show high similarity to 2023 Vietnamese strains and form an emerging lineage of B5 (lineage L) with recent domestic B5 strains. Notably, this emerging lineage predominantly caused infections in older children. Two sub-lineages were further designated as L1 and L2. A faster estimated evolutionary rate (8.50×10^{-3} substitutions/site/year) in lineage L led to a recent increase in the B5 genetic diversity recently. Transmission pathways indicated the Chinese clade of this lineage likely originated from Vietnam, with a reliable Bayes factor (BF) value (32.19). Five substitutions unique to the emerging sub-lineage L2 were mapped to 3C (M/V182E) and 3D (P48S, T75K, P259G, H383D). Two important recombination forms were confirmed within the lineage L2: a small-fragment recombination with Coxsackievirus A6 in the 3ΨTR and a long segment of recombination with Coxsackievirus A2 in the P3 region. Given that this emerging lineage has accumulated extensive mutation and recombination events, it is imperative to maintain rigorous and continuous virological surveillance of EV-A71.



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The Results of Virological Respiratory Pathogen Monitoring among Kyiv Population in 2025-2026 season

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Introduction: For today the world exists in the condition of a post pandemic period. Influenza viruses in the pandemic period caused by coronavirus SARS-CoV-2 showed minimal activity, but didn't disappear. After the end of the COVID-19 pandemic influenza viruses continue to cause epidemic raises of morbidity.

The aim of our work was to conduct the virological monitoring for the circulation of pathogens of respiratory infections in Kyiv in the season of 2025-2026.

Materials and methods: Samples for testing (nasopharyngeal swabs), were collected from patients with respiratory symptoms and tested by a real time PCR method. The testing was conducted for influenza A and B viruses, SARS-CoV-2, RSV, parainfluenza viruses, adenoviruses, metapneumoviruses, rhinoviruses, bocaviruses and seasonal coronaviruses. 1752 respiratory samples were tested by the PCR method and 26 of influenza viruses were sequenced.

Results: Influenza viruses caused an epidemic in Kyiv and amounted to 30% from all tested samples. The biggest quantity of tested samples was collected during 2-8 weeks 2026 (37.8%) the same as the majority of samples (26%). The main pathogen of epidemic was strain of influenza virus A/Singapore/GP20238/2024 A(H3N2). Influenza viruses subtype A(H1N1) was in the minority - the strain A/Missouri/11/2025.

Among all of the tested samples only 7.5% were positive for SARS-CoV-2. Another 11.5% were positive for other respiratory viruses.

Conclusions: Today the etiological factor of epidemics are influenza viruses. SARS-CoV-2 viruses cause seasonal growths of morbidity in the periods when influenza viruses almost don't circulate. There is a necessity of respiratory pathogen monitoring, including genetic monitoring and because of their constant variability and easy way of transmission.

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Population-level effect of hepatitis C direct-acting antivirals (DAAs) and reinfection rate in the 2004–2025 period in Slovenia: a nationwide study

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Introduction: Highly effective treatment for hepatitis C virus (HCV) with direct-acting antivirals (DAAs) has been available for a decade; however, complete eradication remains a challenge. The aim of this study was to assess the changes brought by the introduction of DAAs.

Material and Methods: This retrospective observational study analyzed all HCV RNA data routinely collected in Slovenia during the 2004–2025 period. HCV RNA-positive events (HCVPEs) (defined as all consecutive HCV RNA-positive results per patient) and HCV RNA-negative events (HCVNEs) were extracted and analyzed. Reinfection was defined as an additional HCVPE with at least two consecutive HCV RNA-positive results following at least 12 weeks of consecutive HCV RNA-negative results.

Results: A total of 47,836 HCV RNA results were included, and 4,511 HCVPEs (20,578 HCV RNA-positive results) were observed in 3,756 patients. The majority of patients were men (70.4%) with a median age of 37 years at the first HCV RNA-positive result. A significantly lower mean peak viremia (5.94 ± 0.93 vs. 6.03 ± 0.87 log₁₀ IU/ml; $p=0.0117$) was observed in the DAA period (2015–2025; 1,518 HCVPEs) compared to the pre-DAA period (2004–2014; 2,993 HCVPEs). HCVNE was detected after 3,313 HCVPEs and was achieved in a median of 31.3 ± 54.6 months and 10.0 ± 19.2 months following the first HCV RNA-positivity in 2004–2014 and 2015–2025, respectively (hazard ratio 2.98, 95%CI 2.75–3.22, $p<0.001$). Reinfection was identified in 199/3,756 (5.3%) patients, corresponding to a reinfection rate of 1.64 per 100 person-years. Reinfection occurred at an additional time point in 19/199 (9.5%) patients.

Conclusions: Time to HCV RNA negativity was nearly three-fold faster in the DAA period. Using strict criteria, we observed a reinfection rate of 1.6 per 100 person-years. Safe practices should be reinforced.

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Anti-EBV microblots detect EBV infection stages with high sensitivity and specificity

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Introduction: Epstein-Barr virus (EBV) infects ~90% of the global population and persists as a life-long infection. EBV diagnostics rely on specific biomarkers indicating different stages of infection. VCA-IgM is the primary indicator of acute or recent infection. VCA-IgG provides broader diagnostic utility by appearing during the acute phase and persisting for life. The distinction between recent or past infection relies on detection of anti-EBNA-1 IgG antibodies, which typically appear 3-6 months post infection. Here, we illustrate performance data of microblots, representing a significant technological advancement in EBV serodiagnostics.

Methods: The EUROMicroblot Anti-EBV (IgG, IgM) contains miniaturized immunoblots in a 96-well microtiter plate format for detection of IgG antibodies to VCA p19, VCA gp125, EA-D, EBNA-1, and p22, and IgM antibodies to VCA p19, VCA gp125, and EA-D.

Diagnostic sensitivity and specificity for detection of anti-VCA IgM and IgG, and anti-EBNA-1-IgG antibodies were calculated using 389 serum samples from serologically precharacterized patients (51 seronegative for anti-EBV IgM/IgG antibodies, 46 with acute EBV infection, 292 with past EBV infection).

Results: The overall diagnostic sensitivity and specificity for the detection of specific antibodies were: 98.6% and 94.8% for anti-EBNA-1 IgG, 95.7% and 96.5% for anti-VCA IgM, and 100% and 98% for anti-VCA IgG, respectively.

Antibody detection at 6 timepoints during EBV infection in 2 patients showed the benefit of comprehensive antibody detection for infection staging.

Conclusion: The results indicated that the EUROMicroblots allow precise detection of EBV biomarkers for EBV infection staging. The microblot technology enables simultaneous detection of multiple antibody responses in one procedure and is easily automatable at high throughput on ELISA devices.

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STAT1 signaling regulates cholesterol homeostasis in lung epithelial cells and RSV-induced syncytia formation

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Respiratory syncytial virus (RSV) remains a leading cause of severe respiratory illness in infants, older adults, and immunocompromised individuals. While STAT1 signaling is central to interferon-driven antiviral immunity, its role in host lipid metabolism during infection has only recently emerged. We investigated how STAT1 controls cholesterol homeostasis and RSV-induced syncytia formation using CRISPR/Cas9-mediated STAT1 knockout HEP-2 epithelial cells. Specifically, we characterized STAT1-associated responses through transcriptomics, live-cell imaging, and biochemical assays. RNA-Seq analysis revealed that STAT1 deletion impaired regulation of the SREBP-SCAP-INSIG pathway, including SREBF2, SCAP, and INSIG1, leading to significant intracellular cholesterol accumulation. RSV-infected STAT1-deficient cells exhibited strongly increased syncytia formation compared to wild-type cells, despite identical viral replication kinetics, demonstrating that enhanced cytopathology was independent of viral load. Pharmacological intervention with 25-hydroxycholesterol and gemfibrozil significantly reduced syncytia size by suppressing cholesterol biosynthesis. Mechanistically, cholesterol depletion disrupted RSV fusion protein trafficking and membrane stability. These findings reveal an immune-metabolic checkpoint whereby STAT1 constrains RSV cytopathology through cholesterol regulation. Given widespread statin use in elderly populations and rising metabolic syndrome rates, host cholesterol status may be an underappreciated determinant of RSV disease severity, opening avenues for repurposing lipid-lowering therapeutics as adjunctive interventions

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Respiratory Syncytial Virus G Protein Facilitates Viral Spread via TLR2-NFkB-Mediated NLRP3 Priming and Pyroptotic Cell Death

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Respiratory syncytial virus (RSV) is a leading cause of severe lower respiratory tract infections worldwide, impacting infants, immunocompromised individuals, and older adults. Disease burden arises from direct cytopathogenicity and dysregulated inflammation characterized by excessive IL-6 and IL-8 production, alongside secondary complications including superinfections and cardiovascular events. Despite recently approved vaccines and monoclonal antibodies, understanding molecular mechanisms driving RSV pathogenesis remains essential for developing targeted therapeutics. The RSV attachment glycoprotein G binds via a CX3C-like chemokine motif and exists in a secreted form (sG) with immunomodulatory functions. Pulldown experiments with recombinant sG demonstrated binding to TLR2, triggering NF κ B activity and secretion of IL-6, IL-8, VEGF, and CCL2. sG pretreatment enhanced RSV titers in airway epithelial supernatants, revealing a novel role in promoting viral replication distinct from canonical receptor-binding activity.

We identified a two-phase inflammasome activation process: sG-TLR2-MyD88-NF κ B signaling primes uninfected cells via NLRP3 upregulation and ROS accumulation, while subsequent RSV infection triggers caspase-1 activation and pyroptosis. This explains how sG facilitates viral spread by preconditioning neighboring cells for inflammasome-driven lysis and viral egress, potentially explaining prolonged viral shedding and hyperinflammation in severe cases.

Targeting the sG-TLR2 interface could simultaneously reduce inflammatory damage and viral spread. These results support evaluating CX3C motif-targeted vaccination strategies and NLRP3 inhibitors in high-risk populations.

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Substitutions affecting the HA-NA-receptor balance preceded the emergence of the pandemic 2009 H1N1 virus

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Introduction/Aim: Swine are a crucial species in Influenza A virus (IAV) ecology. They can support replication and consequently evolution of human, avian and swine strains and are the source of the 2009 pandemic H1N1 virus (H1N1pdm09). Understanding how swine IAV breach the animal-human species barrier is essential for early detection of potentially pandemic viruses, but is often overlooked compared to avian IAV. We hypothesize that the HA-NA balance of (pre)pandemic swine viruses is a key feature that needs to be adapted as swine and humans differ in their sialoglycome (receptor availability).

Material: We focused on a set of (pre)pandemic swine viruses collected in Mexico following the emergence of H1N1pdm09.

Methods: We performed phylogenetic analysis combined with engineering of recombinant PR8 viruses with/without substitutions in swine HA and NA, primary Air-Liquid-Interface (ALI) cultures and Biolayer Interferometry (BLI).

Results: The emergence of H1N1pdm09 was preceded by HA and NA substitutions predicted to affect their function. HA acquired an A/T227E substitution previously shown to reduce receptor binding in H1N1pdm09, while NA obtained substitutions in the 2nd sialic acid binding site (2SBS) predicted to reduce NA cleavage. Substitutions in the 2SBS negatively affected replication in primary human and swine airway ALI cultures. Interestingly, efficient replication was restored by the HA A227E substitution. The effect of these substitutions, on the HA-NA balance, was confirmed using BLI, highlighting that NA proteins with a mutated 2SBS are less efficient in cleaving glycosidic bonds.

Conclusions: We showed that the emergence of H1N1pdm09 was preceded by substitutions in HA and NA that adjusted the HA-NA balance in agreement with our hypothesis that this is a critical step for crossing the host-species barrier.

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Performance evaluation of a CE IVD automated PCR molecular platform for the syndromic testing of Herpes Simplex Virus, Varicella Zoster Virus and Adenovirus

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Background/Aim: Herpes Simplex Virus (HSV), Varicella Zoster Virus (VZV) and Adenovirus (ADV) are batch-tested molecular analyses performed by NHS Fife Medical Microbiology and Infection Control. Altona Diagnostics UK Ltd supply pathogen panel assays which cover these target pathogens and are compatible with the AltoStar® Molecular Diagnostic Workflow.

The aim of this verification and validation is to critically evaluate the AltoStar® AM-16 platform (Altona Diagnostics UK Ltd) and compare the performance against the Qiagen QiaSymphony platform currently used by the department.

Methods/Material: 63 samples previously tested for HSV, VZV and/or ADV (patient and EQA samples) were tested using the AltoStar AM-16 methodology and qualitative results compared. Sample types included in the verification include oral-facial swabs, genital swabs and non-genital cutaneous skin swabs all collected in Copan eNAAT medium.

20 control material replicates for each target pathogen were tested to enable calculation of measurement uncertainty for each assay.

Results: For the HSV/VZV (alphaHerpesvirus) and Adenovirus assays, there was 96% accuracy achieved for HSV1, 96% accuracy for HSV2, 100% accuracy for VZV and 100% accuracy for ADV. The MU for each assay determined by the replicate testing of standardised control material lies between 0.400 and 0.606.

Conclusion: The AltoStar® AM-16 and pathogen assays produced results that were high in specificity, sensitivity and accuracy with measurement uncertainty values that are highly acceptable for a molecular assay intended for syndromic testing.

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Antiviral activity of the dHODH inhibitor MEDS433 on Parvovirus B19 replication

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Aim of the Study: Parvovirus B19 (B19V) is a human ssDNA virus with selective tropism for erythroid progenitor cells and associated with hematological and systemic disorders. Currently, no specific antivirals are available. MEDS433 is a potent inhibitor of human dihydroorotate dehydrogenase (dHODH) which catalyzes the oxidation of dihydroorotic acid (DHO) to orotic acid (ORO), a critical step in the biosynthesis of uridine and cytidine required to fulfill cellular nucleotides demand. This study aimed to evaluate its effect on B19V replication.

Materials and Methods: The antiviral activity of MEDS433 (0.001–10 µM) was evaluated in UT7/EpoS1 cells and erythroid progenitor cells (EPCs) derived from peripheral blood mononuclear cells. Viral replication was quantified by qPCR and expressed as EC50. Cell viability was assessed as CC50. In addition, to verify that the antiviral activity of MEDS433 derives from specific inhibition, infected cells were treated with MEDS433 in the presence of exogenous uridine, orotic acid (ORO), or dihydroorotic acid (DHO).

Results: MEDS433 markedly inhibited B19V replication in a dose-dependent manner, with EC50 values of 0.05 µM in UT7/EpoS1 cells and 0.04 µM in EPCs. Cytotoxicity occurred at higher concentrations (CC50: 5–10 µM and 0.5 µM, respectively). The antiviral effect was reversed by uridine and ORO supplementation, but not by DHO, even at high concentration.

Conclusions: MEDS433 inhibits B19V replication by targeting dHODH and blocking pyrimidine biosynthesis downstream of DHO. Its high antiviral potency, combined with a favorable selectivity profile, supports its potential as a promising candidate for the development of targeted therapies against B19V infection.

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Implementation of the Qiagen QuantiFERON human cytomegalovirus assay for management of renal transplant patients

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Human cytomegalovirus (CMV) infection is a major cause of morbidity in renal transplant recipients and is associated with CMV disease, graft dysfunction, increased hospitalisation and reduced graft survival. Current CMV management relies on assessment of donor (D) and recipient (R) CMV IgG serostatus, immunological organ compatibility and serial CMV DNA viral load monitoring. While viral load testing identifies active replication, it does not assess CMV-specific cell mediated immunity (CMI) and cannot reliably predict which patients are at risk of disease, relapse, or late-onset CMV following antiviral prophylaxis. Multiple studies in solid organ transplant (SOT) populations demonstrate that absence of a robust CMV-CMI is strongly associated with increased risk of CMV complications. A positive QF-CMV response is associated with immune protection. The value of CMV-specific immune monitoring for CMV outcomes across SOT recipients has been previously highlighted.

In this project, we have included as per CMV IgG donor/recipient status, 12 low risk, 22 intermediate risk and 11 high risk renal transplant recipients (RTxR) to pilot the introduction of the Qiagen QuantiFERON®-CMV (QF-CMV) in a large diagnostic laboratory. QF-CMV is a CE-IVD marked, whole-blood interferon-γ release assay and could be used as an addition to existing CMV viral load monitoring in RTxR. This assay provides a functional assessment of CMV-specific CD8⁺T-cell immunity or CMI. Its feasibility and a preliminary evaluation of its clinical role in the management of CMV infection and reactivation in RTxR an ethnically diverse population of East London was also performed.

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Viral Kinetics and Risk Factors associated with Refractory CMV Infection Post-Kidney Transplantation in the UK

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Introduction: Refractory (ref) CMV infection is associated with poor clinical outcomes after kidney transplantation (KTx). We investigate risk factors associated with ref CMV or needing >1 anti-viral therapy (AVT).

Methods: Univariable logistic regression was performed on 268 adult KTx patients in UK RaDaR registry, treated for CMV DNAemia or developing CMV post-prophylaxis in first year after KTx.

Results: Median age 55 yrs, 56% male, 66% White ethnicity
79% deceased donors

34% D+R+, 42% D+R-, 22% D-R+ at KTx

54% had valganciclovir prophylaxis

Induction: 22% ATG/Campath, 78% Basiliximab

Immunosuppression: 92% tacrolimus, 89% MMF, 73% prednisolone

Median(IQR) CMV viral load iU/mL (VL) at: CMV diagnosis 1955 (285,25285); 1st treatment 7107 (1573,46485); 2 weeks post treatment 976 (79, 9776); 2nd treatment 42831 (9746,163540)

In first 2 weeks post diagnosis, median peak VL was 6069 (1663,46752) with AUC (area under curve) VL of 57645 (11270,539928). Median VL doubling time 14 d (7,25).

Odds ratios (95% CI) for risk factors associated (P<0.01) with ref CMV or needing >1 AVT v nonrefractory CMV were:

CMV mismatch at KTx (D+R-): 4.1 (2.2-7.7)

CMV management strategy, Prophylaxis v Surveillance: 3.9 (2.0-7.8)

Induction therapy: 2.92 (1.5-5.6)

Increasing time from KTx to CMV [median:4.4 v 2.4 mths]: 1.25 (1.1-1.4) per mth

Higher VL at diagnosis [median:12834 v 1266 iU/mL]: 1.2 (1.1-1.3) per doubling

Higher max VL in first 2 weeks of infection [median:136006 v 5037 iU/mL]: 1.3 (1.2-1.5) per doubling

Increased AUC for VL in first 2 weeks of infection [median:726351 v 41900 iU/mL]: 1.1 (1.1-1.3) per doubling

Conclusions: This first UK real-world analysis reveals risk factors associated with ref CMV and/or >1 AVT. Enhanced surveillance and early therapy escalation in high-risk patients to optimise outcomes should be considered.

Study funded by Takeda UK Ltd.

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Dynamics of intact and total HIV-1 DNA-reservoirs in Relation to plasma viral load levels

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Introduction/Aim: The Intact Proviral DNA Assay (IPDA) is a high-precision multiplex digital PCR (dPCR) technique that distinguishes intact, hyper-mutated, and 3' defective HIV-1 proviruses. By providing a granular view of the viral reservoir, IPDA holds promise for routine monitoring and therapeutic decision-making in people living with HIV.

Material/Methods: CD4⁺T-cells were isolated by immunomagnetic enrichment from 350 participants. Genomic DNA was extracted and quantified using the QIAcuityDx digital PCR platform (Qiagen) with a triplex IPDA. The assay targeted the psi and env regions to identify intact proviruses, while the LTR (RU5) region measured total HIV-1 DNA (intact + defective). Cell equivalents and DNA integrity were assessed using the RPP30 housekeeping assay with primers positioned equidistant as the viral amplicons (shearing-control). Participants (100 per VL stratum) were stratified as follows: G1 <50 c./mL, G2 50–200 c./mL, G3 200–1000 c./mL (N=50), and G4 >1000 c./mL.

Results: Total HIV-1 DNA increased stepwise across VL groups (copies per 10⁶ CD4⁺T cells: G1=140, G2 253, G3=356, G4=644; p<0.001). Intact proviral DNA showed a parallel rise (G1=8.1, G2=11, G3=45, G4=38 c./10⁶ cells; p<0.01), despite considerable inter-individual variability. The increase in intact proviruses relative to proviral DNA was most pronounced between the suppressed viral load group and the groups with viral loads greater than 200 c./mL.

Conclusions: Simultaneous quantification of total and intact HIV-1 DNA delineates reservoir size and composition, revealing significant differences among VL strata. These metrics may serve as biomarkers for identifying patients at heightened risk of therapeutic failure. Future studies that take clinical variables into account could shed light on the factors influencing the persistence of the reservoir.

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Vaccinia and Monkeypox Virus-Neutralizing Antibodies in People Living with HIV: A Serological Study in an Orthopoxvirus-Endemic, Low-Income Region in Brazil

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Introduction/Aim: People living with HIV (PLHIV) are at increased risk of severe outcomes from emerging and re-emerging viral infections. The Diamantina/Serro region, Minas Gerais, Brazil, is endemic for Orthopoxvirus vaccinia (VACV) and Orthopoxvirus monkeypox (MPXV), where zoonotic outbreaks affect rural communities. This study characterized the frequency of OPV neutralizing antibodies (NABs) and cross-immunity between VACV and MPXV in PLHIV.

Material and Methods: Cross-sectional seroepidemiological study with 177 PLHIV from the Specialized Care Service of Diamantina, Minas Gerais (December 2021 to August 2022). NABs against VACV and MPXV were measured by plaque reduction neutralization test (PRNT). Sociodemographic and clinical-laboratory data were obtained from medical records.

Results: Overall OPV NAB seropositivity was 27.7%, significantly higher among individuals born before 1978 (53.3%) than after (1.1%), reflecting the impact of smallpox vaccination cessation. Among VACV-seropositive individuals, 40.8% also neutralized MPXV, indicating cross-immunity. All anti-MPXV NAB-positive individuals were born before 1978, suggesting vaccination rather than natural MPXV exposure as the antibody origin. A positive correlation was observed between VACV and MPXV PRNT reduction, with all samples showing higher neutralization against VACV than MPXV. No significant associations were found between seropositivity and sex, residence area, viral load, or CD4⁺ T cell count.

Conclusions: PLHIV retain partial OPV cross-immunity when previously vaccinated, but individuals born after smallpox eradication show near-complete absence of NABs, highlighting increased susceptibility in this vulnerable population. These findings reinforce the importance of OPV surveillance in immunocompromised individuals in endemic regions.



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Imported Chikungunya Virus Strains in 2025, Taiwan

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Introduction: Chikungunya is a mosquito-borne viral disease characterized by symptoms of fever, rash, myalgia, and polyarthralgia. Chikungunya is not endemic to Taiwan, but the transmitted vector *Aedes* mosquitoes are present throughout Taiwan. Infected travelers with viremia could lead to local transmission. To reduce the risk for importation and spread of chikungunya virus (CHIKV) in Taiwan, fever screening at airports and hospital-based surveillance systems were implemented.

Material and Methods: The complete structural protein genes of CHIKV were sequenced by the Sanger method, and MEGA v6 was used to construct the phylogenetic trees.

Results: A total of 39 laboratory-confirmed imported chikungunya cases were recorded in 2025. Nucleotide sequences of complete structural protein genes of 28 CHIKV strains from these cases were successfully sequenced, including 15 from Indonesia, 8 from the Philippines, 2 from Cuba, 1 from Thailand, 1 from China, and 1 from Sri Lanka. All CHIKV strains from the Philippines and 13 strains from Indonesia belonged to the Asian genotype; 2 strains from Indonesia and the strains from Cuba, Thailand, China, and Sri Lanka belonged to the ECSA genotype. The 2025 large-scale chikungunya outbreak in China has drawn our attention because people frequent contact between countries. Sequence analysis revealed that the CHIK/China/2510aTw/2025 strain from China harbored mutations, including E1-A226V, E2-L210Q, and E2-I211T substitutions that have been demonstrated to significantly enhance viral replication efficiency in *Ae. Albopictus*. That has the potential to cause autochthonous transmission in Taiwan.

Conclusions: Continuing to monitor newly introduced CHIKVs can provide essential information on global expansion and genetic evolution that is useful for disease surveillance and laboratory diagnosis.

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Extending a meningitis/encephalitis PCR panel to plasma for HSV detection: a proof-of-concept study

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Introduction/Aim: Herpes simplex virus (HSV) is a major cause of meningitis and encephalitis, requiring rapid molecular diagnosis on cerebrospinal fluid (CSF). Plasma HSV DNA detection is also critical in severe presentations (neonatal infection, immunosuppression, disseminated disease, acute liver failure), where early antiviral therapy is lifesaving. However, plasma testing often relies on batch-based assays with prolonged turnaround times. The BioFire® FilmArray® Meningitis/Encephalitis (ME) Panel enables rapid detection of HSV-1 and HSV-2 within one hour but is currently validated for CSF only. This study evaluated its performance in plasma.

Material and methods: Forty-seven plasma samples from two virology laboratories were retrospectively included. These comprised 26 HSV DNA-positive clinical plasma samples, 10 HSV-negative plasma samples spiked with HSV-positive mucocutaneous material, and 11 HSV-negative plasma samples from patients with fulminant hepatitis used to assess specificity. Plasma samples (200 µL) were tested using the ME Panel, and HSV-1/HSV-2 results were compared with reference PCR assays routinely used in each laboratory. Both qualitative (detected/not detected) and semi-quantitative results (Ct values) were analyzed.

Results: Specificity was 100% (11/11). The ME panel detected and discriminated HSV-1 and HSV-2 in 31/36 samples (86.1%). All discrepant results were ME-negative/reference-positive and corresponded to low viral loads (Cycle threshold ≥37).

Conclusions: In severe HSV infections, usually associated with high plasma viral loads (Ct <37), rapid HSV DNA testing using the ME Panel may be suitable as a first-line approach. Yet, when clinical suspicion remains high and the ME Panel result is negative, confirmation using a validated assay is warranted.



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Performance of new ELISAs for the detection of anti-Oropouche virus-specific IgM and IgG antibodies

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Introduction: This study assessed the performance of two newly developed ELISAs for the determination of antibodies against Oropouche virus (OROV).

Methods: Testing was performed using Euroimmun Anti-Oropouche Virus ELISAs (IgM, IgG; IVDR-pending; ANVISA-registered). Longitudinal antibody responses were analyzed using sequential samples from a PCR-confirmed OROV case from Brazil (n=13). Seroprevalence was determined in sera from blood donors in Manaus/Brazil, collected during the 2023-2024 OROV epidemic (IgM, n=1872; IgG, n=1965). In a subset (n=676), IgG ELISA results were compared to FRNT. Sensitivity was evaluated using PCR-confirmed OROV cases from Peru (n=15), and specificity using control panels (IgM, n=847; IgG, n=849), including blood donors, children, and pregnant women from non-endemic areas, as well as patients with serologically confirmed CHIKV, DENV, or ZIKV infection. Malaria samples (n=31) were analyzed separately to assess potential interferences. Borderline results were excluded from calculations.

Results: Longitudinal analysis demonstrated typical antibody kinetics, with anti-OROV IgM peaking around 10dpo, followed by IgG seroconversion. Seroprevalence in Manaus was 1.8% (IgM) and 16.9% (IgG). The IgG ELISA showed very high overall agreement with FRNT (97.7%, $\kappa=0.953$). Sensitivity reached 100% for IgM and 80.0% for IgG, while specificity was 97.7% and 99.9%, respectively. A small subset of malaria samples showed reactivity (IgM, 17.2%; IgG, 3.3%).

Conclusion: The new Euroimmun Anti-OROV ELISAs are standardized and easy-to-use tools that support seroepidemiological surveillance and provide reliable detection of acute OROV infections. Reduced IgG sensitivity observed in the applied sample panel likely reflects early sampling prior to IgG seroconversion. Potential interference by acute malaria cannot be excluded.

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Improved and customized dengue serodiagnostics through combined NS1/IgM testing and novel dual-cut-off IgG ELISA

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Introduction: This study examined the diagnostic accuracy of four Euroimmun ELISAs, including a newly developed dual-cut-off anti-dengue virus (DENV) IgG ELISA.

Methods: The Dengue Virus NS1 ELISA, Anti-Dengue Virus Type 1-4 ELISA (IgM), Anti-Dengue Virus Type 1-4 ELISA (IgG; native antigen/gE-based), and the novel Anti-Dengue Virus NS1 ELISA 2.0 (IgG; recombinant NS1-based, with alternative higher cut-off for flavivirus-endemic regions) were analyzed. Sensitivity was determined using sera from 22 Vietnamese patients with RT-PCR-confirmed DENV infection, collected during acute (t1, 1-6dpo), early convalescent (t2, 4-9dpo), and late convalescent (t3, 13-19dpo) phases. Specificity was assessed with samples from 500 German blood donors (HBD) and 40 patients each with WNV or ZIKV infection.

Results: Sensitivities were 90.5%/70.0%/0% (t1/t2/t3) for NS1, 33.3%/85.0%/77.3% for IgM, 66.7%/100%/100% for IgG, and 33.3%/65.0%/100% vs. 19.1%/50.0%/100% for IgG 2.0 (standard vs. alternative cut-off). Combined NS1/IgM testing achieved 100% sensitivity in single acute-phase samples. Overall specificity was 85.7% (HBD/WNV/ZIKV: 95.0%/50.0%/5.0%) for IgG, compared to 95.7% (98.2%/95.0%/65.0%) vs. 99.5% (99.8%/97.5%/97.5%) for IgG 2.0 (standard vs. alternative cut-off).

Conclusion: Euroimmun ELISAs support customized strategies applicable to various dengue testing contexts. Combining NS1 and IgM ELISAs offers an alternative to molecular assays in the acute phase. The native antigen/gE-based IgG ELISA enables early sensitive IgG detection, although with limited specificity. With minimal cross-reactivity, the NS1-based dual-cut-off ELISA 2.0 (IgG) reliably captures DENV-specific IgG dynamics and enhances differentiation from other flaviviruses, which could be advantageous for convalescent-phase diagnostics, surveillance, and serostatus determination.



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Evaluating CMV-RNA as a marker of active replication in allo-HSCT patients during and after letermovir prophylaxis

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Introduction: Cytomegalovirus (CMV) infection remains a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Letermovir (LTV), inhibiting the viral terminase and preventing processing of CMV genomic concatemers, has reduced CMV infection and reactivation, but the optimal virological monitoring strategy in the LTV era remains uncertain. CMV-DNA is the standard marker, though it may reflect genomic fragments or concatemers rather than active replication. CMV-RNA, targeting UL21.5 mRNA packaged in virions, has been proposed as a marker of CMV replication. This study evaluates CMV-RNA and CMV-DNA kinetics during and after LTV prophylaxis in allo-HSCT patients.

Methods: We enrolled 42 allo-HSCT patients receiving LTV for ≥ 2 months; 30 (71.4%) were monitored post-LTV for a median of 87 days. Overall, 464 plasma samples were tested for CMV-DNA and CMV-RNA using Elitech assays (LOQ 112 IU/ml and 30 cp/ml).

Results: During LTV prophylaxis, 5 patients (11.9%) showed CMV-DNA > 112 IU/mL (median 296 IU/mL), mostly isolated or recurrent blips; none had detectable CMV-RNA, indicating absence of virion production. After LTV discontinuation, 16/30 patients (53.3%) developed CMV-DNA > 112 IU/mL (median 536 IU/mL); CMV-RNA was positive in 5/16 (31.2%). RNA positivity correlated with higher DNA levels (median 12196 vs 78 IU/mL, $p=0.0005$) and prolonged DNA detectability (median 129 vs 10 days, $p<0.0001$). CMV-RNA predicted clinically significant CMV infection (csCMVi) more accurately than DNA (PPV 100%, NPV 68% vs 80% and 48%). During anti-CMV therapy, CMV-RNA declined more rapidly, indicating suppression of virion production despite persistence of DNA fragments.

Conclusions: CMV-RNA improves CMV monitoring in allo-HSCT by distinguishing active replication from residual genomic material and may support more appropriate antiviral stewardship.

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Hybridisation-capture metagenomic next generation sequencing for viral detection in plasma: can a protocol using the Oxford Nanopore Technologies (ONT) platform achieve sensitivity equivalent to targeted PCR?

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Introduction: Hybridisation-capture methods for viral metagenomic next generation sequencing (mNGS) increase sensitivity for viral detection. We assessed a published protocol for hybridisation-capture on the Oxford Nanopore Technologies (ONT) platform, which allows real-time analysis, giving the potential to reduce the time to result compared to the fixed run time of the Illumina platform, and be more economical for small sample numbers.

Materials and Methods: 18 remnant plasma samples were processed using the Twist Comprehensive Virus Research Panel combined with a post-hybridisation 4-primer PCR to allow sequencing on an ONT MinION. 6 samples were multiplexed on each run, alongside a negative and positive control. All samples were also spiked with an internal control. Following human read removal, EsVirus was used for viral identification. Performance was evaluated against routine testing.

Results: Using a threshold of >1 mapped read after 24 hours of sequencing, all viruses identified by routine testing were detected in 17/18 samples, including chikungunya (Ct 35), dengue (Ct 32), sindbis (Ct 33), CMV (< 53 IU/ml), and rubella (Ct 33). Dengue (Ct 35) was not detected. Excluding Anelloviridae and Pegivirus, there were additional RNA virus detections in 1 sample (HIV in a PLWHIV), and DNA virus detections in 9 samples. All additional DNA viruses detected (EBV, CMV, HSV-1, hAdV-2, MCPyV and HPV) establish latency and orthogonal testing is in progress.

Conclusions: In initial assessment, the protocol demonstrates excellent sensitivity and specificity for RNA and DNA viruses. Further assessment with an analytical limit of detection and more clinical samples is ongoing.



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Distinct patterns of HBsAg isoform display viral activity and characterize disease stage in the setting of chronic HDV infection

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Background and aims: Hepatitis B surface antigen (HBsAg) circulates as three isoforms (small[S],medium[M],large[L]), reflecting distinct steps of viral life cycle and morphogenesis. In HBV/HDV co-infection, we investigated the relationship between total HBsAg and its isoforms with markers of viral activity and liver injury.

Method: This study analyzed 366 HDV-RNA+patients from the HDV Describe study, mostly males (62.6%) and Italians (59.0%) with a median age of 56 years. Serum total, S-, M-, and L-HBsAg levels were quantified by ELISAs (Beacle Inc.) and were correlated with HDV-RNA (Robogene_v2), HBcrAg (Fujirebio), ALT, and liver stiffness measurement (LSM). Patients were classified into distinct clinical phenotypes according to liver cirrhosis status.

Results: Median HDV-RNA was 5.1 Log IU/mL and total HBsAg was 3331(675–9055) ng/mL, while median levels of S-, M-, L-HBsAg were 2686, 538 and 0.75 ng/mL, respectively. Total, S-, M-, L-HBsAg correlated positively with HDV-RNA and HBcrAg (all p<0.001). Total, S-, M-, and L-HBsAg levels were significantly higher in patients with ALT>40U/L (all p<0.001), whereas only L-HBsAg showed a modest correlation with LSM (p=0.007). Total, S-, M-, L-HBsAg levels varied according to patients' clinical phenotype, with the highest levels observed in high-viremic CHD, followed by cirrhosis and mild disease. In multivariate analysis, higher S-HBsAg levels were significantly associated with high-viremic CHD (OR=1.58;p=0.003), higher M-HBsAg with liver cirrhosis (OR=1.78;p=0.035), while lower L-HBsAg levels were associated with low-viremic CHD (OR=0.38;p<0.001).

Conclusion: Both total HBsAg levels and HBsAg isoforms correlated with HDV activity. Notably, HBsAg isoforms showed distinct associations across clinical phenotypes, suggesting a differential regulation of HBsAg in HDV-driven liver disease.

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Evaluation of the QIAstat-Dx Meningitis-Encephalitis Syndromic Test Results in a University Hospital

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Background: Central nervous system (CNS) infections are critical conditions requiring rapid pathogen identification to guide treatment and improve outcomes. The QIAstat-Dx Meningitis/Encephalitis (ME) Panel is a CE-IVD-marked multiplex PCR assay that enables rapid detection of 15 pathogens directly from cerebrospinal fluid (CSF). We evaluated our 39-month real-world experience with the QIAstat-Dx ME panel in a university hospital setting. We also aimed to explore whether routine CSF parameters may help guide more targeted use of syndromic testing.

Methods: Between January 2023 and March 2026, 389 CSF samples (mean age is 32.29, 55.7% male) were analyzed using the QIAstat-Dx ME Panel alongside conventional methods (microscopy, biochemistry, and cultures). Data were analyzed using SPSS v32.0.

Results: Pathogens were detected in 30 patients (7.7% positivity rate). The study population spanned a wide age range (0–90 years). Detected pathogens included HHV6 (n:6), Streptococcus pneumoniae (n:6), HSV1 (n:5), enterovirus (n:5), VZV (n:3), human parechovirus (n:3), Neisseria meningitidis (n:1) and Cryptococcus neoformans (n:1). Comparative analyses showed distinct distribution patterns of CSF parameters (leukocytes, erythrocytes, total protein, glucose, sodium, chloride, lactic acid) between the positive and negative cohorts across different age groups. PCR panel positivity was significantly associated with lower glucose, elevated protein, and increased leukocyte levels in cerebrospinal fluid ($p < 0.05$, AUC > 0.658). Among the CSF parameters evaluated, low glucose level demonstrated the highest diagnostic accuracy for distinguishing bacterial from viral meningitis cases, while elevated leukocyte and total protein levels also provided strong discriminatory power. ($p < 0.006$, AUC > 0.914)

Conclusion: The QIAstat-Dx ME Panel enables rapid diagnosis of CNS infections. Low positivity rates, despite test requests being limited to certain departments, suggest that integrating CSF glucose, protein, and leukocyte thresholds into ordering algorithms may improve cost-effectiveness.

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Evolutionary trajectory of the HIV-1 Tat protein

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Introduction/Aim: The Tat protein plays a pivotal role in HIV-1 pathogenesis, orchestrating both viral transactivation and host cell penetration via its Basic Domain. Unlike many essential proteins, mutation rate of Tat-1 is unusually high. We analyzed how sequence variations affect Tat functional capacities. Our study aims to unravel the evolutionary trajectory of the Tat protein in relation to its pathogenic extracellular role.

Material and Methods: We integrated in vitro functional assays with phylogenetic selective pressure analysis. Transactivation and penetration of rationally designed Tat mutants were evaluated in T- and B-cell lines (Jurkat, RPMI8866/BLAS). Extracellular Tat internalization and host gene regulation are being mapped via RNA sequencing. Parallely, BEAST tracing and Datamonkey dN/dS estimations identified specific structural loci under significant selection.

Results: Crucially, experimental validations identified residue R57 as strictly essential for optimal cellular uptake, whereas substitutions such as Q54H and R52W enhanced penetration. Furthermore, temperature-independent uptake profiles suggested dual endocytic and non-endocytic entry mechanisms, with confocal microscopy confirming the successful nuclear and cytoplasmic translocation of internalized variants.

Conclusions: By correlating evolutionary selection metrics with in vitro functional efficacies, we mapped a profound cell-type-specific functional adaptation: evolutionary selection scores negatively correlated with cellular penetrance across tested cell lines, a trade-off that notably extended to transactivation capacity specifically in B-cells. These findings reveal how sequence polymorphisms dictate extracellular function and intracellular persistence, establishing a foundation for tailored therapies based on HIV subtype-specific Tat properties.

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Hepatitis C Virus Downregulates SERPINA1 Expression via the NS4B Protein

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Introduction/Aim: Alpha-1 antitrypsin (AAT), encoded by the SERPINA1 gene, is a major plasma serine protease inhibitor known to modulate immune responses to viral infections. This study investigates the interaction between the hepatitis C virus (HCV) and SERPINA1.

Materials: The suppression of SERPINA1 expression by HCV was examined in cell culture models—including HuH7, HuH7.5, and HCV subgenomic replicon cells—as well as in serum samples from HCV patients and healthy control subjects.

Methods: SERPINA1 mRNA levels were quantified using real-time RT-PCR, while AAT protein levels were detected via Western blot analysis.

Results: HCV downregulates SERPINA1 expression through its NS4B protein. Specifically, NS4B facilitates the degradation of the AAT protein, a process that is blocked by the proteasome inhibitor MG132. Conversely, the expression of SERPINA1 was found to suppress HCV replication in both stable and transient expression systems.

Conclusions: Hepatitis C virus downregulates SERPINA1 expression to create a more favorable environment for its own replication.

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Epidemiological Shifts and Seasonal Forecasting of Hand, Foot, and Mouth Disease Outbreak Clusters in Beijing, 2019–2024: From Kindergartens to Schools

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Introduction: Hand, foot, and mouth disease (HFMD) frequently causes outbreak clusters in Beijing, disrupting child education. This study characterizes the spatiotemporal epidemiology of HFMD outbreak clusters (2019–2024), focusing on setting shifts, and forecasts 2025 trends.

Materials and Methods: Data on HFMD outbreak clusters in Beijing (2019–2024) were extracted from the Beijing CDC database. Demographic, clinical, setting, spatial, and virological characteristics were analyzed using Kruskal-Wallis, chi-squared, and Fisher's exact tests. A SARIMA model was developed to forecast monthly clusters for 2025.

Results: Of 4,265 clusters (12,396 cases), annual clusters decreased by 74.4% during 2020–2022 (COVID-19 NPIs) versus 2019, then rebounded sharply in 2023 to 1,719 clusters (+42.5% vs. 2019). In 2023, schools became the predominant setting (44.9% of clusters), a 4.2-fold increase versus the pandemic period, surpassing kindergartens. Median case age rose from 4 years (2019–2022) to 6 years in 2023. CV-A6 dominated (60.2%), peaking at 90.7% in 2023; EV-A71 was rare (0.12%). Clusters concentrated in near-suburban areas (>50% annually). The SARIMA(2,0,0)(0,1,1)₁₂ model (AIC=724.54, Ljung-Box p=0.682) forecasts 716 clusters for 2025 with a bimodal pattern: a primary autumn peak (299 clusters) and a secondary summer peak (165 clusters).

Conclusions: HFMD outbreak clusters in Beijing exhibited a biennial pattern with NPI-induced suppression followed by a rebound exceeding pre-pandemic levels. A significant epidemiological shift from kindergartens to schools as the primary transmission setting was identified, alongside sustained CV-A6 dominance. The SARIMA model forecasts a similar bimodal pattern. Findings underscore the need for setting-specific strategies prioritizing schools in high-risk areas and continued pathogen surveillance.



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Reciprocal Antagonism Between HCV and Serpin D1 Defines a Host–Virus Regulatory Axis

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The interplay between the Hepatitis C virus and host restriction factors is a key determinant of viral persistence. In this study, we identify Serpin D1, also known as heparin cofactor II (HCII), as a novel host factor modulating HCV replication. Differential gene expression analysis using ddRT-PCR revealed that HCII is markedly downregulated in HCV replicon cells. This suppression occurs at the transcriptional level and is reversed upon interferon- γ -mediated clearance of viral RNA. Consistently, reduced HCII protein secretion was observed in both HCV replicon systems and sera from infected patients.

Functional studies demonstrated that HCII acts as a negative regulator of HCV replication: HCII knockdown enhanced viral replication, whereas ectopic expression significantly suppressed replication in both replicon and infectious systems. Mechanistically, HCV nonstructural proteins NS3 and NS5A mediate transcriptional repression of HCII. HCII directly interacts with NS3, and its antiviral effect can be antagonized by NS3 overexpression, but not NS4B, indicating a specific viral counter-defense mechanism. Interestingly, HCII-mediated inhibition of HCV replication occurs independently of its canonical protease inhibitory activity, suggesting a non-classical antiviral function.

In conclusion, our findings uncover a previously unrecognized host–virus regulatory axis in which HCII restricts HCV replication, while the virus actively suppresses HCII expression to facilitate its persistence

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Co-Circulation of respiratory pathogens that Cause Severe acute Respiratory Infections among hospitalized patients in Beijing, China

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Objective: To explore the co-circulation of respiratory pathogens among hospitalized patients from severe acute respiratory infections (SARI) in Beijing, multi-pathogen detection analyses were performed.

Methods: We conducted a retrospective analysis using targeted Next Generation Sequencing (tNGS) to assess infection patterns of SARI patients during the autumn and winter of 2023 in Beijing, China. Oropharyngeal swab samples were collected for detection of multiple pathogens. A total of 107 pathogens were detected in the swab samples, including 74 viral pathogens and 33 bacterial pathogens.

Results: Etiological and epidemiological characteristics of SARI were explored to inform treatment and prevention strategies. Among all SARI cases, 572 (90.1%) had at least one positive result. The most frequently identified bacterial pathogen was *S. maltophilia* (31.4%, 231/735). The most frequently identified viral pathogen was the Epstein-Barr (EB) virus (20.6%, 81/394). Viral-bacterial co-infections were present among all cases. The most common viral-bacterial co-infection was between *S. maltophilia*, EB virus, and HSV-1. The most common bacterial co-infections were *S. maltophilia*–*M. pneumoniae*, *S. maltophilia*–*H. influenzae*, *S. maltophilia*–*A. baumannii*, and *S. maltophilia*–*K. pneumoniae*.

Conclusion: These findings might help to better identify predominant respiratory pathogens, apply differential and more accurate diagnoses, and improve treatment and prevention. The findings suggest that we need to draw our attention to some neglected pathogens, and improve the coverage rates and effectiveness of vaccines, and improve the treatment of pathogens. Key words: Severe acute respiratory infections; targeted Next Generation Sequencing; co-circulation; infection patterns; *S. maltophilia*.

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Epidemiological features and genomic characteristics of the imported cases of Lassa fever in Beijing, China

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Objective: Lassa fever (LF) is an acute viral hemorrhagic disease endemic to West Africa, with sporadic imported cases reported in non-endemic regions in recent years. This study aimed to investigate the epidemiological features, viral shedding kinetics, and genomic characteristics of two imported LF cases in Beijing, China.

Methods: The clinical information was collected from the hospital and field investigators adopted a standardized surveillance and reporting form to collect epidemiological and clinical data. To investigate the viral shedding kinetics of Lassa fever, blood and urine specimens were collected at different time points and used to PCR testing for cycle threshold (Ct) value determination. The genes of Lassa fever virus were amplified by NGS.

Results: Both cases were male workers employed in Kankan, eastern Guinea, with unsanitary working environments involving frequent exposure to rodents. Clinical samples were collected for real-time polymerase chain reaction detection, next-generation sequencing, and phylogenetic analysis. The results showed that both imported cases presented typical LF symptoms, with prolonged viral shedding in urine (58 days and 43 days, respectively). Phylogenetic analysis based on L and S genes revealed that the viral strains belonged to Lineage IV, closely related to Guinea-derived strains.

Conclusion: This study highlights the occupational exposure risk of LF in endemic regions and the importance of strengthened surveillance, timely diagnosis, and strict infection control measures for imported LF cases in non-endemic areas to prevent secondary transmission.

Keywords: Lassa fever; epidemiological features; genomic characteristics; imported cases; surveillance

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Ultra-deep Next-Generation-Sequencing to investigate rapid Development of Aciclovir-Resistance Mutations in Herpes Simplex Viruses type 1

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Aim: Severe manifestations of HSV-1 are commonly treated with potent antiviral agents, particularly aciclovir (ACV) and its derivatives. However, the emergence of drug-resistant HSV-1 strains represents a significant risk, especially for immunocompromised patients. In this study, we aimed to elucidate the genotypic basis underlying the development of phenotypic ACV resistance.

Methods: We compared the replication kinetics of (i) bacterial artificial chromosome (BAC)-derived viruses and (ii) clinical HSV-1 isolates in the presence of increasing ACV concentrations up to 62 µM and determined their IC50 values. Whole genome sequencing (WGS) was performed on treated samples, and ultra-deep sequencing was applied to the parental virus stocks. Bioinformatic analyses were conducted to assess whether resistance-associated mutations originated from preexisting viral variants. In addition, BAC-derived viruses were serially passaged to evaluate the impact of replication on resistance acquisition.

Results: Within seven days all viruses replicated in the presence of ACV to same titers as untreated controls. They showed an increase in their IC50 value due to antiviral treatment. WGS revealed multiple emerging mutations in UL23, the gene encoding the thymidine kinase. Ultra-deep sequencing of the parental viruses showed the origin of these resistance associated mutations stem from minority variants. The rescued BAC derived virus was able to grow under ACV treatment after ten untreated passages.

Conclusion: Our findings demonstrate that ACV-sensitive HSV-1 populations rapidly adapt under antiviral pressure, primarily through the selection of preexisting resistant minority variants, particularly in genes such as UL23 and UL30. Increased genetic diversity generated through passaging further facilitates resistance development.

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High Proportions of GAP43 Positivity in the Cerebrospinal Fluids of Patients with Sporadic and Certain Types of Genetic Creutzfeldt-Jakob Diseases by Western Blot Analysis

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Introduction: Growth-associated protein-43 (GAP43) is a neuronal protein essential for synaptic function and plasticity, and its reduction has been observed in brains of prion diseases (PrDs) and rodent models. However, its status in the cerebrospinal fluid (CSF) of patients with PrDs remains unclear.

Methods: CSF samples from 140 PrD cases, including 48 sCJD, 35 T188K-gCJD, 22 E200K-gCJD, 35 D178N-FFI, and 36 non-PrD controls, were analyzed for GAP43 by Western blot. The results were compared with 14-3-3 and calmodulin (CaM) detected by WB, and associated with clinical features.

Results: GAP43 positivity was significantly higher in sCJD (70.83%), T188K-gCJD (65.71%), and E200K-gCJD (72.73%) than in non-PrD controls (27.78%). The sensitivity and specificity of GAP43 (around 70–75%) were comparable to 14-3-3 and CaM, though inferior to RT-QuIC and total tau reported elsewhere. CSF GAP43 positivity correlated with sCJD-associated MRI changes, periodic sharp-wave complexes (PSWC) on EEG, and with 14-3-3 and CaM positivity.

Conclusions: Our data here indicate the feasibility of usage of GAP43 by Western blot analysis as a diagnostic, at least as a screening, biomarker for sCJD and certain types of gPrDs.

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Baseline etiological data from a randomised trial to investigate the effect of nasal sprays versus usual care in non-hospitalised patients with respiratory infections (ECRAID-Prime)

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Introduction: COVID-19 and COVID-like-illness cause significant ill-health. Since the greatest burden is in the community, it is in primary care (PC) and community settings where early treatments will be of most overall benefit. ECRAID-Prime is a platform randomised, multicountry trial evaluating investigational products for treatment of patients in PC with respiratory infections.

The aim was to determine the baseline etiology in the recruited patient population.

Methods: 812 patients were recruited. A combined throat-nose swab was taken at baseline (D0) placed in universal transport media and frozen till shipment to the central laboratory in Antwerp, Belgium. Nucleic acids were extracted by using the MagMAX Viral/Pathogen II Nucleic Acid Kit on the KingFisher Flex and analysed by the TrueMark Respiratory Panel 2.0, TaqMan Array Card (ThermoFisher Scientific) to detect presence of viruses and atypical bacteria.

Results: Samples from 565/812 patients enrolled between 09/10/2024 – 20/03/2026 (Belgium n=145, France n=62, Germany n=33, Ireland n=40, Poland n=71, Spain n=73, United Kingdom n=97 and Georgia n=44) were analysed (male n=220, female n=345; 18 – 87 years).

In 373/565 (66.0%) ≥1 respiratory virus was detected: 20.0% influenza virus (INF), 20% rhinovirus (HRV), 14.2% seasonal coronaviruses (HCoV), 6.8% respiratory syncytial virus (RSV), 4.8% SARS-CoV-2, 2.3% parainfluenza virus, 2.3% enterovirus-D68. In 5.5% ≥2 respiratory viruses were detected. The most frequent combinations were HRV + HCoV, INF + HCoV, HRV + SARS-CoV-2 and INF + RSV.

C. pneumoniae and M. pneumoniae were detected in 0.2% and 0.4% of samples respectively.

Conclusion: In 2/3 patients presenting with COVID-like-illness to PC, a respiratory virus was detected. INF, HRV, and HCoVs were most often detected. In 5.5% of samples multiple respiratory viruses were detected.

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Torque Teno Virus in Bronchoalveolar Lavage Fluid of Hematological Patients and Association with Pathogens

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Introduction/Aim: Torque teno virus (TTV) replication is tightly modulated by the host immune response. As such, TTV viremia has been investigated as a biomarker of immune competence for monitoring the risks of allograft rejection and opportunistic infections in solid organ transplant recipients. We aimed to investigate the associations between TTV levels in bronchoalveolar lavage fluid samples (BALF) and pathogen detection in patients with hematological diseases with suspicion of lower respiratory tract infection (LRTI).

Material/Methods: We conducted a retrospective monocentric study on patients admitted to the hematology department between January 2021 and July 2024, who underwent BALF sampling and microbiological investigations for suspicion of LRTI. TTV DNA was quantified in BALF using qPCR. Linear and logistic regressions were used to study associations between TTV levels in BALF and patients' characteristics, cytological patterns and pathogen detection.

Results: A total of 330 BALF from 272 patients with hematological malignancies were included. TTV DNA was detected in 44.5% of samples. The median TTV viral load (VL) in positive samples was 6.24 log copies/mL (IQR: 4.68–7.66). Strong positive associations were found between high TTV levels in BALF and a diagnosis of lymphoma (OR= 4.61), a neutrophil fraction >3% (abnormal proportion cut-off) of BALF cells (OR= 3.57), and the detection of at least one respiratory virus (OR=3.51). To a lesser extent, allogeneic hematopoietic stem cell transplantation, significant bacterial growth in BALF culture, and cytomegalovirus detection in BALF were also associated with TTV levels.

Conclusions: These findings suggest that high TTV levels in BALF could reflect an impaired local immune status, and correlate with the risk of viral LRTI in immunocompromised patients.

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Performance of serological and molecular markers to predict congenital CMV infection (cCMV) in offspring's of pre-immune pregnant women

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Introduction/Aim: In Europe and the USA, cCMV infection occurs in 30 to 50% of cases in pregnant women with preconceptional immunity. We aimed to assess the performance of serological and virological markers in this population.

Materials and Methods: Pregnant women with preconceptional immunity were enrolled between October 2022 and November 2024 during their first visit (11 - 14 weeks of gestation) at Necker Hospital (Paris, France). Serum samples for anti-CMV IgG, IgM, and IgG avidity were collected during the first trimester (T1). Blood and urine samples were collected at T1 and at delivery (T3). Neonatal saliva samples were collected within the first 15 days after birth. Quantitative CMV PCR was performed on blood, urine and saliva specimens.

Results: 1299 with pre-existing immunity were followed throughout pregnancy. Saliva samples were positive for CMV DNA in 2/1074 newborns; corresponding to a cCMV rate of 0.2%. These 2 children have been followed up to 12 and 17 months and did not develop any sequelae. One pregnancy was terminated at 27 weeks' gestation due to severe cCMV infection. The sensitivity to predict cCMV of IgM and CMV DNA in blood at T1 and T3 was 33.3%, whereas CMV DNA detection in urine at T1 reached 66.6%. The positive predictive value (PPV) of these assays did not exceed 5.8%. When CMV DNA was detected in both urine and blood at T1, PPV reached 16.7% with a sensitivity at 50.0%. The rate of vertical transmission in women with CMV replication was 2.6%.

Conclusion: In this population of women with pre-existing immunity, both vertical transmission rate and cCMV rate were low (2.6 and 0.2%). Virological markers showed low sensitivity and very limited PPV, highlighting their poor diagnostic value. Our findings underscore the need to develop new markers to identify pregnant women at risk of cCMV.

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Serum Torque Teno Virus Levels Are Strongly Associated with Stages of Non-Advanced HIV-1 Infection at Diagnosis

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Introduction/Aim: Torque teno virus (TTV), a ubiquitous component of the blood virome, is tightly controlled by the immune system. TTV levels have therefore been proposed as a marker of immune competence in immunocompromised individuals. In people with HIV-1 (PWH), high TTV levels have been associated with advanced infection.

We assessed TTV viral load (VL) in non-advanced HIV-1 infection at diagnosis and compared primary and chronic stages to determine whether TTV levels could reflect immune dysfunction not reflected by CD4⁺T-cell counts.

Methods: This monocentric cohort study included adults newly diagnosed with HIV-1 infection, antiretroviral therapy-naïve, viremic, and without advanced disease. Infection stage was defined using immunoblot-based Fiebig classification. Serum TTV DNA was quantified by qPCR. Associations with HIV-1 infection stage were assessed using logistic regression.

Results: A total of 126 PWH were included. Primary HIV-1 infection accounted for 42.1% of diagnoses and chronic infection for 57.9%. TTV DNA was detected in 61.1% of participants and was more frequent in chronic than primary infection (82.2% vs 32.1%, $p < 0.0001$). Median TTV VL was significantly higher in chronic infection (4.82 vs 2.65 log copies/mL, $p < 0.0001$), despite similar CD4⁺T-cell counts. In multivariable analysis, only HIV-1 VL and TTV VL were independently associated with HIV-1 infection stage. TTV VL ≥ 4 log copies/mL was strongly associated with chronic infection (OR= 8.09, $p < 0.0001$).

Conclusions: In non-advanced HIV-1 infection, TTV levels differ markedly between primary and chronic stages despite comparable CD4⁺T-cell counts. TTV VL may therefore reflect immune alterations not captured by standard markers and could serve as an additional biomarker of immune competence in PWH.

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POST PANDEMIC REBOUND OF ENTEROVIRAL MENINGITIS IN POLAND SUPPORTS A DELAYED SUSCEPTIBLE COHORT EFFECT

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Introduction/Aim: Enteroviral meningitis displays marked seasonality and is highly sensitive to changes in population contact patterns. We assessed whether national enteroviral meningitis surveillance in Poland supports a delayed susceptible cohort effect after COVID-19 non-pharmaceutical interventions (NPIs).

Material and Methods: We performed a retrospective ecological analysis of monthly enteroviral meningitis notifications in Poland from January 2012 to March 2026 using national surveillance data. A pre-pandemic seasonal baseline was defined from 2012–2019 as the mean number of cases for each calendar month. Post-pandemic monthly and annual notifications were compared with expected baseline values to account for seasonality.

Results: Pre-pandemic enteroviral meningitis incidence was variable but strongly seasonal, with a mean expected annual burden of 116 cases. During the NPI period, notifications fell to 14 cases in 2020 and 5 in 2021, representing a marked suppression relative to baseline. Activity remained below expected in 2022 (54 observed vs 116 expected; excess -62), followed by sustained excess transmission in 2023 (244 vs 116; +128), 2024 (347 vs 116; +231), and 2025 (174 vs 116; +58). Relative to the pre-pandemic baseline, Poland experienced approximately 213 fewer than expected cases during 2020–2021, followed by a cumulative excess of 356 cases in 2022–2025. Seasonality-adjusted monthly anomalies showed prolonged positive deviation from baseline beginning in 2023 and persisting through 2025.

Conclusions: Enteroviral meningitis in Poland showed marked suppression during COVID-19 followed by sustained multi-year excess transmission. These findings support a delayed susceptible cohort effect consistent with post-pandemic enterovirus immunity debt.

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From pediatrics to geriatrics: clinical and genomic approach to respiratory syncytial virus (RSV) infections

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Introduction: Since 2023, RSV burden in pediatrics has decreased thanks to nirsevimab. In 365-year-old, morbidity/mortality remain comparable to influenza, despite available but not reimbursed vaccines in France. This study compared RSV infections in children and in older adults, focusing on morbidity/mortality and genomic data.

Methods: This single-centre retrospective study included 81 RSV infections confirmed by RT-PCR (Seegene Allplex) during the winter of 2024–2025. Data were collected and high-throughput RSV sequencing (NextSeq550, Illumina) enabled phylogenetic analysis (Nextclade).

Results: Forty-one <1-year-old (median age: 2 months; IQR: 0.7–3.0; sex ratio: 0.7) patients were included of whom 21 (51%) had received nirsevimab. In parallel 40 unvaccinated adults (median age: 80 years; IQR: 74–84; sex ratio: 0.9) were enrolled: 19 (48%) had risk factors for severe disease including chronic obstructive pulmonary disease (12%; 5/40) or immunosuppression (30%; 12/40). Many children exhibited respiratory distress (80%; 33/41) compared with adults (40%; 16/40; $p=0.02$). Invasive ventilation was required in 10% (4/40) of adults and 5% (2/41) of children, all non-immunized. ICU admission was common in children (46%; 19/41), especially in unprotected, premature or <2-month-old infants (OR=0.049; $p=0.0012$), compared with adults (18%; 7/40; $p=0.0002$). No deaths occurred in children, but adult mortality reached 8% (3/40), consistent with reported data. Despite clinical differences, RSV-A and B clades distribution was age and prophylaxis-independent.

Conclusion: Adults and children are infected regardless of RSV clade. Infection varies by age: early symptoms and frequent ICU admission in young children, prolonged hospitalization due to comorbidities in older adults. While effective in pediatrics, prophylaxis would likely benefit older adults.

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When Is Adenovirus Clinically Relevant? Distinguishing Etiologic from Incidental HAdV Detection in Pediatric URTI and LRTI

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Introduction/Aim: Human adenovirus (HAdV) is frequently detected by PCR in children with respiratory tract infection (RTI), but etiologic relevance is uncertain due to prolonged shedding and frequent viral codetections. We aimed to identify clinical and virological patterns, distinguishing likely etiologic HAdV infection from incidental detection, with focusing on differences between upper (URTI) and lower RTI (LRTI).

Material & Methods: In a prospective hospital cohort in Mid-Norway (2006–2017), nasopharyngeal aspirates from 4,881 children admitted with RTI were tested by in-house real-time PCR (high viral load defined as $C_t \leq 30$), standard viral culture, and a broad panel of 18 respiratory viruses. A comparison group of 673 children admitted for elective surgery was included. Among HAdV-PCR-positive children, logistic regression adjusted for age and sex compared URTI and LRTI with HAdV-positive controls.

Results: HAdV was detected by PCR in 6.6% (322/4,881) children with RTI (144 URTI, 178 LRTI) and 7.4% (50/673) controls. URTI was strongly associated with high viral load ($C_t \leq 30$; OR 10.3, 95% CI 4.2–25.5), culture positivity (OR 10.5, 4.7–23.3) and single HAdV detection (OR 3.6, 1.4–8.9). LRTI showed weaker association with $C_t \leq 30$ (OR 3.8, 1.6–9.1) and culture positivity (OR: 2.3, 1.1–4.8), but a strong association with co-detected severe viruses (OR 8.3, 2.6–26.6), particularly RSV (OR 13.0, 1.7–98.4), but no association with single detection (OR 1.0, 0.4–2.7).

Conclusions: In hospitalized children with RTI and HAdV detection, URTI accompanied by high viral load, culture positivity or single detection strongly supports HAdV as the primary etiologic pathogen. In LRTI, frequent codetection of severe respiratory viruses, especially RSV, suggests HAdV is often an incidental bystander unless supported by markers of active replication.

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Testing the Tests: Performance of Norovirus Antigen Assays in New EQA Scheme

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Introduction: Norovirus, a common cause of acute gastroenteritis, spreads mainly via the faecal-oral route. Its high infectivity leads to frequent outbreaks, especially in healthcare and other closed settings. Although RT-qPCR is the preferred diagnostic method for norovirus diagnosis, rapid antigen detection assays are widely used because they provide fast and practical results. Due to variable assay sensitivity, external quality assessment (EQA) is important to ensure reliable antigen test performance. This study presents the successful results of a newly developed EQA scheme for norovirus antigen detection by EQA provider Labquality.

Material and Methods: Sample sets, including 3 swab samples, one negative and two norovirus-positive samples containing recombinant norovirus GI.1 protein and GI.4 protein, respectively, were distributed to 469 participants in 13 countries over six rounds. Participants were instructed to handle swabs as stool samples, elute material into test buffers, and follow test instructions. Qualitative results, genogroup, and methods were reported via Labquality's electronic LabScala platform.

Results: In total, 1384 results were reported (response rate 94%) using 19 commercial tests. Most assays were lateral flow-based, including single- and multi-analyte formats, some distinguishing genogroups GI and GII. The overall success rate was 98% (97% for GI-positive, 97% for GII-positive, and 99% for negative samples). Genogrouping was reported in 682/923 positive results; 12 reported the wrong genogroup and 7 reported a negative result for the GI sample, while 5 reported the wrong genogroup for the GII sample.

Conclusions: Due to the highly contagious nature of norovirus, there is a need for rapid antigen tests and, consequently, for the corresponding EQA scheme. Based on the EQA results presented, norovirus antigen tests demonstrated a consistent overall performance.

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Testing the Tests: External Quality Assessment for Nucleic Acid Detection of HSV-1, HSV-2, VZV and *Treponema pallidum*

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Introduction: Labquality, a provider of External Quality Assessment (EQA), has developed a new EQA scheme for molecular assays detecting herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus (VZV), and *Treponema pallidum* (TP) in lesion samples. The increasing availability of multiplex molecular assays enables simultaneous detection of multiple ulcerative pathogens, supporting faster and more comprehensive diagnosis. As these infections may present similarly but require different treatments, reliable testing is essential. There is a growing need for EQA schemes that support diverse testing approaches and allow comparison across multiplex and single-target methods.

Materials and Methods: Sample sets of three swabs, each containing 0–4 pathogens, were distributed to 213 participants in 15 countries over six rounds. Participants eluted swabs, performed nucleic acid extraction and NAAT according to routine protocols, and reported results via LabScala platform. Each participant's test selection was considered in the evaluation.

Results: In total, 1699 results were reported (response rate 90%) using 45 methods, with an overall success rate of 99%. Of the results, 368 were obtained using tests detecting all four targets and 284 using tests detecting both HSV and VZV. Most results (62%) came from single-target assays. Performance was excellent for all pathogens (98–100%), with most deviations observed for VZV. No performance differences were observed between samples containing one, multiple, or no pathogens (99–100%).

Conclusions: Test methods used in different laboratories vary in target combinations and workflow characteristics, reflecting a rapidly evolving diagnostic landscape. The results of Labquality's multiplex EQA for ulcerative pathogens indicate overall good and consistent performance across methods.



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Salivary detection of Rotavirus A in household contacts as a potential marker of transmission dynamics

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Rotavirus A (RVA) is a leading cause of acute gastroenteritis in children, yet adult infections are often asymptomatic and the role of saliva in transmission remains poorly characterized. This study investigated RVA detection and viral load in saliva of patients and their household contacts.

During the 2024–2025 period, households in continental Croatia with PCR-confirmed RVA-positive index cases were included. Stool and saliva samples were collected from index patients (median age 2), while saliva was collected from household contacts (median age 33). RVA was detected and quantified using real-time RT-qPCR. Preliminary VP7/VP4 genotyping was performed using multiplex RT-PCR, and detection rates and viral loads were compared using appropriate statistical tests.

Among 141 RVA-positive households (141 index cases and 232 contacts), RVA was detected in saliva of 69.5% of index cases and 50.0% of contacts. Viral load (median, copies/mL) differed significantly between sample types: highest in stool (1.38×10^9), followed by index case saliva (1.39×10^3) and contact saliva (6.23×10^2). Households with saliva-positive index cases had a higher proportion of saliva-positive contacts. While fecal viral load significantly decreased after day 8 since symptom onset, salivary viral load showed higher levels at days 4–7 compared to days 0–3. Among saliva-positive contacts, 64.7% were asymptomatic, with no difference in viral load compared with symptomatic contacts. Preliminary genotyping of saliva indicated predominance of G3 and P[8] genotypes, with others detected at lower frequencies.

RVA is frequently detected in saliva of infected patients and household contacts. The association between salivary positivity in index cases and household contacts suggests that salivary RVA may reflect household exposure dynamics rather than symptomatic infection.

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Seroprevalence and molecular surveillance of Enterovirus A71 after the 2016 outbreak in Spain

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Introduction/Aim: Enterovirus A71 (EV-A71) causes neurological and mucocutaneous pathologies. Following a 2016 paediatric encephalitis outbreak in Spain driven by the C1v subgenogroup, this study characterises EV-A71 infections (2017–2025). Pre- (2015) and post-outbreak (2018) seroprevalences were determined to assess immunity dynamics.

Materials and Methods: Through the national surveillance system, 4,083 Enterovirus-positive clinical samples from Spanish hospitals were analysed. Genotyping utilised 3'-VP1 RT-PCR, sequencing, and phylogenetic analysis. Microneutralisation assays were conducted on age-stratified sera from 2015 (n=119) and 2018 (n=137) against historical C1 and emerging C1v EV-A71 strains to quantify neutralising titres.

Results: From 2017–2025, 145 EV-A71 infections (3.5%, 145/4,083) were identified. Annual prevalence (0.5–10%) remained lower than in 2016 (22%). Children ≤5 years comprised 89% of cases, primarily presenting encephalitis (48%) or febrile syndrome (30%). Phylogenetic analysis showed C1v predominance (89.8%). In 2015, seroprevalence was lower in children aged 1–5 (34.5%) than in those aged 6–14 (69.6%, $p < 0.05$) or adults (55%). Overall seroprevalence increased from 51.3% (2015) to 69.3% (2018) ($p < 0.01$). However, geometric mean titres (GMTs) did not increase commensurately, remaining consistently lower for the 2016-C1v variant than the 2012-C1 strain.

Conclusions: EV-A71 C1v is now endemic in Spain, predominantly impacting naive paediatric populations. Despite significant post-outbreak seroconversion across the population, the attenuated magnitude of neutralising antibodies against the C1v variant suggests potential sub-immunogenicity. It is hypothesised that this could confer an adaptive evolutionary advantage, facilitating partial immune evasion and persistent community transmission.

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Characterization of HIV-1 Subtypes and Drug Resistance Mutations at ULSSM using Next-Generation Sequencing (May 2023 – December 2025)

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Introduction: HIV-1 genotypic resistance testing has shifted from Sanger sequencing to NGS, which provides higher sensitivity and detects low-frequency variants. This study aims to characterize HIV-1 drug resistance results obtained by NGS and identify the most frequent drug resistance mutations in treated and naïve patients.

Materials and Methods: Samples submitted for HIVDR testing between May 2023 and December 2025 were analyzed, and viral load was measured using Roche COBAS 6800 system. Library preparation was performed using Longwood HIV-1 Arrow kit, followed by sequencing on Illumina iSeq 100. Data were analyzed using Smartvir software and Stanford HIV DR database, evaluating mutations associated with resistance to NRTIs, NNRTIs, PIs and INSTIs.

Results: Samples corresponded to the first HIVDR test per patient during the study period (n=600). Median age was 39 years; 68,3% male. The most prevalent subtypes were B(36%), CRF02_AG(14,3%), G(11,7%) and C(11,2%). Patients were grouped as Naïve, Treated, NoTARV (at test date), and Unknown Status. Significant differences were observed between Naïve and Treated groups in age, gender, logCV, and subtypes (p<0,01). Resistance mutations were compared between Naïve (n=171) and Treated (n=195). 16,4% of naïve and 38,5% of treated patients presented major RM in at least one class of ARVs. The most frequent Drug Resistance Mutations in treated patients were M184I/V for NRTIs (12,3%), K103N/S for NNRTIs (16,9%), L90M for PIs (3,6%), and S230R for INSTIs (4,1%). In naïve patients the most frequent mutation was K103N/S (7%).

Conclusions: In naïve patients, associations were found with younger adults and higher proportion of CRF02_AG and subtype F; in treated patients, associations with older adults, female gender and subtypes G and B. Clinical information is essential for accurately characterizing all groups.

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Adjusting internal entero/parecho PCR diagnostics into the direction of IVDR

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Background: Enteroviruses are a large group of RNA viruses (genus Enterovirus) comprising over 100 distinct serotypes that cause a wide range of diseases. They are characterized by transmission via the fecal-oral or respiratory routes, causing mild respiratory illnesses, Hand-Foot-and-Mouth Disease (HFMD), and severe infections like meningitis or polio.

Aim: The aim is to replace the current in-house test with a commercial test that is compatible with our laboratory workflow and whose controls have a higher stability than our currently laboratory-developed controls.

Material and Methods: Enterovirus and parechovirus are tested in patients with respective symptoms in stool samples, respiratory secretions, swabs from bladder, wounds, blisters, and the upper respiratory (Flow solution by Roche: extraction MP96, amplification ABI7500 FAST).

The validation of the AltoStar® EV & RV & HPeV RT-PCR Kit 1.5 focuses on CSF, respiratory swabs and stool samples as the most common routine sample matrices. The internal sample bank was searched to create dilution series (analytical sensitivity) or perform point measurements in clinical material (diagnostic sensitivity). Almost all important enterovirus were found except enterovirus A71 and coxsackie virus B4.

Conclusion: The commercial AltoStar® EV & RV & HPeV RT-PCR Kit 1.5 (RUO) combined with the MP96 /ABI7500 Fast show equal or better results for the detection of Enterovirus and Parechovirus (Rhinovirus not tested) in CSF, respiratory swabs and stool samples as the internal validated in-house assay.



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Detection of Human Bocavirus 1 (HBoV1) in Respiratory Samples

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Introduction/Aim: Human bocavirus 1 (HBoV1) is a parvovirus frequently detected in children with respiratory tract infections (RTIs), often alongside other respiratory pathogens. While HBoV1 is well described in pediatric populations, data across all age groups are limited. This study aimed to describe the epidemiology, age distribution, viral load patterns, and codetection rates of HBoV1 in respiratory samples analyzed between 2023-2025.

Material and Methods: Results from 223,065 nasopharyngeal swabs submitted for RTI diagnostics from patients of all ages were included. Samples were analyzed using a multiplex panel (14 viruses and 3 bacteria). 165,966 swabs had full panel analysis. HBoV1 positivity was defined as cycle threshold (Ct) <36, inconclusive results were excluded. Viral load was categorized as high (Ct ≤30) or low. Demographic data and code-detections were recorded and analyzed descriptively.

Results: HBoV1 was detected in 0.67% (1,501/223,065) of samples. Median age was 2 years (IQR 1–4). Positivity was highest in children <10 years (72.6%, 1,089/1,501). High viral load (Ct ≤30) was observed in 35.4% (532/1,501) of positive samples and occurred predominantly in young children. 30.0% (450/1,501) were <10 years (median age 1 year, IQR 1–2). A slight male predominance was observed among HBoV1-positive children, while adults aged 30–45 years, showed a modest female predominance. Among positive samples with full panel analysis (n=1,031), codetections were common, most often with rhinovirus (30.0%), adenovirus (11.0%), and RSV (10.8%). HBoV1 monodetection accounted for 32.5% of cases, mainly in children <10 years.

Conclusions: HBoV1 was detected across all age groups but showed clear predominance and higher viral loads in children <10 years. These findings support inclusion of HBoV1 in routine pediatric respiratory viral panels.

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Lessons to be Learned from HHV6: A Case Report of a Nine-Year-Old Girl

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Background: Following a visit to the paediatrician, who diagnosed a streptococcal infection with skin manifestations and prescribed amoxicillin/clavulanic acid and ibuprofen for a nine-year-old female patient, her condition deteriorated, resulting in admission to a peripheral hospital. The exanthema was characterised by blisters spreading across the entire body. HHV-6 DNA was detected by PCR in blister fluid. As the patient's condition worsened, she was transferred to the intensive care unit at the Medical University of Innsbruck. The mother reported that her daughter had been treated for sinusitis with ibuprofen and oxymetazoline one month earlier. Consequently, toxic epidermal necrolysis was considered as an additional differential diagnosis.

Methods: Clinical chemistry laboratory values and microbiological tests were performed in laboratories in Vorarlberg, at the Medical University of Innsbruck, and at the Center for Virology at the Medical University of Vienna.

Results: Although the blood count and inflammatory markers remained relatively stable, liver and bilirubin values increased significantly. As HHV6 was present in the plasma, foscarnet was administered for 12 days. During this time, the dermatological condition improved with topical therapy and prednisone administration. Finally, the patient's hair follicle was tested for HHV-6 nucleic acid by PCR. The result revealed chromosomally integrated HHV-6 (ciHHV-6).

Conclusions: Based on the detection of ciHHV-6, the severe epidermal necrolysis with liver involvement was presumably not caused by HHV-6, but rather by a toxic trigger, most likely related to ibuprofen. This case report underscores the importance of timely exclusion of ciHHV-6 in severely ill patients with suspected HHV-6 infection to avoid unnecessary antiviral therapy and allow for the search of alternative diagnoses.



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Readiness for Future Testing: CMV Non-blood EQA as a Model of Real-World Complexity in Pediatric Infection

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Introduction: Cytomegalovirus (CMV) is a leading cause of pediatric infection, with diagnosis frequently relying on non-blood specimens such as swab, urine, and amniotic fluid (AF). These matrices present distinct challenges compared to blood. External Quality Assessment (EQA) schemes incorporating non-blood matrices, to model analytical complexity, give an opportunity to assess laboratory performance. This study evaluates data from the QCMD CMV non-blood (CMVNB) EQA pilot scheme and explores its value in assessing diagnostic performance in pediatric infection.

Materials and Methods: The CMVNB EQA pilot, introduced in 2025, distributed panels comprising simulated urine, AF, and swab samples containing CMV between 2.0 and 4.7 log₁₀ digital copies/ml alongside negative controls. Participating laboratories reported qualitative results via the dedicated ITEMS online platform.

Results: Across 159 datasets (1,060 results), specificity remained high (98%) with minimal false positives (0.4%). Sensitivity varied by matrix, with urine demonstrating reduced detection (75%) compared to simulated swab and AF (each 99%). Performance was dependent on viral load, increasing from 26% to 93% between 10² and 10⁷ copies/ml respectively, with most false negatives occurring in urine below 3 log₁₀ copies/ml.

These findings demonstrate that performance in non-blood CMV testing is driven by matrix and viral load effects.

Conclusions: The CMVNB EQA provides a structured framework for evaluating diagnostic performance in specimen types central to congenital and neonatal infection. The observed variability across matrices and viral loads highlights limitations that are not captured by conventional blood-based EQA schemes. It reveals how diagnostic performance changes under clinically relevant conditions, supporting a realistic appraisal of laboratory performance.

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Bridging Variability in Viral Load Measurement: A Digital PCR Approach to Harmonisation in Transplant Virology

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Introduction: Harmonisation of viral load measurement remains a significant challenge in clinical virology, particularly for infections routinely monitored in transplant recipients. Variability in assay design, calibration materials, and reporting units can lead to discrepancies across laboratories, potentially impacting clinical interpretation. This is especially relevant for ADV, HHV6, CMV, EBV, Parvovirus B19, TTV and BKV, where viral load thresholds guide monitoring and intervention.

Methods: We evaluated digital PCR (dPCR) as an absolute quantification approach to support harmonisation of viral load data generated through QCMD external quality assessment (EQA) schemes. Datasets from participating laboratories over three years were analysed, comparing reported viral loads against dPCR-derived measurements as an independent quantitative reference.

Results: Analysis of 3,592 datasets demonstrated substantial inter-assay variability across all viruses. Reported viral load ranged from copies/ml: 1.7-5.7 log₁₀; IU/ml: 2.0-5.5 log₁₀. The overall mean bias relative to dPCR was low (copies/ml: +0.07 log₁₀; IU/ml: -0.02 log₁₀), but virus-specific differences reached +0.50 log₁₀ (HHV6) and -0.27 log₁₀ (BKV). Conversion to IU/ml did not consistently improve agreement with dPCR. Variability was most pronounced at lower viral loads, particularly for EBV and parvovirus B19, where different thresholds are commonly used to guide clinical intervention.

Conclusions: Significant variability in viral load measurement persists despite standardisation efforts. dPCR provides a robust independent reference that can reveal systematic bias and improve comparability across assays. Its application may enhance harmonisation and support more consistent clinical interpretation, particularly in the low viral load range where management decisions are most challenging.

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Infections with the hepatitis B and D viruses diagnosed for patients managed by the Permanent Access to Healthcare clinical ward of university hospitals of Marseille, South-eastern France

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Introduction/Aim: Infections with hepatitis B and D viruses (HBV, HDV) are global concerns. High endemicity areas include Africa from where often originate migrant patients receiving care in clinical wards providing Permanent Access to Healthcare (PASS) at University Hospitals of Marseille (UHM), southern France. We aimed to describe HBV/HDV epidemiological and virological features for PASS patients, over 12 years.

Materials and Methods: This retrospective study included patients with HBV and/or HDV serological and PCR tests between 06/2013-05/2025. Serologies used Architect Abbott, Cobas Roche, Vidas BioMérieux, Dia. pro, or LiaisonXL DiaSorin assays. PCR used RealTimeAbbott, Veris Beckman, NeumoDx Qiagen, in-house, or Altona assays. Genotyping used Sanger or Illumina next-generation sequencing and MEGA, BioEdit and Geno2pheno for phylogenetic and mutation analyses.

Results: For HBV, 9615 samples from 8887 patients (mean age 38 years, 5505 men (H:F=1.6)) were tested. 498 patients (5.6%) were newly HBV diagnosed; mean age 33 years, 375 men (H:F=3.0). Vaccine immunization, past-HBV infection with anti-HBs, and anti-HB core only profiles accounted for 8.4%, 19.5%, and 4.8% of tested patients, respectively. HBV genotypes were A, D, and E in 11, 37, and 53 cases, respectively (13 genomes). An occult HBV-D (HBsAg escape mutation A128V) infection with anti-HBs only was diagnosed. For HDV, 604 samples from 473 patients were tested. 39 (0.8%) and 22 (0.5%) patients were HDV IgG/total and HDV RNA positive, respectively. HDV genotypes were 1, 5, and 6 in 13, 1, and 1 cases, respectively (7 genomes).

Conclusion: Incidence of HBV/HDV diagnoses were high among PASS patients at UHM and associated with a specific genetic diversity and very atypical serology in one case. Treatments are recommended but require long-term therapies and management.

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Incidence of diagnoses of 17 respiratory viruses in a French university hospital between 2019 and 2024 (5 years)

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Introduction: Weekly surveillance of infectious agents has been conducted for 23 years in the laboratory of our University Hospitals. Here, we aimed to describe the weekly incidence of 17 respiratory viruses (RespV) over the period 2020-2024 (5 years) at our center.

Materials and Methods: This retrospective study was based on weekly numbers of tests and positive diagnoses of RespV for respiratory samples sent to our laboratory at University Hospitals of Marseille, France. The study period was from 07/2019 to 06/2024. qPCR tests used commercial assays including Xpert Xpress (Cepheid), BioFire FilmArray (BioMérieux), QIAstat-Dx RPV2 (Qiagen), FTD 21 (Fast Track Diagnosis), or Anatolia Bosphore (Launch Diagnostics), and in-house techniques.

Results: Numbers of tests were 1390019, 156794, and 120412 for SARS-CoV-2, influenza A (IAV) and B (IBV) viruses/respiratory syncytial virus (RSV), and all other RespV, respectively. Positive were 114462 (8.2%), 9806 (6.2%), 2799 (1.8%), and 7047 (4.5%) for SARS-CoV-2, IAV, IBV and RSV, respectively, and 18233 (12.9%), 5011 (3.6%), 4464 (3.0%), 4200 (3.2%), and 2860 (2.0%) for rhinovirus (HRV), endemic coronaviruses (HCoV), parainfluenza viruses (HPIV), adenoviruses (ADV), and metapneumovirus (HMPV), respectively. Incidence was abnormally low for IAV/IBV (n=5) between 05/2020-10/2021, and RSV (2) and HPMV (1) between 05/2020-12/2020. Incidence was higher (p<0.001) in 2021-2022 vs 2020 and 2023 for RSV (6.7% vs 2.6%), ADV (4.8% vs 2.0%), HPIV (4.3% vs 1.6%), and HRV (15.9% vs 9.6%). The majority of RespV circulated all year's months, with significant year-to-year incidence variability, and high incidence duration-periods.

Conclusion: These data warrant characterizing genomes and genotypes for all these 17 RespV, including to describe more precisely the emergence, evolution, and disappearance of lineages.

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Evaluation of alternative specimen types using the Cobas Eplex respiratory pathogen panel 3

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Introduction: The Cobas® Eplex respiratory pathogen panel 3 (RP3) is a CE-marked molecular syndromic panel that was validated using nasopharyngeal swab (NPS) samples from patients presenting with respiratory tract infection (RTI). However, clinical labs may receive additional respiratory samples, which are perceived as either less invasive or more appropriate for lower RTI. The present study evaluates the feasibility of several alternative sample types with RP3.

Material and Methods: Nasal swabs, nasal washes, throat swabs, bronchoalveolar lavage (BAL), and sputum samples that were positive for at least one pathogen by local standard of care methods were enrolled at two geographically distinct sites in Europe and tested with the RP3 test. All samples were tested fresh without prior freezing. Sputum and BAL samples were pretreated by diluting 200 µL of specimen into 1 mL of TRIS/EDTA buffer and bead beating for 5 minutes prior to loading supernatant into the RP3 cartridge.

Results: The study processed 218 specimens, of which 188 (86.2%) yielded valid, evaluable results. Of these, 167 (88.8%) were positive for one or more pathogens by the index test. Validity for throat swabs, nasal swabs, sputum, and BAL was ≥ 85.0%, while nasal wash validity was 75.0% (n=12). Positive percent agreement (PPA) for throat swabs, nasal swabs, and nasal wash ranged from 83.6% to 90.0%. In comparison, the PPA for more viscous specimens was lower at 78.4% for BAL and 73.1% for sputum. Overall Negative Percent Agreement (NPA) across all specimen types remained high, ranging from 98.0% to 100%, with an overall pooled NPA of 98.7% (95% CI: 98.2% - 99.0%).

Conclusions: This study demonstrates the feasibility of using alternative sample types with the RP3 test and highlights the need for pre-analytic considerations with viscous specimens.

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Evolution of respiratory virome profile in children under 5-years with persistent low respiratory symptoms

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Introduction/Aim: To characterize short-term respiratory virome dynamics in hospitalized children with persistent low-respiratory-symptoms (LRTS).

Methods: Nasopharyngeal swabs were collected at admission (Day0) and on day 3 of hospitalization (Day3) from 20 under-five children with LRTS. Virome was processed using QIAseq-xHYB-Kit. Reads were taxonomically classified with Kraken2, validated by de-novo assembly with metaSPAdes, and retained only when contigs aligned with RefSeq had length ≥300 bp, average coverage ≥2, and ≥90% length covered. Differences in Alpha-diversity (Observed, Shannon, Simpson) indexes and virus relative abundance (RA) between Day0 and Day3 were assessed with Wilcoxon-test.

Results: The 20 children (median age: 0.9 years) had a median admission SpO₂ of 93% and symptom onset 4 days earlier. All still had LRTS on Day3. Alpha-diversity did not differ significantly between timepoints (Δ mean±SE: Observed 0.45±0.26, p=0.08; Shannon 0.12±0.11, p=0.41; Simpson 0.05±0.07, p=0.69). Virome composition remained stable in only 5 (25.0%) children, and the number of samples with ≥3 viral species increased from 5 (25%) at Day0 to 11 (55%) at Day3 (p=0.054). Pneumoviridae were detected in 9 samples (45%, RSV-A, n=7; MPV, n=2) with a median RA of 55% but declined to 4 (20%; RSV-A, n=3; MPV, n=1) at Day3 (p=0.063), with a median RA of 19.0% (p=0.008). Although not statistically significant, both Anelloviridae prevalence and RA showed a clear upward trend from Day0 to Day3 (13[65%] to 16[80%]; RA 6.4 to 27.8). Herpesviridae (1 CMV, 1 EBV, 1 HHV7) appeared at Day3 in 3 cases, 2 after RSV infection (0[0%] to 3[15%]; RA 0.0 to 4.0).

Conclusion: The respiratory virome in young children with persistent LRTS can undergo significant short-term modifications, suggesting dynamic viral interactions that may influence clinical recovery.



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Climate Change and Spread of Arboviruses: Long-Term Performance Trends in External Quality Assessment (EQA) for Molecular Vector-Borne Disease Testing

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¹QCMD

Climate change together with other drivers, such as globalisation of trade, travel, land-use changes, or socioeconomic factors coupled with rapid population increases can create favourable conditions for emergence of pathogens such as West Nile Virus (WNV) and dengue viruses (DENV) into new geographical areas. EQAs play a critical role in addressing diagnostic capacity and laboratory preparedness. This review examines EQA trends for molecular testing of arthropod-borne viruses over a 10 year period.

QCMD EQA schemes for WNV, DENV, yellow fever virus (YFV), chikungunya virus (CHIKV) and Zika virus (ZIKV) were distributed annually to registered laboratories. Participants received a 10-sample panel, including strains at a range of concentrations, negative and specificity samples. Data and workflow information were returned via QCMDs EQA management system, results were analysed qualitatively relative to technology peer groups.

Submitted data showed consistently high performance over time, with positive core samples detected correctly in at least 83% of submitted datasets. Datasets ranged from 23 to 159 depending on the respective EQA. Performance remained high for ZIKV, introduced in response to the 2015 epidemic and for the YFV scheme introduced in 2019, with detection rates > 89% for ZIKV and > 84% for YFV. International standard material was included in the WNV, ZIKV and CHIKV schemes to generate data critical for standardisation of viral load. Registration increased by 45% for DENV and 31% for WNV in 2024 in line with increased dengue cases and wider circulation of WNV.

The review of data over time for five arboviral EQAs data shows good performance by laboratories and an increasing uptake in schemes especially in recent years. These findings highlight the value of EQAs in supporting quality assured diagnostics in this field.

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External Quality Assessment for Multiplex Testing of Viral Respiratory Infections

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Introduction: Respiratory infections with symptoms ranging from mild colds to severe cases of influenza or COVID-19, can be a particular threat to young children, elderly or immunocompromised individuals. Infections by some viruses can lead to outbreaks or pandemics such as influenza or SARS-CoV-2. Syndromic molecular diagnostic testing allows clinicians to identify aetiology, often in near patient settings where rapid pathogen identification aids faster clinical decision making, treatment and management, including public health measures. This study examines EQA performance trends over a five-year period.

Methods: QCMD distributes three multiplex EQA schemes aimed at assessment of viral respiratory syndromic testing; RESPI covering influenza virus and RSV, RESPIplus covering influenza virus, RSV and SARS-CoV-2, and RESPII covering metapneumovirus, rhinovirus, seasonal coronavirus, enterovirus, parainfluenza virus and adenovirus. Participants received 10 samples of viral targets at different concentrations in transport medium for blind testing. Data and workflow information were returned via QCMDs EQA management system, results were analysed qualitatively relative to technology peer groups.

Results: From 2021 to 2025, participation in respiratory syndromic EQAs increased, from 420 to 632 for RESPI/RESPIplus and from 239 to 299 for RESPII. Performance in RESPI/RESPIplus was consistently >90% with 97% (25,971/26,772) correct results. Performance in the RESPII scheme which covers a greater range of targets was >90% with 92% (12,281/13,276) results as expected.

Conclusions: The data shows qualitative performance >90% for multiplex molecular workflows. However, continued EQA participation is crucial to ensure ongoing quality of respiratory diagnostics, as epidemiology of infections, viruses and diagnostic technologies change frequently.



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Syndromic Testing for Viruses Associated with the Central Nervous System: Five-year Trends in Molecular External Quality Assessment

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Viral infections of the central nervous system (CNS) are linked with significant morbidity and mortality. The burden on human health is complicated by different neurotropic viruses and diverse routes of entry into the CNS. Outcome is dependent on rapid identification of aetiology and initiation of appropriate therapy. This review examines External Quality Assessment (EQA) performance trends for syndromic molecular testing of viral meningitis and encephalitis over a five-year period.

QCMD distributes a multi-pathogen EQA-scheme aimed at assessment of syndromic testing of viral CNS infections. The CNSI scheme includes targets of enterovirus, parechovirus, herpes simplex virus type 1 and 2, and Varicella-Zoster virus in either transport medium or a synthetic cerebrospinal fluid matrix. Participants received ten samples for blind testing. Data and workflow information were returned via QCMD's EQA management system, results were analysed qualitatively relative to technology peer groups.

Between 2021 and 2025, there was a steady increase in uptake of syndromic EQA panels for viral CNS pathogens, increasing from 173 to 239 registered laboratories. Performance was consistently high with 9547 of 9958 results (96%) reported as expected when the target was included in the workflow. False negative results (189/8870 (~2%)) for workflows including the target, could not be linked to any one workflow type. Approximately 2% of results (23/1065) for the true negative samples were false positives.

We present a five-year review of analysed data for the QCMD CNSI EQA scheme. Good performance with more than 90% detection rates on positive samples and few false positive results was seen over the review period, showing that the need for quality assured diagnostics is being met.

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A Pragmatic Approach to Managing Suspected Cases of MERS-CoV: Nine-Year Experience from an East London NHS Trust

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Intro: Middle East respiratory syndrome coronavirus (MERS-CoV) may cause an airborne high consequence infectious disease. Most human cases are linked to camels, particularly in Saudi Arabia. Barts Health NHS Trust serves many returning travellers meeting the UK Health Security Agency MERS case definition. Isolation requirements place significant pressure on emergency departments (ED). We introduced in-house MERS-CoV testing (2017) and criteria for home isolation (2025) aiming to improve patient care.

Methods: A retrospective evaluation was conducted of in-house MERS-CoV testing at Barts Health NHS Trust between September 2017 and March 2026. Electronic notes were reviewed for alignment with UKHSA criteria, turnaround times, final diagnoses and consideration of home isolation.

Results: A total of 212 patients were tested for MERS-CoV RNA and all were negative. The case definition was not met in 41 patients who were excluded from further analysis. Of the remaining 171 patients (92 males (54%); median age 49, IQR 39-62), the majority had travelled to Saudi Arabia (151, 88%), mainly for religious pilgrimage. Case definition 1 was met by 126, and 45 met case definition 2. The most common diagnoses were pneumonia (61, 36%) and influenza (49, 29%). The median time to result was 30 hours (IQR 23-42). Around one fifth (33, 19%) were discharged to isolate at home, saving on average 22.5 hours in hospital. A further 11 patients were identified as likely to have been suitable for home isolation.

Conclusions: In-house MERS-CoV testing enables prompt deisolation of hospitalised patients. In low prevalence settings, home isolation is a sensible approach to support patient flow in ED whilst maintaining public safety. Pragmatic public health guidance would be welcome to standardise practice nationally.

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Evaluation of the clinical performances of the new Access HBeAg assay* on the Dxl 9000 Access Immunoassay Analyzer

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Introduction: Beckman Coulter has recently developed a fully automated assay for the qualitative detection of HBeAg on the Dxl 9000 Immunoassay Analyzer (Dxl 9000). The objective of this European multi-centre study was to evaluate the performance of the Access HBeAg assay on the Dxl 9000.

Methods: The clinical specificity of the Access HBeAg assay was determined using HBeAg negative samples from blood donors and hospitalized patients and clinical sensitivity was determined using presumed HBeAg positive samples. Samples were tested with the Access HBeAg assay and a CE-marked HBeAg comparator assay for concordance, and discordant samples further tested with a CE-marked HBsAg assay + HBsAg Confirmatory, if applicable, to determine final sample status. Five seroconversion panels were tested to assess the sensitivity for early detection of HBV infection.

Results: Clinical specificity of the Access HBeAg assay on 1100 blood donors and 300 hospitalized patient samples with final sample status negative was 100.00% (95%CI: 99.73 – 100.00%). Clinical sensitivity on 218 final sample status positive HBeAg samples was 98.62% (96.03 – 99.53%). Evaluation of seroconversion panels demonstrated similar detection in three panels and earlier detection in two panels versus a comparator assay.

Conclusions: The newly developed Access HBeAg assay for use on the Dxl 9000 demonstrated clinical specificity and sensitivity performances equivalent to currently marketed HBeAg assays.

*For investigational use only. Pending achievement of CE compliance; not yet available for in vitro diagnostic use.

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Detection of JC Polyomavirus in Genital Secretions of University Students: Implications for Sexual Transmission?

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Introduction: JC polyomavirus (JCPyV) is a ubiquitous human virus and the causative agent of progressive multifocal leukoencephalopathy. The main mode of JCPyV transmission remains unclear. However, its clarification would be important for developing prevention strategies. Sexual route is a well-documented transmission mode for several microorganisms. University students, representing a young and sexually active population with an increased risk of acquiring sexually transmitted infections (STIs), were selected to assess the hypothesis that genital secretions may contribute to JCPyV transmission.

Material and Methods: A total of 672 samples, comprising 644 vaginal, 12 anal and 16 ureteral exudates were collected from university students (age range: 18-35 years, median age: 22 years) attending a STIs screening program at a Portuguese university. Anal and ureteral exudates were obtained from men, and vaginal exudates from non-pregnant women. The presence of JCPyV genome was evaluated by real-time PCR.

Results: JCPyV DNA was detected in 37 of the 672 analysed exudates (5.5%), including 36 vaginal exudates (5.6%) (median age: 22 years) and one anal exudate (8.3%) (man, 23 years). Detection of JCPyV in vaginal exudates did not significantly differ with age ($p=0.865$). None of the ureteral exudates presented the studied viral genome.

Conclusions: These promising results suggest that vaginal secretions could represent a source of viral particles for JCPyV transmission. Results on detection of JCPyV in anal exudates, despite preliminary could suggest that these secretions may also be involved in JCPyV transmission. Nevertheless, further studies should be performed, particularly including a higher number of anal exudates, to clarify the potential impact of sexual route in the transmission of JCPyV.



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Characterization and quality control of biological samples collected during and after the COVID-19 pandemic

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The UNIFESP Biobank was inaugurated in 2021, during the COVID-19 pandemic, aiming to fill the gap of this type of biological collection at the university. This collection of samples from patients diagnosed with COVID-19 were stored between 2021-23. Given the health system crisis during the pandemic, with hospital overload, high demand for diagnosis, and limited space for appropriate sample storage, samples were kept at room temperature until the patient's informed consent was obtained, which may affect molecular integrity. A partnership between the Biobank and the NB3 Retrovirology Lab. resulted in the collection of circa 6,000 blood samples are processed into serum/plasma and buffy coat/PBMC. Nasal swab samples were collected for SARS-CoV-2 isolation and virus characterization. This project aims to establish criteria to guarantee the quality and ethical reliability of stored samples, even under adverse conditions for current and future projects. Buffy coat samples were selected from 104 participants, from whom RNA was extracted to test seven reference genes: GAPDH, B2M, RNA18S1, ACTB, TBP, PPIA, and HPRT1. Four participants were selected for viral isolation in Vero-E6 cells and sequencing using Nanopore MinION Mk1D. Positive results were obtained for ca. 75% of participants. SARS-CoV-2 viral strains were isolated from four participants and are undergoing characterization. The partnership is ready to maintain biological collections for high-quality research. Results showed that, even under unfavorable conditions, it was possible to extract RNA with adequate yield and integrity. Both formed a qualified partnership to expand biological collections with ethical and technical reliability, supporting current and future research.

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A comparison between Monospot and Epstein Barr Virus serology for the diagnosis of infectious mononucleosis

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Introduction: Infectious mononucleosis (IM) is a viral infection usually caused by Epstein Barr Virus (EBV). Although IM is usually self limiting, serological diagnosis is often sought. There are two main serological tests used in the diagnosis: A heterophile antibody test (monospot) and EBV antibody profile. UK guidance suggests monospot should be used as a first line investigation for IM over 4 years of age. There is limited research directly assessing the agreement between monospot and EBV serology. This retrospective audit set out to compare the two serological investigations.

Methods: Data was collected through the North Bristol Trust Laboratory Information Management System from 03/01/23 to 29/10/24. Patients with monospot and EBV serology collected on the same day were included.

Sensitivity, specificity, diagnostic accuracy and Cohen's kappa coefficient were used to analyse the agreement between the two investigations.

Results: 415 patients were included in the study. 39.2% (n=163) were acute EBV serology positive and 34% (n=142) were monospot positive. EBV serology had a 91.3% sensitivity and 85.4% specificity in comparison to monospot. Monospot had a sensitivity of 77.3% and a specificity of 94.8%. Accuracy of both tests were the same at 87.50%. There was good agreement between the two investigations (K=0.69).

Discussion: This study provides a contemporary, large-cohort comparison of monospot and EBV serology. Earlier research cited in expert opinion articles reported lower specificity for heterophile antibody tests, but was published over 30 years ago.

The results of our study support the use of monospot testing as a first line investigation for infectious mononucleosis, in view of lower cost, and in line with current UK guidelines.

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Performance of CMV PCR Alinity M® on Dried Blood Spots (DBS) for retrospective diagnosis of congenital CMV infection (cCMV)

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Introduction: CMV PCR on DBS collected in the neonatal period (Guthrie cards) is the recommended method for retrospective diagnosis of cCMV beyond the neonatal period. Current methods are often time-consuming and manual. This study evaluates CMV PCR on DBS using the Alinity M® (Abbott, USA), a fully automated, random access platform.

Objective: To compare the analytical performances on DBS samples of CMV Alinity M® to those of the laboratory reference technique (Qiagen® (Qiagen, France) DNA extraction followed by PCR with CMV R-gene® (BioMérieux, France)).

Methods: We conducted a prospective, monocentric study from November 2024 to November 2025. Guthrie cards sent to our laboratory for cCMV retrospective diagnosis were tested in parallel with both assays. Weakly positive results (<2.7 log IU/ml and ≤ 2.2 log IU/ml for the reference technique and the Alinity M® respectively) were retested and considered as positive only if the repeat test was positive.

Results: 236 DBS were analyzed. Compare to the reference method, the Positive Percent Agreement was 94.7% (18/19) [95%CI: 75.4–99.7] and the Negative Percent Agreement was 98.6% (214/217) [95%CI: 96.0–99.6]. Viral load comparison for the 18 positive samples showed no significant difference (Δ log IU/mL < 0.75) in 6 (33.3%), higher loads with the CMV R-gene® (median difference: 0.8 log IU/mL) in 11 (61.1%) and higher load with Alinity M® in 1 (5.6%) (difference 1.1 log IU/mL).

Conclusion: Overall, 1.7% (4/236) of samples were discordant. One sample was positive by R-gene® but negative by Alinity M® (2.6 log IU/mL). Conversely, 3 samples were positive by Alinity M® but negative by R-gene®. All 3 had viral load below 2.2 log IU/mL. The Alinity M® system offers a reliable and streamlined automated alternative for cCMV screening on DBS.

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Temporal dynamics and genomic evolution of influenza viruses in northern Italy (2023–2026): insights from whole-genome sequencing

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Introduction: Influenza viruses undergo rapid genetic evolution, affecting transmission and vaccine effectiveness. We investigated genomic diversity, temporal distribution, and mutational landscape of seasonal influenza A (H1N1, H3N2) and B viruses in northern Italy across three seasons (2023–2026), and evaluated the added value of whole-genome sequencing (WGS) for phylogenetic inference.

Materials and Methods: A total of 393 influenza-positive respiratory samples (October 2023–March 2026) were analyzed by WGS. High-quality genomes (breadth $\geq 95\%$, depth $\geq 100\times$) were obtained for 355 samples and used for variant calling. Variants were classified by type and functional impact, normalized per sample, and analyzed across seasons and genomic segments. Phylogenetic analysis was performed using hemagglutinin (HA) alone and concatenated whole genomes (mirror-tree approach).

Results: A(H1N1) predominated in 2023–2024 and 2024–2025, while A(H3N2) dominated in 2025–2026. Influenza B circulated at low levels and was absent in 2025–2026. Mutational burden increased over time, especially in A(H1N1). Synonymous mutations were most frequent, followed by missense variants; nonsense mutations were rare. Most changes were SNPs, mainly A>G and T>C. PB2, NP, and HA were the most variable segments, with increasing antigenic diversification, especially for A(H3N2). Missense mutations showed heterogeneous distribution with high-frequency clusters, consistent with emerging variants. Phylogenetic comparison showed concordant topology between HA and whole-genome trees, indicating limited impact of single-segment variation.

Conclusions: Influenza viruses showed progressive variant replacement and increasing genetic diversity. WGS improves resolution of viral evolution, supporting genomic surveillance and public health strategies.



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Cytomegalovirus drug-resistance genotyping: 10-year evolution through QCMD External Quality Assurance Assessment

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Introduction: Cytomegalovirus (CMV) antiviral resistance remains a major challenge in immunocompromised treated patients. International guidelines recommend genotyping in cases of treatment failure to detect resistance to ganciclovir, maribavir, foscarnet, and cidofovir by full-gene sequencing of UL97 and UL54, and UL56 for letermovir. In 2014 QCMD launched the 1st international CMV genotyping drug resistance (CMVDR) External Quality Assessment (EQA) to support laboratories. We report 10-year (2016–2025) evolution of methods and results.

Materials and Methods: CMVDR panels include the wild-type Davis strain for reproducibility and various resistant strains (viral load >3log₁₀ copies/mL). Participants submitted workflow, FASTA sequences and mutation sites through the dedicated ITEMS online platform. Scores for each full-length gene are based on comparison with participant consensus (1 point for each site that matched the consensus sequence and 0 point for a mismatch).

Results: Laboratories (~35/year) from 5 continents participated in each challenge. Performance scores for UL97, UL54, and UL56 were stable (CV~13%) or improved, depending on the strain tested. Viral load did not impact sequencing success. NGS use increased progressively, mainly with in-house PCR full-gene amplicons. Mutation ID scores were similar for NGS and Sanger sequencing. By 2025, UL97 NGS and Sanger performance were indistinguishable. UL54 NGS reached near-maximal performance since 2018 earlier than Sanger.

Conclusion: The CMVDR EQA provided participants with a consistent reference point for performance. Laboratories performance met current CMVDR sequencing recommendations, with improving drug-resistance mutation scores and commutability to new drug resistance patterns. The impact of workflow on reproducibility, coverage, and scores is still being evaluated.

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Persistent Viral Infection of Tubular Epithelial Cells and MPGN-Type Immune Complex Glomerulonephritis Following Primary Parvovirus B19 Infection in a Kidney Transplant Recipient: A Case Report

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Background: We recently reported that parvovirus B19 (PVB19) establishes latent infection in P antigen-positive renal tubules after primary infection and is detectable in approximately one-third of donor kidneys (Virchows Archiv, 2025). Here, we describe longitudinal clinicopathological findings in a kidney transplant recipient who developed renal dysfunction after primary PVB19 infection.

Case Presentation: A 49-year-old woman underwent a living-donor kidney transplant for end-stage renal disease due to IgA nephropathy. Graft function remained stable (sCr 1.3–1.5) until Year X, when she developed polyarthralgia, leg edema, and renal dysfunction (sCr 2.0). Laboratory tests showed hypocomplementemia, immune complexes (ICs), and anti-S-S-A/SS-B antibodies, suggesting Sjögren's syndrome with or without systemic lupus erythematosus. However, symptoms resolved spontaneously within weeks.

Four months later, PVB19 IgM antibodies were tested to be positive, indicating that the preceding symptoms were due to primary PVB19 infection. Despite clinical improvement, IgM positivity, ICs, hypocomplementemia, and mild renal dysfunction persisted for >1 year, prompting biopsy at Year X+1.5. Light microscopy showed no rejection but revealed glomerular basement membrane duplication, foam cells, and endocapillary proliferation. Immunofluorescence (IF) showed C1q, C4d, IgG, and IgM deposition, and electron microscopy identified subendothelial deposits, consistent with IC-mediated membranoproliferative glomerulonephritis.

PVB19 was undetectable in blood by PCR, but viral DNA and protein were present in renal tissue. A prior biopsy (Year X–6) showed negative IF and no PVB19 DNA.

Conclusions: This case demonstrates dynamic changes in autoantibodies and renal histology before and after primary PVB19 infection, providing insight into PVB19-associated glomerulonephritis.

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Characterization of BK polyomavirus serologic responses in kidney transplant recipients and healthy subjects

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Background: Kidney transplant recipients (KTRs) frequently experience persisting BK polyomavirus (BKPyV) replication and a subsequent decrease in allograft function, leading to BKPyV-associated nephropathy. In this study, we aimed to assess the antibody-mediated immune response in patients under immunosuppression with different BKPyV replication kinetics based on the duration of virus replication and viral load in serum or urine.

Methods: Anti-BKPyV IgG and IgM response to serotypes I and IV in patients and healthy controls was determined using a Luminex Multi-Analyte Profiling (xMAP) assay. The sensitivity of the xMAP IgG assay was validated using two methods, a commercial qualitative and semiquantitative ELISA assay (VIDITEST, VIDIA; n=70) and a quantitative pseudovirus-based neutralization assay (n=50). The thresholds indicating viral reactivation based on IgM and IgG levels were calculated using results from BKPyV DNA-negative KTRs.

Results: Luminex xMAP IgG assay showed good correlation with ELISA ($r^2=0.96$) and neutralization assay ($r^2=0.88$). KTRs were classified into four groups based on their viral kinetics: BKPyV DNA detected only in urine (n=28), low-level DNAemia (in blood; n=18), and high-level DNAemia with >104 copies/ml (transient-resolved, n=26, or persisting DNAemia, n=7). All groups with BKPyV+ DNA significantly increased IgG and IgM antibody levels compared to BKPyV DNA-negative controls. At the end of the study period, IgG and IgM levels exceeded the viral reactivation threshold in 66 out of 79 and 42 out of 78 BKPyV DNA+ patients, respectively.

Conclusion: In this study we demonstrated that levels of anti-BKPyV IgG and IgM were significantly increased in all KTRs with detectable levels of BKPyV DNA in plasma or urine, and that the serologic response was proportional to the level of virus replication.

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Epidemiology of RSV infections in the 2025/26 Season in Tyrol, Austria: Characterizing RSV infection among children visiting three hospitals in Tyrol

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Introduction/Aim: Following the introduction of Nirsevimab, the clinical and molecular epidemiology of RSV infection has gained increasing attention. This study aimed to characterize RSV-positive paediatric patients and their respiratory samples during the 2025/26 RSV season.

Material/methods: We included n=162 patients admitted at three tyrolean hospitals characterising the demographics and immunization status and obtained oropharyngeal swab samples for whole-genome sequencing.

Results: Regional hospital data showed significantly lower admissions in 2025/26 compared to 2023/24. The median age at admission increased significantly in the post-Nirsevimab era (469 vs. 238 days, $p<0.0001$), while the mean length of hospital-stay decreased (3.19 vs. 3.4 days, $p=0.0008$). In the 2025/25 season RSV A (clade A.D.3) predominated. RSV B infections were associated with a higher likelihood of hospitalization. Out of 49 RSV-positive infants (<12 months) included in the study some 21 had received Nirsevimab prior to infection. No significant differences in subtype, clade distribution, or mutation patterns were observed between breakthrough and non-breakthrough cases. No novel mutations were detected in the Nirsevimab-binding site.

Conclusions: The decline in hospitalization rates, coupled with a higher mean age at admission and reduced length of hospital stay in the post-Nirsevimab era, underscores the high effectiveness of the immunization. In addition, the lack of emerging mutations, especially at the Nirsevimab binding site, indicates no molecular evidence of selective pressure on the virus.

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RdRp Inhibitors for Respiratory Virus Infections in the Immuno-suppressed: is it time to combine?

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Community acquired respiratory viruses (CARVs) are a significant cause of morbidity and mortality in immunocompromised patients which include patients with haematological malignancies (HM), recipients of haematopoietic stem cell transplantation (HSCT), B-cell targeting therapies, immunomodulatory monoclonal antibodies, chimeric antigen receptor T cell therapies (CAR-T), and solid organ transplantation (SOT). (Ison and Hirsch 2019; Von Lilienfeld-Toal et al. 2026; Rubin and Hirsch 2008)

While several antiviral drugs are licensed for treatment of SARS-CoV-2 and Influenza, we focus here on treatment of non-SARS-CoV-2 and non-Influenza RNA respiratory viruses, including Respiratory Syncytial virus (RSV), Rhinovirus (RV), seasonal human coronaviruses (sCoVs) (OC43, HKU1, 229E, NL63), Parainfluenza virus (PIV), and human Metapneumovirus (hMPV).

We review the evidence supporting current guidelines for treating these viruses in immunocompromised populations and review the data on in-vitro and in-vivo efficacy of repurposed and other drugs reported to be active. Given the success with combination antivirals for treatment of HIV, and hepatitis C we address the potential for this strategy in the management of severe respiratory infections.

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The Niche of Time: Convergence of SARS-CoV-2 and Human Seasonal Coronavirus Infection Age Profiles in the Post-Pandemic Period

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Introduction: It is important to learn from the epidemiological changes of SARS-CoV-2 as it transitions from a novel pandemic virus, for planning public health interventions and increasing pandemic resilience. There is currently a lack of nuanced quantitative frameworks to define that transition, so we apply established statistical tests from the ecological sciences to compare SARS-COV-2 and human seasonal coronaviruses (sCoVs).

Methods: We analyse results from 82,646 specimens enrolled in the representative Oxford-Royal College of General Practitioners Research Surveillance Centre (RCGP-RSC) sentinel surveillance network in England between 2019 and 2025 and perform a time-series analysis, an age-specific incidence analysis, and coinfection analysis using Generalised Additive Models (GAM) to account for non-linear relationships, and compare the epidemiology of SARS-CoV-2 and the sCoVs during, and after the pandemic periods.

Results: While median age shifts remained static (61 to 60 years), SARS-CoV-2's demographic transition toward infants was captured by an EMD of 7.6 years, an increase in Schoener's D from 51% to 82% and a decline of Standardized Rate Ratios from 2.42 to 1.12 over successive seasons. Additionally, sCoV infection was strongly associated with reduced odds of SARS-CoV-2 coinfection (OR 0.09, 95% CI: 0.06-0.14, $p < 0.001$).

Conclusions: Applying ecological methods to surveillance data reveals with great nuance that the age-specific epidemiological distribution of SARS-CoV-2 infection is converging towards that of sCoVs and expresses the hallmarks of endemicity.



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Decreased susceptibility of herpes simplex virus 1 (HSV1) to acyclovir in connection with the presence of certain natural polymorphisms in the viral thymidine kinase: role of polydes

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Introduction: HSV1 resistance to acyclovir (ACV) may emerge, particularly in immunocompromised patients. The resistance to ACV is mainly linked to the presence of mutations in UL23 gene encoding the thymidine kinase (TK). Sequencing of UL23 gene allows the identification of resistance mutations and natural polymorphisms, i.e. mutations that do not confer resistance. We identified 7 natural polymorphisms that decrease susceptibility of HSV-1 to ACV, referred as to polydes: A192V, G240E, G251C, V267L, P268T, D286E, and N376P/T/H.

Material and methods: A total of 605 UL23 sequences from refractory HSV1 (French NRC for Herpesviruses database: 2008-2025) were included: 301 without and 304 with ACV resistance mutations. The 50% effective concentration (EC50) towards ACV was evaluated for 31 HSV1 isolates by phenotypic assay. In-silico 3D models of TK, with either ACV or thymidine and ADP, were generated with SWISS-MODEL.

Results: The presence in HSV1 TK of at least 4 polydes was significantly associated with the presence of an amino acid change conferring ACV resistance ($p=0.006$) preferentially localized within conserved sites 3 to 5 of the viral enzyme ($p=0.034$). The median EC50 towards ACV was statistically higher for HSV1 isolates with 3 to 6 polydes than for those with 0 to 2 polydes: 1.7 μM versus 0.1 μM ($p=0.0003$). Therefore, polydes were associated with a decreased susceptibility to ACV, but not with ACV resistance (defined as EC50 $>7 \mu\text{M}$). Polydes in TK did not affect viral growth. No significant structural differences between TK with or without the 7 polydes explaining reduced susceptibility to ACV could be identified.

Conclusion: The presence of polydes in TK confers a reduced susceptibility of HSV1 to ACV and could potentially constitute an early stage in the process of the emergence of a mutation conferring ACV resistance.

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Deciphering the role of coinfection by herpes simplex viruses (HSV) and human papillomaviruses (HPV) in pseudotumoral anogenital herpes

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Introduction: HSV and HPV are sexually transmitted infections. Anogenital HSV-HPV coinfections can complicate the diagnosis and management of patients as they may be responsible for atypical presentations or resistance to standard treatments. We investigated anogenital HSV-HPV coinfections in 2 groups of patients.

Patients and methods: Group 1: 199 patients (65 men, 134 women, median age 46 years) with typical anogenital herpes. Forty-four (22%) patients were infected with HIV. Group 2: 14 patients (11 men, 3 women, median age 55 years) with pseudotumoral anogenital herpes. All were immunocompromised (HIV infection: 13/14) and infected with HSV2. HSV2 genotypic resistance testing and HPV detection were performed.

Results: Group 1: anogenital HSV-HPV coinfection was detected in 98 (49%) patients, more frequently in immunocompromised ones (59% vs 46%; $p=0.033$). High-risk/potentially high-risk oncogenic (HR/pHR) HPV53, 52, 66 and 16 were the most common. Patients infected with HR/pHR HPV were younger (36 vs 53 years, $p=0.019$). HIV infection was associated with a higher frequency of HSV-HPV coinfection ($p=0.018$), infection with multiple HPV genotypes ($p=0.003$), and multiple HR-HPV genotypes ($p<0.001$). The CD4/CD8 ratio in HIV-infected patients was lower in those coinfecting with HSV-HPV than in those infected with HSV alone (0.38 vs 0.71; $p=0.040$). Group 2: anogenital HSV-HPV coinfection was detected in 10 (71%) patients, and infection with multiple HPV genotypes in 6 (43%) patients. At least one HR/pHR HPV was detected in 7 (50%) patients. HSV2 resistance to antivirals was diagnosed in 12 (86%) patients.

Conclusion: Anogenital HSV-HPV coinfections are common, particularly in HIV-infected patients. A better understanding of the role of HPV in pseudotumoral anogenital herpes is needed to optimize patient management.

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The clinical relevance of HHV6 DNA detection in CSF – prevalence of iciHHV6 and frequency of HHV6A vs. HHV6B infection

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Human herpesvirus 6 (HHV6) DNA is commonly detected by PCR in cerebrospinal fluid (CSF) samples of patients with neurological symptoms, but its clinical significance remains unclear, especially in immunocompetent adults. Inherited chromosomally integrated HHV6 (iciHHV6) occurs in about 1% of the population, leading to persistent HHV6 DNA PCR positivity in cell-containing patient material, and can influence the significance of HHV6 detection in CSF. The aim of this analysis was to assess the proportion of iciHHV6 and the frequency of HHV6A vs. HHV6B in patients with HHV6 positive CSF samples.

We performed a retrospective data analysis on diagnostic CSF samples tested positive for HHV6 DNA at our center between 01/2020 and 04/2026. iciHHV6 was defined by the detection of HHV6 DNA in patient hair follicles or fingernail. PCR analysis and genotyping were performed following in-house protocols.

Over the observation period, 56 CSF samples were tested positive for HHV6 DNA. Median patient age was 17 years, and 28 patients (50%) were female. Thirty patients were tested for chromosomal integration of HHV6. Of these, 21 (70%) were iciHHV6 positive, and displayed a significantly higher median HHV6 load in CSF ($4.11 \log_{10} \text{ c/ml}$) than non-iciHHV6 patients ($2.54 \log_{10} \text{ c/ml}$, $p=0.004$). iciHHV6 patients displayed HHV6A vs. HHV6B integration in 68% and 32%, respectively. In cases where both hair follicles/fingernails and CSF were genotyped, agreement in detected species was 100%. Among non-iciHHV6 patients, all were under the age of 4 years and/or immunocompromised. Only HHV6B was detected in the CSF of non-iciHHV6 patients.

Taken together, iciHHV6 is common among neurological patients with HHV6 DNA positive CSF, and should be excluded in respective cases, especially in adult, immunocompetent patients.

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Assessment of the Elecsys® Anti-HEV IgM Assay Performance in acute hepatitis E Diagnosis

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Introduction: Hepatitis E virus (HEV) infection is the leading cause of acute viral hepatitis. Diagnosis is based on detecting anti-HEV IgM antibodies (HEV-M), with RT-PCR confirmation in blood or stool recommended, especially for immunosuppressed (IS) patients. Reliable serological assays are essential. We evaluated the performance of the Elecsys® HEV-M assay.

Methods: A prospective study (March–May 2025) analysed 157 patient sera tested for HEV-M using Elecsys® (E®) and Diasorin® (D®) assays. HEV RT-PCR (AltoStar®) was performed on blood or stool in 33 cases. A retrospective study included 54 sera previously positive for HEV-M using D®, which were retested using the E®. RT-PCR results were available for 51 patients.

Results: In the prospective cohort, HEV-M prevalence ranged from 3.2% (E®:5/157) to 4.5% (D®:7/157). Agreement between assays was excellent ($\kappa=0.82$), with an overall concordance of 98.7% (155/157). Discrepant results corresponded to two false-positive D® results. Among the 33 PCR-tested samples, both assays showed a sensitivity (Se) of 100%. Specificity (Spe) was 93.3% for E® and 86.7% for D®. Positive predictive values (PPV) were 60% and 42.9%, respectively, while negative predictive values (NPV) were 100% for both assays. In the retrospective cohort, concordance between assays was 53.7% (29/54). Only 11/51 patients were confirmed HEV RNA-positive. E® showed a higher PPV than D® (37.0% vs 21.7%), suggesting reduced cross-reactivity. One case of hepatitis E with neurological manifestations was missed by the E® assay.

Conclusion: Elecsys® HEV-M assay demonstrates very good Se and slightly improved Spe compared with DiaSorin® assay, resulting in a higher PPV. Discordant cases highlight that, in the presence of strong clinical suspicion, viral genome detection remains the gold standard, notably in IS patients.

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The incidence and mortality rates for tick-borne encephalitis remain unsatisfactory in the Czech Republic

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Introduction: Tick-borne encephalitis (TBE) is a severe acute neuroinfection transmitted predominantly by tick bite or consumption of raw milk. The incidence of TBE is particularly high in Central Europe. The disease is expanding into new areas and higher altitudes, with climate change acting as a contributing factor.

Material and Methods: Retrospective descriptive analysis of case-based TBE data from the Czech nationwide infectious disease reporting system.

Results: On average, 600 to 800 cases of the disease are reported annually in Czechia; the mean incidence of TBE from 2007 to 2025 was 6.1 per 100,000 inhabitants. In 2025, 707 TBE cases were reported in Czechia with an incidence of 6.5 per 100,000 inhabitants. Extremely high incidence in some regions could be compared to an outbreak in the most affected areas. The disease affects all age groups, including children. The proportion of children and adolescents among KE cases increased from 10.8% in 2011 to 19.3% in 2025.

During previous 15 years (2011-2025), there were 57 reported TBE death in Czechia, i. e. reported case fatality rate was 0.59%. 45 deaths (nearly 79%) were recorded among people over 60 years.

Conclusions: Czechia ranks among European nations with the top TBE incidence, experiencing a long-term rise in cases, including a growing proportion of children and adolescents. Given the availability of a safe and effective vaccine, immunization against TBE is recommended for all age groups.

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Evolve: Enterovirus OnLine tools for Viral Epidemiology

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Introduction: Enteroviruses (EV) are a genetically diverse group of RNA viruses associated with a broad spectrum of clinical outcomes. Effective genomic surveillance is essential to monitor viral evolution, emergence, and transmission dynamics. However, limited data sharing restricts large-scale analyses and hampers public health responses.

Methods: We developed the EVOLVE (<https://evolve.vhir.org>), an open-access and collaborative platform designed for the storage and sharing of EV genomic sequences, as well as for the analysis of EV next-generation sequencing (NGS) data and FASTA files. Developed in collaboration with Nostrum Biodiscovery, the platform integrates bioinformatics Nextflow pipelines for sequence quality control, assembly and annotation. It also incorporates structured associated metadata enabling contextual interpretation of genomic data. The system supports secure data submission and provides integrated tools for phylogenetic and epidemiological analyses.

Results: The platform acts as a centralised and scalable resource integrating enterovirus genomic data and associated metadata from clinical laboratories, surveillance systems, and research groups. Combining repository functionality with embedded NGS analytical capabilities streamlines the processing of sequencing data. Initial implementation demonstrates improved accessibility and enhanced sequence characterization, facilitating downstream analysis, such as viral evolution or geographic spread.

Conclusions: The repository EVOLVE is a comprehensive solution for EV genomic surveillance and NGS data analysis. It delivers an integrated analytical environment that enables end-to-end processing of sequencing data with associated metadata. It is vital that scientific and public health communities participate to ensure maximum representativeness and impact.

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Updates on the development of new and replacement WHO International Standards for viral infectious disease diagnostics

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The WHO, primarily through the MHRA National Institute for Biological Standards and Control (NIBSC), has a long and successful history of establishing biological reference preparations intended for calibration of diagnostic nucleic acid amplification (NAT) and antigen tests for viral infectious diseases, in International Units. The availability of such materials for high-profile viral markers, such as HIV, Hepatitis B & C, has greatly improved the global harmonisation and comparability of assays used to diagnose and monitor infections.

Here, we will present updates on various ongoing projects to establish new and replacement WHO International Standards. This will include the candidate 4th WHO IS for Hepatitis B Surface Antigen (HBsAg), which is intended for calibration of HBsAg assays, and defines the minimum acceptable sensitivity for such assays as stipulated in the EU Common Technical Specifications (EU 2022/1107). The candidate standard, coded 25/120, was recently evaluated in an international collaborative study, and is expected to be established in late 2026. We will also describe progress on the development of the 2nd WHO IS for human Cytomegalovirus (hCMV-VHCMV), intended for calibration of NAT assays, for which extensive characterisation of the fragmented nature of the virus has been performed in order to provide a replacement WHO IS with improved commutability with patient samples.

Finally, we will summarise progress in our efforts to establish new WHO International Standards for Influenza, RSV and Mpox. These standards will be intended for calibration of NAT assays for the different viral markers and thus will be aimed at improving harmonisation of assays from different manufacturers.

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Characterisation of liver pathological processes in children with AAV2-associated acute severe hepatitis and after AAV gene therapy

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Introduction: Primary infection with adeno-associated virus 2 (AAV2) has been linked with acute hepatitis and liver failure in children. Liver toxicity and fulminant liver failure have also occurred in children after receiving AAV gene therapies (AAV-GT). The mechanisms linking AAV uptake to liver toxicity are poorly understood. To better understand the pathogenesis of these conditions, we undertook detailed characterisation of samples from affected children.

Materials & Methods

We analysed liver and serum samples from children with severe AAV2-associated hepatitis, and children who received AAV-GT for spinal muscular atrophy. Histological, metagenomic, transcriptomic and proteomic approaches were used to study pathological processes.

Results: Histological appearances of the liver were similar between AAV2 hepatitis cases and in a case of AAV-GT hepatitis, with ballooning degeneration of hepatocytes and a lymphocytic inflammatory infiltrate. Metagenomics revealed frequent presence of co-infecting viruses alongside AAV2, including human herpesviruses and adenovirus. Liver RNA-seq demonstrated a distinct altered transcriptome in AAV2-infected liver (n=6) when compared to normal liver (n=20), hepatitis B infection (n=18), and autoimmune hepatitis (n=5). Liver from a child with severe hepatitis after AAV-GT clustered within the AAV2 hepatitis group. Serum proteomic analysis of inflammation-associated proteins identified eight universal hepatitis biomarkers shared between the groups when compared to controls, reflecting an acute phase response and impaired liver synthetic function. There were also distinct serum proteomic signatures: AAV2 outbreak cases had altered extracellular matrix remodelling, cell adhesion, and complement activation, while AAV-GT cases had perturbations in coagulation and fibrinolysis pathways which persisted more than two months after infusion.

Conclusions: We show some similarities in liver pathology in AAV2 and AAV-GT-associated hepatitis, while distinct serum proteomic signatures between the groups may indicate key differences in disease mechanisms.

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Whole blood versus plasma in CMV DNA monitoring: clinical implications of sample choice in transplant patients

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In our laboratory, laboratory-developed tests (LDTs) have been essential for keeping pace with advances in molecular diagnostics. With the implementation of the In Vitro Diagnostic Regulation (IVDR), many LDTs must transition to commercially available, IVDR-compliant assays. In this context, we initiated the validation of the Cobas 5800 for the detection of cytomegalovirus (CMV) DNA.

Our hospital manages a substantial transplant cohort, of mainly heart transplant patients, for whom CMV monitoring is essential. Historically at our hospital, CMV DNA has been assessed in whole blood using an LDT qPCR. In contrast, the Cobas 5800 assay detects CMV DNA in EDTA plasma. Current literature does not demonstrate a clear preference for either sample type.

Over a monitoring period of more than one year, both methodologies and sample types were applied in parallel. A total of 706 samples were analyzed with both techniques: 55 % tested negative with both methods, while 25 % were positive with both. Discordant results were observed in 140 samples, with 20 % of all the samples testing positive only in plasma using the Cobas 5800 (of which 68 % were below the limit of quantification), and 1 sample was positive only in whole blood using the LDT (this sample was below the limit of quantification).

Notably, for most patients, CMV DNA was detected earlier and persisted longer in plasma than in whole blood with these methods. This may enable earlier initiation of antiviral therapy but could also prolong treatment duration. These findings raise an important clinical question: does earlier and more prolonged detection improve patient outcomes, or does it increase the risk of overtreatment? Is there a need for implementation of specific thresholds? We plan to initiate a study to investigate this in greater detail.

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Implication of the environment in JC Polyomavirus transmission: an emerging public health problem?

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Background: Environmental change, poor sanitation and new lifestyle habits are emerging problems in the transmission of human viruses. The human JC polyomavirus (JCPyV) has a worldwide distribution, despite the mode of transmission remains unknown. Since near half of JCPyV-infected individuals continuously excrete the virus in urine, its presence in wastewaters is expected. Based in a One-Health approach, and aiming to clarify the implication of the environment in JCPyV transmission, we evaluated the presence of this virus in untreated influents, treated effluents, and surrounding aquatic organisms.

Methods: Influent and effluent samples were collected at 15 Wastewater Treatment Plants (WWTP) across Portugal in two different periods. In addition, 18 composite samples of riverine and estuarine bivalve molluscs (digestive gland), and 15 samples of fishes (gut content), were also considered. All samples were evaluated for the presence of JCPyV DNA by real-time PCR.

Results: JCPyV genome was detected in 27 (90%) of the analysed influents, proving the virus ubiquity. WWTPs were only effective in removing JCPyV genome from 14 (52%) of those samples. JCPyV genome was detected in five of the 18 composite bivalve samples (28%), all of them obtained from species that feed in the water column. None of the samples from fishes revealed the presence of JCPyV DNA.

Conclusion: JCPyV is frequently detected in influents, but only half of the evaluated WWTPs can effectively remove it, which contribute to its dissemination to the aquatic environment. The detection of JCPyV in filter-organisms that feed in the water column confirms the dissemination of human viruses to surrounding aquatic organisms. These results suggest that the environment may play a role in JCPyV transmission, through the ingestion of contaminated water or aquatic organisms.



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Selection and verification of a dengue rapid diagnostic test for use in an Infectious Diseases ambulatory care unit in the UK

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Introduction: Dengue is the most common arboviral infection in UK travellers and autochthonous transmission in Europe is increasing. Dengue rapid diagnostic tests (RDTs) are used in endemic settings, but most UK hospitals rely on reference laboratory testing, with attendant delays and cost. We identified RDTs appropriate for use in an ambulatory care unit which diagnoses around 50 dengue infections a year.

Methods: RDTs were identified through manufacturer information and published literature. Predefined selection criteria included: detection of NS1 antigen (Ag), compatibility with whole blood for implementation at point of care, regulatory approval, and UK availability.

30 plasma samples were used for verification, comprising 20 dengue RT-PCR positive samples (median Ct 23, IQR 20-28) and 10 samples with relevant alternative diagnoses.

Results: We identified 24 manufacturers producing dengue RDTs. NS1 Ag RDTs and combined NS1 Ag/IgM/IgG RDTs from two manufacturers, Standard Q (SD Biosensor) and Bioline (Abbott), were assessed. The sensitivity of NS1 Ag for detecting RT-PCR confirmed dengue was higher with the Standard Q RDT (80%, 16/20), than the Bioline RDT (60%, 12/20).

The addition of IgM increased sensitivity, with IgM or NS1 Ag positive with the Standard Q RDT for 19/20 samples (95%) and Bioline RDT for 17/20 samples (85%). There were no false positive NS1 Ag results, but a Zika RT-PCR positive sample gave a false positive IgM on both manufacturers RDTs. The Standard Q NS1 Ag RDT was the cheapest product assessed.

Conclusions: The Standard Q Dengue NS1 Ag RDT was selected based on better performance and lower cost. Prospective assessment of the RDTs implementation using whole blood is ongoing, including review of performance against reference laboratory testing, user acceptability, and impact of faster diagnosis on the patient pathway.

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Genetically diverse Mumps detected in Leiden, The Netherlands between 2019 and 2025 by a novel pan-genotypic amplicon sequencing method

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Introduction: Mumps is a notifiable and vaccine-preventable respiratory disease yet frequently overlooked in comparison to measles. Genome sequencing can facilitate greater understanding of population-level mumps virus circulation and can also support epidemiological outbreak investigation in community or healthcare settings. However, limited sequencing, generally only targeting the short SH gene for basic genotyping, is currently undertaken. Published whole genome strategies may be restricted in genotype or offputtingly complex. Therefore, a simple pan-genotypic multiplex PCR whole genome sequencing protocol was designed to target mumps and applied to a retrospective patient cohort.

Methods: 40 nasopharyngeal swabs, 12 saliva and 2 urine samples diagnosed positive for mumps between 2019 and 2025 in Leiden, The Netherlands were re-extracted. cDNA was initially validated by qPCR, then subject to tiled-multiplex PCR spanning the near-complete genome with primers designed against a pan-genotypic reference alignment. Amplicons were qualitatively inspected, then processed for Nanopore sequencing. Data outputs were processed by the ARTIC pipeline and phylogenetic relationships interrogated.

Results: 50 samples passed re-extraction QC and near-complete genome coverage was achieved in 42 (84%) of mumps samples respectively. Incomplete sample amplification was related to initial diagnostic Ct, specimen type and potentially long-term storage as primary specimen. Whilst the majority of mumps sequences were of the expected genotype G, multiple sub-lineages and three other genotypes were also shown to be in circulation. Sequencing additionally revealed some variability in regions targeted by oligonucleotides in globally deployed RT-qPCR assays that may impact diagnostic detection.

Conclusions: Pan-genotypic amplicon sequencing of mumps can be straightforward and revealed hidden genomic diversity at a single European diagnostic centre. The increased resolution afforded by whole genome sequencing can inform understanding of transmission and may help focus interventions.

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Impact of Human papillomavirus vaccination on High-Risk HPV Epidemiology: A 13-Year Experience from a Portuguese Healthcare Center

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Introduction: Human papillomavirus (HPV) infection is the leading cause of cervical cancer worldwide. Despite effective vaccination programs (currently based on the nonavalent vaccine targeting HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) continuous monitoring of genotype distribution is essential. This study aimed to assess long-term epidemiological trends of high-risk HPV (hrHPV) infection and to evaluate the impact of vaccination in a Portuguese population.

Material and Methods: A retrospective analysis of 7,008 HPV DNA tests (6,970 women; mean age 43 years) performed between 2012 and 2025 was conducted. HPV16 and HPV18 were individually identified, while other hrHPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), were detected as a pooled group. For vaccination impact, women aged ≤30 years were stratified by birth year into a likely vaccinated cohort (≥1995) and a likely unvaccinated cohort (<1995).

Results: Overall hrHPV detection rate was 15.2%. Non-16/18 hrHPV types were the most frequent (69%), followed by HPV16 (14.1%) and HPV18 (~7%). HrHPV detection was higher in younger women and decreased with age. A significantly lower incidence of HPV16 was observed in the likely vaccinated group (1.5% vs 5.0%, $p=0.025$), supporting vaccine effectiveness. No significant reduction was observed for HPV18, possibly due to limited sample size. Conversely, non-16/18 hrHPV types showed a higher incidence in the vaccinated cohort (17% vs 9%, $p<0.001$). This may reflect sampling bias or type replacement dynamics.

Conclusions: HPV vaccination shows a clear protective effect against HPV16 in this population. However, the increased incidence of hrHPV types highlights the importance of continuous epidemiological surveillance. In the era of nonavalent vaccination, comprehensive genotyping is essential to distinguish HPV types included and not included in the vaccine, to better understand shifting genotype distribution and to optimize cervical cancer prevention strategies.

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A novel pan-genotypic amplicon sequencing method for measles viruses applied to contemporary clinical cohorts from Leiden, The Netherlands

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Introduction: Despite a longstanding global vaccination campaign towards elimination, measles has resurged in recent years. Genome sequencing can facilitate greater understanding of measles transmission and support epidemiological outbreak investigation in both community and healthcare settings. However, sequencing is frequently restricted to a 450bp region of the nucleoprotein for basic genotyping, whilst whole genome methods may be restricted in genotype, complex or poor in the phylogenetically informative M/F untranslated region. Therefore, a simple pan-genotypic multiplex PCR whole genome sequencing protocol was designed and validated.

Methods: 41 respiratory swabs and 9 urines diagnosed measles-positive between 2022 and 2025 at Leiden University Medical Centre, NL were re-extracted. cDNA was initially validated by qPCR, then subject to two PCR reactions containing primer pairs spanning the target genome in c.1200bp amplicon tiles designed against a pan-genotypic reference alignment. Amplicons were qualitatively inspected by gel-electrophoresis, then processed for Nanopore sequencing. Data outputs were processed by the ARTIC pipeline, assessed for completeness and phylogenetic relationships.

Results: Complete genome coverage was achieved in 96% of measles samples, with an upper limit of approximately Ct30. Sample amplification failure was related to initial diagnostic Ct, urine specimen type and potentially long-term storage as primary specimen. Measles was predominantly of the globally prevailing B3 and D8 subtypes, although clinical disease caused by the replicative, yet attenuated genotype A vaccine strain was also detected. Sequencing additionally revealed some variability in oligonucleotide sites targeted by the global gold-standard detection assay that may impact diagnostic detection.

Conclusions: The presented pan-genotypic amplicon sequencing of measles is straightforward, sensitive, and capable of providing near complete genomes that may be of increasing utility in public health epidemiology through both periods of disease resurgence and elimination.



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Preclinical Safety and Toxicokinetic Evaluation of KNIH-88 LP, a Neutralizing Human Monoclonal Antibody Against MERS-CoV, in Cynomolgus Monkeys

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Introduction/Aim: Middle East Respiratory Syndrome (MERS) remains a highly fatal emerging viral disease with limited therapeutic options. KNIH-88 is a fully human monoclonal antibody targeting the spike protein of MERS-CoV with potent neutralizing activity against viral infection. This study evaluated the preliminary safety and toxicokinetic profile of KNIH-88 in cynomolgus monkeys using a dose-escalation study (DES).

Materials and Methods: KNIH-88 lyophilized formulation (KNIH-88 LP) was administered to one male and one female cynomolgus monkey by intravenous infusion at sequential escalating doses of 25, 100, and 400 mg/kg at approximately 4-week intervals (Days 1, 29, and 57). Clinical observations, mortality, body weight, food consumption, clinical pathology, and gross pathology were evaluated throughout the study. Toxicokinetic assessment was performed following the first two administrations (25 and 100 mg/kg) using a qualified ligand-binding assay. Necropsy was conducted on Day 71 following the final administration.

Results: No mortality or treatment-related clinical signs were observed. No treatment-related effects were identified in body weight, food consumption, clinical pathology, or gross pathology at any dose level. KNIH-88 LP was well tolerated following repeated intravenous administration up to 400 mg/kg. Toxicokinetic assessment demonstrated measurable plasma exposure after dosing, prolonged systemic persistence with quantifiable pre-dose concentrations at Day 29, and approximately dose-proportional increases in exposure from 25 to 100 mg/kg. Slightly elevated exposure after the second administration suggested limited accumulation, with no meaningful sex-related differences.

Conclusions: KNIH-88 LP demonstrated favorable tolerability and predictable toxicokinetic behavior following repeated dose-escalation intravenous administration in cynomolgus monkeys. These findings support further GLP-compliant toxicity studies and development of KNIH-88 LP as a therapeutic neutralizing antibody for MERS-CoV infection.

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Impact of the virome in pediatric transplantations: transplantation outcomes after persistent viral infections

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Paediatric solid organ transplantation (SOT) recipients are at substantial risk of post-transplant complications, including graft rejection, immune dysregulation, and lymphoproliferative disorder. Viruses such as cytomegalovirus and Epstein-Barr virus are well-established contributors to these complications, yet the role of the broader DNA virome remains poorly characterised.

In this retrospective study, we aimed to identify viral markers predictive of graft complications and post-transplant lymphoproliferative disorder. We analysed 120 formalin-fixed paraffin-embedded (FFPE) biopsies from 24 paediatric SOT patients with verified post-transplant complications. The cohort included kidney transplant recipients (n=14) with chronic allograft dysfunction and heart transplant recipients with recurrent biopsy-proven rejections (n=5). Five patients with acute liver failure followed by bone marrow aplasia of unknown aetiology were also included.

Comprehensive clinical data, including demographics, history of viral infections, immunosuppressive regimen, and HLA typing, were collected for all patients. Tissue samples were processed using a targeted hybrid capture enrichment panel covering the full length of 41 DNA viruses, followed by short-read sequencing on Illumina platform. Sequencing data were analysed using our custom bioinformatic pipeline, TRACESPipe. Minor variant analysis was performed with ViroMino and viral integrations into the host genome with ViroJoin.

Preliminary data show distinct viral profiles across graft and complication type and prove that viral material can be successfully recovered from FFPE samples. Targeted qPCR validation supports the sensibility of the approach.

This study represents one of the first comprehensive DNA virome analyses in paediatric SOT recipients. Our findings highlight the potential of virome sequencing to improve detection of clinically relevant viruses and support more informed clinical decision-making, potentially improving transplantation outcomes.



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Clinical Characteristics of Influenza A(H1N1)pdm09, A(H3N2), and B Infections Detected During the 2024-25 Influenza Season at Ankara Bilkent City Hospital

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Introduction/Aim: Influenza viruses cause approximately 1 billion cases worldwide each year. This study compared disease severity among patients with laboratory-confirmed influenza A(H1N1)pdm09, A(H3N2), or B infection.

Materials and Methods

A total of 1,015 patients (604 children, 411 adults) with influenza A(H1N1)pdm09, A(H3N2), or B detected in respiratory samples in the 2024-25 influenza season at Ankara Bilkent City Hospital were included. Hospitalization was the primary outcome; composite "severe course" was secondary.

Results: Hospitalization rates were 12.6% in children and 55.0% in adults. In both age groups, hospitalization was highest in the A(H1N1)pdm09 group and lowest in the influenza B group. Compared with influenza B, hospitalization risk was approximately fourfold higher for A(H1N1)pdm09 and threefold higher for A(H3N2) in children; in adults, it was approximately threefold and twofold higher, respectively. Although the lower hospitalization rate among adults with influenza B may be partly explained by younger age and fewer comorbidities, multivariable analyses showed that influenza A subtypes increased hospitalization risk independently of age and comorbidities.

Severe course was most common in A(H1N1)pdm09 infection in both age groups. Because severe course was uncommon in children (n=15), multivariable analysis was not performed. In adults, severe course was independently associated with older age, comorbidity, previous hospitalization, and A(H1N1)pdm09 subtype. Although influenza B appeared to have a milder course, its high association with pneumonia in children was notable (46.3%). Complication rates did not differ significantly among children, whereas they were approximately twice as high in adults with influenza A subtypes compared with influenza B. Complications were strongly associated with comorbidities rather than influenza type/subtype in both age groups.

Conclusion: Influenza infections do not present a uniform clinical pattern by type/subtype. Evaluating influenza type/subtype together with age and comorbidities is important for prognosis.

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Dynamics of HSV2 resistance to antivirals in an HIV-infected patient with recurrent genital herpes

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Introduction: Herpes simplex virus 2 (HSV2) is the commonest virus responsible for genital herpes (GH) in human immunodeficiency virus (HIV) infected individuals and (val)acyclovir ((V)ACV)) remains the gold standard for antiviral therapy so far. ACV has to be primophosphorylated by viral thymidine kinase (TK) to be active. HSV resistance to ACV is mostly mediated by TK alterations (95% of cases), either amino acid substitution or nucleotide insertion/deletion leading to a translational frameshift, sometimes associated with premature stop codon. However, amenamevir (AMNV), a viral helicase-primase inhibitor, has demonstrated efficacy in VACV-refractory infections bypassing TK pathway.

Case report: A 51-year-old HIV1-infected female - with preserved CD4+ T cells - experienced GH recurrences despite suppressive VACV prophylaxis between 2016 and 2024. Apart HIV infection, she has been suffering from rheumatic disease treated by immunosuppressive therapies. Curative VACV was initially effective, but refractory infections were subsequently observed. Genotypic resistance testing including TK gene sequencing was performed whenever necessary to explain antiviral ineffectiveness. Strikingly, distinct TK frameshift profiles accounting for ACV resistance were identified over time: D229Stop (2017), L98Stop (2020), L263Stop (2022), and frameshift without stop codon (2024). As a last resort, AMNV was used as compassionate curative treatment. No adverse effects were observed and lesions healed completely within a few days. Noteworthy, no relapse has been observed under suppressive VACV up to 22 months after curative AMNV and subsequent recurrences have well responded to curative VACV.

Discussion: Prolonged exposure, suboptimal VACV dosing, and high viral replication have likely favoured the emergence of TK variants and the unusual TK frameshift sequential profiles over time. The patient finally achieved to control GH after curative AMNV. AMNV could become a milestone therapy for GH, but further studies are needed to fully define its benefit for curative and suppressive treatments.

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Public and healthcare-professional knowledge of cytomegalovirus (CMV) during pregnancy after the 2025 French screening recommendation: a community-based survey

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Introduction/Aim: CMV is the leading cause of congenital infection, with neurosensory morbidity. In June 2025, the French Haute Autorité de Santé (HAS) recommended systematic first-trimester (T1) CMV serology in seronegative or unknown-status pregnancies. We assessed CMV knowledge in the public and among healthcare professionals (HCPs) to inform community-based prevention strategies.

Materials and Methods: A cross-sectional study using convenience sampling was conducted by 50 interprofessional health students across five sites in Paris, France (2 district town halls, 2 pharmacies, and 1 medical school stand), from February 16 to April 17, 2026. An expert-reviewed 13-item questionnaire (4 demographic, 9 knowledge, 3 confidence items, aligned with HAS 2025/CNGOF) was administrated. Analyses included 716 respondents who answered ≥ 1 knowledge item. Per-item accuracy is reported with Wilson 95% CIs; HCPs vs non-HCPs differences were tested with χ^2 .

Results: Of 716 participants (76% female; 52% <35 years; 33% HCPs), 16% answered all 9 items correctly (HCPs 25%, non-HCPs 11%). The HAS T1 screening rule was the best-known item (89%; 95%CI 86-91) while vertical-transmission risk was the lowest (60%; 56-64). Overall, 22% (34% of HCPs), were unaware that antenatal valaciclovir reduces vertical transmission. HCPs outperformed non-HCPs on every item except T1 screening (80% vs 89%) and treatment availability (66% vs 77%). Non-HCPs often selected correct answers with low confidence.

Conclusion: Our findings highlight insufficient knowledge of congenital CMV infection in this community sample, including among HCPs. The HAS 2025 screening message has reached the public or was already known, yet vertical-transmission risk and antenatal valaciclovir remain misunderstood. These gaps represent clear targets for public health messaging and HCPs refresher training.

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Whole genome sequencing reveals co-circulating lineages within nosocomial respiratory virus clusters despite predominantly clonal transmission

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Nosocomial clusters (NCs) of respiratory viruses are routinely identified in hospital settings, but their comparative genomic characteristics remain poorly described. We analyzed influenza, RSV, and SARS-CoV-2 NCs during the 2024–25 winter season at Rouen University Hospital.

NCs were identified by the Infection Prevention Department based on routine quadriplex PCR (influenza A/B, RSV, SARS-CoV-2) in symptomatic patients and classified by ward and temporal occurrence. For each NC, 2–3 samples ($C_t < 28$) consistent with temporal cluster dynamics were selected. Whole genome sequencing (WGS) was performed using amplicon-based approaches with virus-specific primers (Pasteur for RSV; Zhu et al. panels for influenza; ARTIC V4.1 for SARS-CoV-2) on Illumina MiSeq (RSV and influenza) or ONT MinION (SARS-CoV-2). Consensus sequences were generated using an in-house bioinformatics pipeline. Lineages and mutations were determined using Nextclade and phylogenetic analyses assessed intra-cluster relatedness.

Three RSV NCs (7 genomes) were all subtype B (B.D.E.1, $n=5$; B.D.4.1.3, $n=2$). Six influenza A/H1N1 NCs (18 genomes) were mainly C.1.9 ($n=16$, including C.1.9.3), with two D.3.1. Eight SARS-CoV-2 NCs (22 genomes) were mainly 24F ($n=14$), with 24I ($n=6$) and 24E ($n=2$). Most NCs showed genomic homogeneity, supporting nosocomial transmission. However, one influenza and one SARS-CoV-2 NC included distinct lineages, indicating co-circulation rather than a single transmission event. Clusters of the three viruses overlapped temporally across wards.

Genomic data confirmed nosocomial transmission in most NCs but identified distinct co-circulating lineages in some epidemiologically defined NCs. This supports an integrated approach combining multiplex PCR-based surveillance and WGS for refined cluster interpretation in a multi-virus context.

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Study of Contact Patterns Among Staff and Residents in UK Care Homes

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Introduction: Respiratory diseases are of serious concern in care homes because of their increased transmissibility and increased chance for severe outcomes in these settings. Mathematical modelling is an important tool to help us better understand transmission in them and measure the impact of interventions, which can often be difficult to quantify in real life. Currently there is no data on contact patterns in UK care homes to include in models. The University of Manchester and UKHSA are collaborating to run a study in five care homes in North England starting in May 2026 to collect such data. The study received ethics approval from a national social care research ethics committee.

Methods: Residents and staff will wear a small device (like a wristband or key fob) that uses Bluetooth to record when they are near others wearing a device. There will also be static location markers and hardware to transmit data. This technology allows us to collect data on: who comes into contact with who, in which room, and for how long. The data will be pseudonymised but will differentiate staff and residents. The study will be run for two weeks in three care homes for the elderly and two care homes for people with learning disabilities.

Results and conclusions: We will have findings by the end of July 2026 for all three care homes for the elderly. The primary outcomes are a description of contact patterns broken down by staff and residents and a contact matrix which can be incorporated into models of respiratory disease transmission. This will greatly improve our knowledge of contact patterns in care homes which will help make modelling more meaningful. It will mean the impact of interventions such as testing, vaccination, cohorting, isolation, etc. can be better tested within models. The data will be applicable to a range of respiratory diseases such as SARS-CoV-2, influenza, and respiratory syncytial virus. In turn this will help shape outbreak procedures and pandemic planning in this vulnerable setting.

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Hepatite A e Hepatite E: Evolução entre 2020 e 2025

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Introduction: Hepatitis A (HAV) and hepatitis E (HEV) are viral infections predominantly transmitted through the faecal-oral route, with similar clinical presentations, and may occur sporadically or in outbreaks. Although generally self-limiting, they may develop into severe forms in high-risk groups.

Materials and Methods: A retrospective study was conducted to evaluate the seroprevalence of hepatitis A and hepatitis E, as well as to identify temporal trends, using serum samples from 1899 individuals with laboratory requests for HAV IgG and IgM antibody testing and from 1167 individuals with laboratory requests for HEV IgG and IgM antibody testing.

Results: Between 2020 and 2025, a progressive increase in the number of tested samples was observed.

Regarding HAV, 1137 samples showed IgG positivity (59.9%), predominantly in the 30–39-year age group, while 20 samples were positive for IgM (1.1%), with the highest incidence observed in the 0–9-year age group. Acute (HAV) infection showed similar frequencies between sexes.

For HEV, 277 samples (23.7%) showed IgG positivity, predominantly in the 50–59-year age group while 67 samples (5.7%) were positive for IgM, with the highest incidence observed in adults over 50 years of age. Acute HEV infection showed similar frequencies between sexes.

Conclusions: This study demonstrates distinct epidemiological profiles for the two hepatitis infections studied. While hepatitis A showed a high IgG seroprevalence, indicating established population immunity, hepatitis E stood out due to its current clinical relevance, with a significantly higher IgM positivity rate than HAV. The progressive increase in sample testing throughout the study period reflects enhanced clinical and laboratory surveillance. These findings suggest that in the studied population, HEV is an important aetiological agent in cases of acute hepatitis, reinforcing the need for its systematic inclusion in differential diagnostic protocols to ensure effective public health surveillance.



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Evaluating pre-analytical strategies for fecal virome sequencing: implications for clinical viral diagnostics

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Fecal virome analysis using metagenomic and metatranscriptomic sequencing is a promising tool for clinical viral diagnostics, enabling pathogen detection and microbiome characterization. However, pre-analytical variability limits routine implementation, particularly when optimal stool specimens are unavailable. This study evaluates how different pre-treatment methods affect virome detection and quantitative performance.

Twelve fecal samples from patients with confirmed viral gastroenteritis were analyzed under five conditions: untreated, polyvinylpyrrolidone (PVPP), ethanol, Cary-Blair medium, and fecal swab (FecalSwabTM, COPAN). Nucleic acids were extracted, and DNA/RNA libraries were sequenced (~15 Gb/sample). Taxonomic and functional profiling employed MetaPhlAn, HUMAnN, and BAQLaVa after quality control.

Sequencing generated a mean of 22.3 million DNA reads and 16.8 million RNA reads per sample. Alpha and beta diversity were highly consistent across conditions (<2% variability). Overall, 722 DNA viral species-level genome bins and 262 RNA viral taxa were detected, including unclassified sequences, indicating abundant viral "dark matter". The virome comprised viruses from multiple families, including Peduoviridae and Microviridae, as well as diverse bacteriophages.

Viral detection was concordant across all conditions, indicating high qualitative agreement.

However, PVPP and ethanol showed higher recovery of DNA/RNA viruses and phages, whereas fecal swabs yielded lower viral load and RNA resolution, likely due to reduced fecal input. These findings highlight the impact of pre-analytical workflows on quantitative outputs and RNA-based sensitivity, with direct clinical implications.

PVPP provided the most balanced performance. Alternative sampling and processing strategies proved feasible when optimal specimens were unavailable, supporting the use of genomic diagnostics in real-world settings. Despite the limited sample size, trends were consistent and warrant validation in larger cohorts, contributing to efforts to standardize genomic workflows in clinical virology.

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Comparative Functional and Biochemical Characterisation of Engineered Human ACE2 Decoys for Neutralisation of SARS-CoV-2 Variants

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Introduction: The emergence of novel SARS-CoV-2 variants has reduced the effectiveness of many antibody-based neutralising agents, highlighting the need for antiviral strategies targeting conserved mechanisms of viral entry. Angiotensin-converting enzyme 2 (ACE2), the cellular receptor for SARS-CoV-2, represents such a conserved target, and engineered ACE2 decoys have demonstrated broad neutralisation potential against diverse viral variants.

Methods: Four ACE2 decoys were designed and characterised: WT ACE2, an Fc-fused ACE2 (ACE2-mFc), an engineered ACE2 decoy containing five substitutions (ACE2-5mut), and its Fc-fused counterpart (ACE2-5mut-mFc). Neutralising activity was evaluated using pseudovirus and authentic-virus microneutralisation assays against SARS-CoV-2 variants. Thermal stability analysis and solution-state characterisation were performed.

Results: Engineered ACE2 decoys demonstrated enhanced neutralising activity compared with WT ACE2 in neutralisation assays, with IC₅₀ values in the low-nanomolar range. Relative decoy performance varied in a variant-dependent manner across assay systems. Engineered ACE2 decoys displayed comparable thermal profiles and defined molecular species in solution, whereas WT ACE2 showed reduced stability during storage. Fc fusion significantly enhanced pseudovirus neutralisation potency of WT ACE2 across all tested variants and improved the activity of engineered ACE2-5mut against most tested variants.

Conclusions: Engineered ACE2 decoys achieved broad and consistent neutralisation across SARS-CoV-2 variants while demonstrating favourable biochemical characteristics. These findings support the continued development of ACE2-based antiviral strategies and emphasise the importance of balancing decoy format, neutralising potency, and biochemical stability in the design of ACE2 therapeutics.

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Respiratory virus seasonality and RSV immunization in young children

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Introduction/Aim: Respiratory viruses are a major cause of pediatric emergency admissions, particularly in children under 3 years old. This study aimed to characterize the seasonal circulation of respiratory viruses between 2022 and 2026 and evaluate the impact of RSV immunization on detection trends.

Methods: Nasopharyngeal swabs from 3537 children under 3 years old admitted to the emergency department between May 2022 and April 2026 were analyzed using multiplex RT-PCR. Monthly detections of Adenovirus, seasonal Coronaviruses, Human Metapneumovirus, Rhinovirus/Enterovirus (REV), Influenza A/B, SARS-CoV-2, Parainfluenza viruses, and Respiratory Syncytial Virus (RSV) were evaluated.

Results: REV and RSV were the most frequently detected viruses. REV circulated throughout the year with increased activity during colder months. Influenza A and Human Metapneumovirus also demonstrated winter seasonality, although with lower detection rates. RSV showed marked autumn–winter seasonality, with peaks in November of 2022 (n=83; 35,3%) and December of 2023 (n=60; 33,3%). From 2024 onwards, RSV detections progressively declined during peak months (n=39; 25% - December 2024; n=37; 26,8% - January 2026). This reduction was not observed for other respiratory viruses. In contrast, SARS-CoV-2 progressively showed higher circulation during August (n=18; 52,9% - 2023; n=17; 47,2% - 2024; n=30; 53% - 2025).

Conclusions: Respiratory viruses maintain well-defined seasonal patterns in children under 3 years old, with RSV and REV as predominant pathogens. SARS-CoV-2 appears to have evolved toward a predominantly summer circulation pattern. The reduction in RSV detections matched the implementation of RSV vaccination. The immunization highlights the importance of preventive strategies in reducing pediatric disease burden. Continuous epidemiological surveillance remains essential to monitor seasonal respiratory virus dynamics and evaluate the long-term effectiveness of vaccination programs.

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Assessment of BK polyomavirus viral load by qPCR in urine, plasma, and whole blood: analytical comparison of the R-gene® and Panther Fusion™ BKV Quant Assays

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Introduction: BK polyomavirus (BKPyV) is a ubiquitous pathogen responsible for infectious complications in immunocompromised patients, particularly in kidney or hematopoietic stem cell transplantation recipients. Routine diagnosis and monitoring rely on the viral loads evaluation in urine and blood samples.

The aim of this study was to evaluate the clinical performance of the Panther Fusion BKV Quant Assay.

Material and methods: In this single-centre prospective study, urine samples (n=200, including 150 positive), EDTA plasma samples (n=102, including 57 positive), and EDTA whole blood samples (off label use for the Panther Fusion BKV assay; n=143, including 93 positive) were analysed using both the BK Virus R-GENE kits (routinely used at Angers University Hospital) and the Panther Fusion BKV Quant Assay. The impact of BKPyV genetic diversity was evaluated using a panel of genotyped samples (n=15) provided by the French National Reference Centre for BKPyV (Strasbourg University Hospital). Agreement between viral load measurements was assessed using Bland–Altman analysis and Pearson's correlation coefficient. The impact of genetic diversity was analysed using the Kruskal–Wallis test.

Results: A high level of agreement was observed for urinary viral load ($r = 0.98$, 95% CI: 0.973–0.986, bias = -0.16). Similar results were obtained for viral load in EDTA plasma ($r = 0.867$, 95% CI: 0.784–0.92, bias = 0.21) and in EDTA whole blood ($r = 0.843$, 95% CI: 0.771–0.893, bias = -1.1).

BKPyV genetic diversity did not significantly affect viral load ($p = 0.39$) and showed a strong agreement between assays correlation ($r = 0.961$, 95% CI: 0.879–0.988).

Conclusion: This study represents the largest evaluation ever conducted on clinical samples testing positive for BKPyV. The Panther Fusion BKV Quant assay demonstrated excellent clinical performance for BKPyV viral load in urine, plasma, and whole blood samples.

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Differential SARS-CoV-2 genomic diversity in patient stools compared to respiratory tract as a risk of variants of concern by digestive shedding

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Aim: The continuous evolution of SARS-CoV-2 can favour variants of concern (VOC). SARS-CoV-2 have shown various tissue-tropisms and viral quasi-species need to be characterized in digestive compartment as an additional spreading pathway.

Methods: From end-2024, nasopharyngeal swabs (NPS) and stools were collected from patients infected by SARS-CoV-2, in Intensive Care Unit (ICU) and Infectious Diseases Department at successive dates. For the first seven patients, Whole Genome SARS-CoV-2 Sequencing (WGS, ARTIC) analyzed the variants, while Shannon entropy and diversity ratio explored a potential viral compartmentalization between respiratory and digestive tracts assessing associated risks of VOC.

Results: Thirty-six samples with 13 respiratory samples and 23 stools showed for NPS at Day 0 (inclusion) viral loads (VL) at 16.1-29.6 Ct, while for stools DO Ct at 27 Ct to not detected, with global VL decline before the patients' discharge.

WGS identified from NPS and stools successive strains as 24C, XEC and 25C variants as expected in France. For compartmentalization: (i) diversity ratio showed a higher diversity in stools than in NPS (p-value<0.05) only in ORF8 gene, (ii) protein domain analysis indicated a tendency for greater Spike protein diversity in stools, (iii) some of the mutations identified in the spike protein, modifying the surface properties, can impact its binding to the human host receptor and its immunogenicity.

Conclusions: SARS-CoV-2 differential dynamics and genetic diversity in respiratory tract and stools showed that viral load and sequence diversity were time-/patient- dependent. A higher genomic diversity in stools than in NPS was retained in ORF8 gene, a key actor of viral immunopathology. In Spike, diversity showed a tendency to be higher in stools and highlighted mutations able to modify its binding to the human host receptor and its immunogenicity. Further research will inform on how this compartmentalization can influence viral persistence and VOC emergence.

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Utility of Dried Blood Spot (DBS) PCR in the Retrospective Diagnosis of Congenital Cytomegalovirus Infection Among Infants Presenting After the Neonatal Period: A Single-Center Experience

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Introduction/Aim: Congenital cytomegalovirus (cCMV) infection is the most common congenital infection and the leading non-genetic cause of neurodevelopmental impairment and sensorineural hearing loss (SNHL). Definitive diagnosis of cCMV requires the detection of CMV DNA in a sample collected within the first 21 days of life; beyond this 21-day window, it is difficult to distinguish congenital from postnatally acquired CMV infection. In infants with suspected cCMV, retrospective testing may be performed using dried blood spots (DBS) collected at birth as part of national newborn screening programs.

This study aimed to evaluate the diagnostic utility of DBS CMV PCR testing for the detection of CMV DNA in infants diagnosed beyond the 21st day of life.

Methods: A retrospective cohort study was conducted among consecutive infants referred for cCMV evaluation at a tertiary pediatric hospital in Warsaw between January 2020 and March 2026. All infants with a positive urine CMV PCR obtained after 21 days of life, whose parents provided written informed consent, underwent DBS testing for cCMV using qualitative real-time PCR. Infants with other TORCH infections were excluded. Clinical, laboratory, neuroimaging, audiological, and ophthalmological data were analyzed according to DBS CMV PCR results.

Results: A total of 209 infants were included (mean age: 121 days; range: 26-325 days). cCMV was confirmed by DBS CMV PCR testing in 13 infants (6.2%). Among confirmed cases, five (38.5%) had abnormal neuroimaging findings, four (30.8%) had abnormal neurological examinations, and one infant (7.7%) was diagnosed with SNHL. Antiviral treatment with valganciclovir was initiated in five patients (38.5%). All infants with cCMV were linked to specialized long-term follow-up.

Conclusions: These findings demonstrate that, in only 6% of infants diagnosed beyond the neonatal period, DBS CMV PCR provided definitive confirmation of cCMV and directly influenced clinical management, therapeutic decisions, and long-term follow-up.



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Enterovirus types detected in acute flaccid paralysis cases within the German National Enterovirus Surveillance, 2006–2025

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Introduction: Enterovirus Surveillance (EVSURV) in Germany offers free enterovirus diagnostics for patients with viral meningitis, encephalitis, or acute flaccid paralysis (AFP). Besides exclusion of poliovirus infections, the surveillance provides insights into circulating non-polio enteroviruses (NPEV) associated with neurological symptoms.

Material and Methods: Data from EVSURV collected between 2006 and 2025 were analysed with focus on AFP-associated enterovirus detections and serotype distribution. Mainly stool, but also cerebrospinal fluid and, in selected cases, respiratory samples were examined by enterovirus PCR and subsequent typing within the German Laboratory Network for Enterovirus Diagnostics (LaNED).

Results: Between 2006 and 2025, 44,113 samples were analysed within EVSURV, including 941 AFP cases. Enteroviruses were detected in 80 AFP patients. The number of AFP cases with confirmed enterovirus infection ranged from one to seven per year. No polioviruses were detected during the entire surveillance period. Overall, 23 different enterovirus types were identified among AFP-associated infections. The predominant serotypes included Enterovirus A71 (EV-A71), Echovirus 18 (E18), Coxsackievirus A4 (CVA4) and Coxsackievirus A9 (CVA9). In 23 AFP cases, enterovirus screening PCR was positive, but no typing result could be obtained. However, poliovirus infection was ruled out in each case. In 2024, one AFP sample tested enterovirus-positive and showed simultaneous detection of EV76, E3 and E25. EV-D68 was detected only rarely in stool samples, supporting the recommendation to additionally test respiratory specimens in AFP/acute flaccid myelitis presentations.

Conclusion: The EVSURV in Germany demonstrates the diversity of NPEV associated with AFP. Continuous molecular surveillance remains essential for maintaining polio-free status and for monitoring emerging neurotropic enteroviruses such as EV-A71. The option to test respiratory specimens in addition to stool samples in cases of AFP is still not frequently used but could improve the Data on EV-D68.

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Genotypic characterisation of norovirus circulating in Ireland, 2024 - 2026

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Background: Norovirus causes acute gastroenteritis (AGE) across all age groups. Since the mid-1990s, variants of GII.4 have been predominant, though an increased prevalence of norovirus GII.17 has recently been reported. We present here Irish data characterising the change in viral epidemiology.

Methods: This retrospective study analysed results from samples received for viral gastroenteritis investigation at the National Virus Reference Laboratory, Dublin, from 1st January 2024 to 31st March 2026. All samples underwent qRT-PCR for detection and genogrouping of norovirus RNA. A subset of samples was tested by Sanger sequencing for genotyping and a further cohort processed for whole-genome sequencing (WGS) using the NetoVIR viral enrichment methodology.

Results: Norovirus RNA was detected in 1677/13938 (12%) samples. Ninety-six (6%) were genogroup (G) I, 1582 (94%) were GII, with one GI & II co-infection.

GII samples from 2024 to 2026 (n=287) underwent Sanger sequencing to determine genotypes. Of these, 88 (31%) were GII.4 and 149 (52%) GII.17. Other genotypes detected were GII.2 (1%), GII.3 (7%), GII.6 (2%), GII.7 (5%), GII.9 (2%), and GII.10, GII.13 and GII.14 (all n=1). Of 15 samples undergoing WGS, 10 (67%) yielded near-complete genomes (range 7148-7701 nt), and were genotype GII.4_Sydney.P16 (n=6) GII.17.P17 (n=2), GII.13.P16 (n=1), and GII.3.P12 (n=1). Of 26 genotyped GI samples, all from 2025, most were GI.7 (n=11), followed by GI.3 (n=9), GI.5 (n=3), GI.4 (n=2) and GI.6 (n=1).

Conclusion: GII.17 accounted for the majority of circulating norovirus in Ireland during 2024 to early 2026, displacing the historically dominant GII.4. The clinical significance of increasing GII.17 prevalence has yet to be established but, as prior exposure to GII.4 strains provides little cross-protective immunity, it has been proposed that GII.17 may lead to larger, more sustained outbreaks. WGS is an essential tool for monitoring outbreaks, tracking recombination events and to understanding the evolution of this dynamic virus.

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Mutational signatures of SARS-CoV-2 across multiple clinical cohorts: drug mutagenesis, host processes and viral clearance

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Introduction/Aim: Mutational signature analysis can decompose viral sequence variation into biologically distinct processes. We applied this framework to characterise mutational signatures in SARS-CoV-2 genomes across multiple clinical cohorts spanning different treatments and immune contexts, to identify signatures associated with viral clearance.

Material and Methods: De novo non-negative matrix factorisation (NMF) of 96-channel mutational spectra was performed on five cohorts: PANORAMIC trial (two arms: participants receiving molnupiravir or usual care and participants receiving paxlovid or usual care), and immunocompromised patients from the LUNAR cohort receiving sotrovimab. Peak timepoint mutations were decomposed into biologically distinct signatures and compared against COSMIC v3.5 and published reference spectra.

Results: NMF consistently identified a conserved background signature across all cohorts and arm-specific signatures reflecting treatment mechanism. In molnupiravir, drug-associated G>A+C>T signatures were consistent with RdRp mutagenesis but did not independently predict clearance after compositional adjustment. A T>C-enriched signature was identified across cohorts and independently associated with faster viral clearance in the usual care arm, confirmed by raw T>C fraction analysis. This signal was observed across cohorts regardless of treatment mechanism, suggesting a consistent biological process independent of drug action.

Conclusions: Mutational signatures differ between treatment arms, reflecting distinct drug and host processes. A conserved T>C-enriched signature, absent from drug-induced mutagenesis, is observed across multiple cohorts and warrants further investigation as a potential correlate of viral clearance. Mutational signature analysis provides a novel framework for dissecting host and drug contributions to within-host viral dynamics.

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Antibody detection of human parechoviruses in HuTu80 and HT29 cells

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Human parechoviruses (PeV-As) are common pathogens associated with a wide clinical spectrum ranging from mild respiratory and gastrointestinal symptoms to severe, life-threatening neurological infections, particularly in infants. Currently, there are nineteen parechovirus types classified within the family Picornaviridae. Most parechovirus types have been identified by genetic analysis due to lack of a universal cell model for parechovirus cultivation. This study aimed to compare the permissiveness of intestinal HuTu80 and HT29 and VeroE6 cell lines for PeV-A genotype replication and to identify suitable detector antibodies. In vitro growth characteristics of PeV-A1 to PeV-A6 strains in HuTu80 cells were assessed using cytopathic effect (CPE) analysis, immunofluorescence assay (IFA) and RT-qPCR. The results demonstrated that isolates representing PeV-A types 1-6 replicated efficiently in HuTu80 cells within 24 hours post-infection. Five in-house antibody preparations (monoclonal antibody P5C4 and polyclonal antisera K8873, K8851, K306, and K316) successfully detected PeV-A types; however, P5C4 was selected for further analyses due to its specificity. Comparative analyses showed that HuTu80 and HT-29 cells were permissive to PeV-A1-6 infections and compatible with P5C4 immunofluorescence-based detection of viral antigens, whereas Vero E6 cells were not suitable for this application due to high background fluorescence. Overall, these findings demonstrate that HuTu80 and HT29 cell lines are suited for cultivation of most parechovirus types and thus enable isolation of parechoviruses for further analyses such as genome sequencing and structural analysis. In addition, virus-specific antibodies, particularly P5C4, provide effective tools for detecting parechoviral antigens and may contribute to development of novel laboratory strategies for parechovirus detection.



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Formulation of reference reagents for nucleic acid amplification test assays for Toscana and Usutu viruses

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Introduction: Nucleic Acid Amplification Testing (NAAT) underpins modern viral diagnostics by enabling sensitive and specific detection of pathogens however assay development and implementation require access to well characterised reference reagents. Since their initial isolation Toscana virus and Usutu virus have expanded geographically across Europe and continue to present diagnostic challenges. Both show strong seasonality linked to vector activity and can cause neurological disease, they are also likely to be under diagnosed. As laboratories adopt or develop new RT-qPCR and broader NAAT workflows to improve detection, external physical reference materials are essential to support assay development, calibration, quality control, and harmonised interpretation between testing centres.

Methods: To address this gap we have developed inactivated NAAT reference materials for Toscana virus (TOSV Phleb.3) and Usutu virus (Strain 3345, Isolate Arb276), designed for use in molecular analyses. Virus stocks were propagated in house and heat inactivated, with inactivation validated by the absence of cytopathic effect in permissive cells following three serial passages.

Results: Heat inactivation had little impact on the NAAT performance of the virus stocks. The resulting materials were diluted to working concentrations in plasma matrix and performance in NAAT assays evaluated. Both reference materials had sufficient linearity ranges for utility as reference materials.

Conclusions: Making these reagents globally available will strengthen innovation in viral diagnostics by enabling more robust assay validation and inter-laboratory harmonisation thereby helping to reduce variability and support surveillance and ultimately improving detection of under-diagnosed infections.

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Clinical Performance of the High-Speed ANCHOR CMV PCR Kit Compared to the cobas® CMV Assay

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Introduction/Aim: Accurate quantification of human cytomegalovirus (CMV) DNA is essential for the clinical management of immunocompromised patients. This study evaluated the clinical performance of the ANCHOR CMV PCR Kit (ANCHOR), a real-time PCR assay with a fast protocol (approx. 30 min), in comparison to the cobas CMV assay (Roche).

Materials and Methods: Remnant diagnostic EDTA-plasma samples (n=307) and contrived samples (n=15) were tested at the University Medical Center Hamburg-Eppendorf. The ANCHOR CMV PCR Kit, traceable to the WHO standard (NIBSC 09/162), was used in combination with the Magna Pure 96 for DNA extraction (500µl, large volume kit), and run on the CFX Opus 96 Dx cycler. The cobas CMV assay (Roche) served as comparator, and discrepant samples were resolved using the GeneProof CMV PCR (NuvinkaDx).

Results: Of 322 samples tested, 152 were CMV DNA positive (CMV DNA range: <LOQ – 6.9 log IU/mL) and 170 CMV DNA negative by the ANCHOR CMV assay, yielding a positive percent agreement (PPA) of 93.79% (95% CI: 88.95–96.59%) and a negative percent agreement (NPA) of 99.38% (95% CI: 96.57–99.89%) versus the comparator assay. All 11 discrepant samples (<LOQ, 18.7, 49.7 and 105 IU/ml respectively) tested negative with the GeneProof assay, consistent with low target concentrations near the detection limit. Quantitative analysis of the 145 concordant positive samples demonstrated excellent agreement, with minimal bias on Bland–Altman analysis (mean difference: –0.06 log₁₀ IU/mL) and Passing–Bablok regression (constant bias: +0.06 log₁₀ IU/mL).

Conclusion: The ANCHOR CMV PCR Kit, despite its short analytical time, is non-inferior to the cobas CMV assay with respect to diagnostic accuracy, as measured by PPA and NPA and a high quantitative agreement allowing adequate patient management decisions.

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Longitudinal Analysis of Persistent Parvovirus B19 Infection and Intra-host Viral Evolution in an Immunocompromised Patient under Continuous IVIG Treatment

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Introduction: Parvovirus B19 (B19V) infections are typically paucisymptomatic and self-limiting. In contrast, immunosuppressed individuals are at risk of persistent infection, posing significant clinical challenges. This study aims to longitudinally characterize persistent B19V infection in an immunocompromised host and to investigate within-host viral evolution under IVIG therapy.

Methods: Plasma collected every 2-4 months was analyzed by quantitative PCR and next generation sequencing based on an amplicon sequencing approach. Analysis was performed using Geneious Prime. Infectivity was assessed using UT-7/Epo-S1 cells.

Results: The patient exhibited persistently high viral loads (10^{11} - 10^{13} copies/ml) over two years associated with recurrent, transfusion-dependent anemia. IVIG administration every 2-4 weeks resulted in transient reductions in viral load (nadir: 10^3 - 10^4 copies/ml). Near full-length B19V genome sequences were successfully generated (mean coverage: 6,000-8,000 reads/nucleotide) that identified B19V genotype 1a. Compared to the reference sequence (NC_00883.2), 18 amino acid substitutions within protein-coding regions were detected and remained stable over time demonstrating limited intra-host viral evolution. Notably, sequence analysis revealed a mutation previously associated with viral persistence (I181M, NS1 protein), and the loss of the first of multiple start codons in the 11 kDa protein due to an M1T substitution. Further, sustained viral infectivity was demonstrated using UT-7/Epo-S1 cells. Samples collected from the patient environment in the ICU were all positive for B19V DNA (mean 1.4×10^7 copies/ml). Evaluation of infectivity is ongoing.

Conclusion: Despite repeated IVIG administration, persistent B19V replication occurred, with limited intra-host sequence diversification while viral particles remained infectious. These findings suggest that, in immunocompromised hosts, mechanisms other than rapid viral evolution contribute to B19V persistence and highlight the importance of humoral immunity for viral clearance.

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An automated bioinformatic workflow for RSV genome surveillance to detect potential nirsevimab resistant variants

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Introduction/Aim: Nirsevimab implementation for prevention of severe infant RSV disease requires surveillance of circulating RSV strains for F-gene mutations that may reduce monoclonal antibody susceptibility. We describe a fully-automated bioinformatic workflow for detecting and prioritizing genomic markers potentially associated with reduced nirsevimab susceptibility in whole-genome RSV sequencing data.

Methods: We adapted a hybrid-capture RSV sequencing protocol to prioritize robust F-gene coverage. Quality-controlled reads are bioinformatically processed in two complementary modules: consensus-genome reconstruction with VADR annotation using RSV-specific gene models, and targeted F-gene surveillance. The latter uses curated RSV references, LoFreq variant calling, depth thresholds, strand-bias filtering, and codon-aware read-based phasing to resolve adjacent nucleotide changes affecting single amino acid positions.

Results: The pipeline generates annotated consensus genomes and F-gene variant tables reporting allele frequencies and predicted amino acid effects. LoFreq variant calling enables detection of intra-sample viral subpopulations. F-gene changes corresponding to manually curated resistance-associated mutations from the literature and other prioritized F-gene changes are flagged for expert review. Sequencing quality, mapping metrics, and F-gene coverage are summarized in interactive HTML reports to ease data interpretation.

Conclusions: We developed a comprehensive and robust workflow linking RSV whole-genome sequencing with targeted F-gene surveillance for potential nirsevimab reduced-susceptibility markers. Automated, quality-controlled reporting supports timely detection and prioritization of relevant F-gene variants, including low-frequency variants; this workflow may inform resistance monitoring as nirsevimab use expands.

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Phylogenetic Analysis of Human Seasonal Coronaviruses in Ghana 2023-2024

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Introduction/Aim: Although human seasonal coronaviruses (HCoVs) NL63, OC43, 229E and HKU1 are endemic, their evolution and persistence within populations remain poorly understood. HCoVs commonly cause respiratory infections affecting children and immunocompromised individuals. In low-resource settings such as in Ghana, respiratory infections contribute substantially to child morbidity and mortality. Understanding HCoV reinfection dynamics is limited by the availability of genomic data, particularly in underrepresented regions. This study aims to explore the diversity of circulating HCoVs by contributing new sequence data from Ghana.

Methods: We extracted RNA from 67 pan-coronavirus PCR-positive nasopharyngeal swab samples collected between 2023-2024 in the Greater Accra and Eastern regions in Ghana, followed by whole genome nanopore sequencing. Real-time, interactive phylogenetic trees based on the whole genome and key structural genes (including spike and nucleocapsid) were built using Nextstrain to analyse the sequences. A novel in-house script was used to assess recombination.

Results: While whole genome sequencing of samples collected in Ghana is still ongoing, preliminary analysis of partial gene sequences indicates the diversity of lineages circulating in Ghana is consistent with global patterns, except for Ghanaian 229E sequences, which appear to cluster separately. Further analysis of mutations in the structural genes will provide insights into the evolution, recombination and antigenic variability of circulating HCoVs, as the proteins they encode are involved in viral entry and transmission, immune evasion and reinfection.

Conclusions: This study expands genomic data for HCoVs in an understudied region and highlights patterns of lineage diversity, including distinct clustering of 229E, which may indicate the presence of a distinct lineage circulating in Ghana. These findings support genomic surveillance efforts and provide insight into the evolutionary dynamics of HCoVs relevant to transmission and reinfection

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Evaluation of the Coyote® Flash Detect™ for simultaneous detection of HSV1, HSV2 and VZV in vesicular fluids in tertiary hospital setting

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Introduction/Aim: Rapid and accurate detection on vesicular fluids of herpes simplex virus (HSV) type 1 and 2 and Varicella Zoster Virus (VZV) is essential for further clinical patients management. The Coyote® Flash Detect™ is a novel molecular point-of-care platform, that enables simultaneous detection of HSV1, HSV2, and VZV, providing both qualitative results and cycle threshold (Ct) values.

This study evaluates the performance of this instrument for HSV1/2 and VZV detection in comparison with routine methods at Ghent University Hospital, Ghent, Belgium.

Methods: Coyote® Flash Detect™ developed by Coyote Bioscience and marketed in Europe by A. Menarini Diagnostics, was compared to Illumigene® HSV assay (Meridian Bioscience) for HSV1/2 and to Allplex™ Meningitis Panel (Seegene) run on the STARlet system for VZV detection, current routine methods in our laboratory. A total of 13 Illumigene®-negative samples were reanalyzed using the Coyote® with discordant results further evaluated on the Allplex™ assay. Also positive routine samples for HSV1 (n=6), HSV2 (n=5) and VZV (n=6) were tested. To assess cross-reactivity, 3 Epstein-Barr virus (EBV)-positive and 2 cytomegalovirus (CMV)-positive samples were also analyzed on the Coyote®.

Results: Among 13 Illumigene®-negative samples, 3 (23%) were HSV-1-positive on the Coyote®, all confirmed by Allplex™. The Coyote® detected all HSV1 and HSV2 positive samples, including one HSV1 sample with additional HSV2 positivity. For VZV full concordance was observed. No cross-reactivity was detected in EBV- or CMV-positive samples.

Conclusions: The results suggest a higher sensitivity for HSV1/2 detection with the Coyote® Flash Detect™ compared to the Illumigene® method. Its ability to simultaneously detect HSV1, HSV2, and VZV represents a significant advantage to our current routine flow. No cross-reactivity with EBV or CMV was observed. The availability of Ct values represents an added value for clinical interpretation. These findings support the Coyote® Flash Detect™ as a valuable addition to routine herpesvirus diagnostics.



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Association Between Torque Teno Virus DNA Plasma Levels and Post-Transplantation Diabetes Mellitus in the First Year After Kidney Transplantation

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Introduction/Aim: Torque teno virus (TTV)-DNA plasma concentration is as a surrogate marker of immune function in renal transplant recipients (RTRs), usually peaking 60-90 days after transplantation. Long-term hyperglycaemia, as indicated by HbA1c, affects the immune response and may influence TTV-DNAemia levels. We aimed to investigate the relationship between TTV-DNA plasma loads and post-transplantation diabetes mellitus (PTDM), a common complication after renal transplantation.

Methods: TTV-DNAemia levels were analysed retrospectively in 303 archived plasma samples collected from 93 RTRs shortly after transplantation and at 3, 6, and 12 months using a quantitative real-time TTV-DNA-PCR assay (R-Gene, bioMérieux). Clinical and laboratory parameters (including glycaemic status, opportunistic infections, allograft rejections and immunosuppressive regimen), were assessed. 17 patients (18.3%) developed PTDM; patients (n=11, 11.9%) with pre-existing diabetes mellitus (DM) were analysed separately.

Results: As expected, TTV-DNA plasma loads peaked at 3 and 6 months in our cohort ($p<0.001$). Upon multivariate analysis, time after transplantation ($p<0.001$), tacrolimus trough levels ($p=0.001$), and, interestingly, HbA1c ($p=0.012$) were associated with elevated TTV-DNA loads. Patients with PTDM, but not with pre-existing DM, showed significantly higher TTV-DNAemia at 3 months ($p=0.011$) and 6 months ($p=0.003$) after transplantation compared to non-diabetic patients. No significant group differences were observed for cumulative prednisolone dose, tacrolimus trough levels and induction therapy. Although patients with PTDM were older, age contributed only marginally to TTV-DNAemia levels. Opportunistic viral infections tended to occur more frequently in patients with PTDM.

Conclusions: PTDM but not pre-existing diabetes is associated with elevated TTV-DNAemia levels early after transplantation and a potentially increased risk of opportunistic infections, suggesting clinically relevant immune dysfunction. Prospective studies are needed to clarify cofactors and clinical impact.

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Lineage-specific virus neutralization assays facilitate diagnostic differentiation of West Nile virus and Usutu virus infections

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Introduction: The mosquito-borne flaviviruses West Nile virus (WNV) and Usutu virus (USUV) co-circulate across Europe and human infections have been detected in Austria since 2009. In Austria, WNV lineage 2 (WNV 2) and USUV are endemic, whereas WNV lineage 1 (WNV 1) is detected occasionally in returning travellers. Due to their close antigenic relationship, infections with these viruses induce cross-reactive antibodies, posing significant challenges for serological diagnosis. Here, we developed virus neutralisation tests (VNTs) for WNV 1 and WNV 2 to differentiate between human WNV and USUV infections.

Materials & Methods: Lineage-specific VNTs were established for WNV 1, WNV 2, and USUV. Briefly, Vero E6 cells were inoculated with 30–100 TCID₅₀ of the respective virus strains, and neutralization titers were determined based on cytopathic effect. Sera from patients with either confirmed WNV 1 (n=8), WNV 2 (n=25) or USUV (n=20) infections were analysed with established VNTs.

Results: Patient sera showed the highest neutralization titers against the infecting viral lineage, with significantly lower cross-neutralization titers against heterologous WNV lineage or USUV. Although cross-neutralization between WNV and USUV sera was observed, the use of lineage-specific VNTs enabled clear differentiation between WNV 1, WNV 2, and USUV, with neutralization titers typically 3–4-fold higher against the corresponding viral lineage. In our cohort, 25% of patients with West Nile fever (WNF) or West Nile neuroinvasive disease (WNND) were PCR-negative and had cross-reactive WNV/USUV titers. In these cases, differentiation required lineage-specific neutralization tests.

Conclusion: Our data show that lineage-specific neutralisation assays are required for serological differentiation of WNV and USUV infections, particularly in patients with WNF or WNND who are often PCR-negative and depend on serological diagnosis. As neutralization titers are lineage-specific, VNTs including both WNV 1 and WNV 2 are essential to improve diagnostic accuracy in regions where WNV and USUV co-circulate.



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HHV-6 in Ocular Disease: Innocent Bystander or Cause of Panuveitis?

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Human herpesvirus 6 (HHV-6) has been implicated in ocular infections, including panuveitis; however, interpretation of HHV-6 DNA detection in ocular fluid is complex. HHV-6 can exist as inherited chromosomally integrated HHV-6 (iciHHV-6), where the viral genome is present in all nucleated cells. Additionally, HHV-6A and HHV-6B are distinct viruses, with HHV-6B more commonly associated with clinical disease.

A 25-year-old male presented with a two-day history of painless blurred vision and floaters. Ophthalmologic findings were consistent with unilateral panuveitis. Extensive investigations, including PCR testing of aqueous humour, were negative for HSV-1, HSV-2, VZV, CMV, EBV and Toxoplasma. Serological tests for HIV, hepatitis B and C, syphilis, Lyme disease, and tuberculosis were negative. Inflammatory markers were normal, but HLA-B27 was positive, and a paternal history of uveitis and arthritis.

Partial clinical improvement was observed with corticosteroids and aciclovir; however, symptoms persisted. Eight months later, further aqueous humour testing using untargeted metagenomics detected HHV-6A, confirmed by RT-PCR. Blood HHV-6 viral load was markedly elevated (20,652,850 copies/mL), with 0.998 HHV-6 copies per human genome, consistent with iciHHV-6. Subsequent reference testing initially detected both HHV-6A and low-level HHV-6B, raising the possibility of active superinfection. Repeat testing confirmed HHV-6B, however there is known cross-reactivity between HHV-6A and HHV-6B and HHV-6B was undetectable by an alternative assay.

The detection of HHV-6B may represent either a false positive or an acute co-infection on a background of iciHHV-6A. This case highlights the diagnostic challenges associated with HHV-6 detection, particularly in distinguishing active infection from chromosomal integration and potential superinfection.

Detection of HHV-6 DNA alone is insufficient to establish causality in ocular disease and interpretation is nuanced by the clinical and virological context.

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Repurposed Lipophilic Statins Combined with Chelerythrine Chloride Exhibit Synergistic Antiviral Activity Against Zika Virus In Vitro

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Introduction/Aim: Zika virus (ZIKV), associated with congenital Zika syndrome and Guillain-Barré syndrome, remains an emerging arboviral threat with no approved therapeutics available. Combination therapy and drug repurposing strategies may improve efficacy and accelerate anti-ZIKV drug development. This study investigated whether chelerythrine chloride (CTC), a PKC inhibitor that reduces ZIKV infectivity through cell-independent mechanisms, could enhance the antiviral activity of lipophilic statins previously shown to inhibit ZIKV replication.

Materials & Methods: CTC and selected lipophilic statins, including atorvastatin (ATO), cerivastatin (CER), mevastatin (MEV), fluvastatin (FLU), and simvastatin (SIM), were evaluated in ZIKV-infected Vero cells using a fixed-ratio combination assay. Synergistic interactions were quantified using combination index (CI) values and isobologram analysis.

Results: Among the tested combinations, CTC-ATO demonstrated the strongest synergistic antiviral activity, particularly at 4:1 and 2:3 CTC:ATO ratios (CI ≤ 0.5). Combinations of CTC with CER and MEV also exhibited partially synergistic antiviral effects across all tested ratios (CI > 0.5-1). However, combinations with FLU and SIM demonstrated predominantly partial synergy, although additive (CI = 1) and partially antagonistic (CI > 1-2) interactions were observed at selected ratios.

Conclusions: These findings demonstrate that the antiviral activity of lipophilic statins against ZIKV can be enhanced through combination with CTC. In particular, the strong synergistic activity observed with the CTC-ATO combination highlights its potential as a lead candidate for further anti-ZIKV drug development. Additional mechanistic and in vivo studies are warranted to evaluate the therapeutic potential and safety of CTC-statin combination therapies.



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Population-Level Human-Animal Contact and Wet Market Use in Thailand and northern Ghana: Implications for Zoonotic Spillover

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Background: Wet markets are frequently at the centre of zoonotic spillover debates, yet there is limited empirical evidence on population-level use and human-animal contact. We investigated important potential correlates of wet market engagement in Thailand and northern Ghana.

Materials and Methods: Cross-sectional, in-person surveys were conducted among adults in Thailand (n=2000) and northern Ghana (n=2343). Surveys captured wet market attendance, frequency of animal contact, species purchased or reared, market characteristics, and infection-related perceptions.

Results: Wet market attendance was higher in Thailand (85.4%; n=1690) than Ghana (29.3%; n=686). Ghanaian respondents reported more frequent animal contact at markets (29.4% touching animals three or more times per visit, 8.8% in Thailand). In both countries participants most frequently reported visiting markets several times weekly. In Thailand, 2.9% of marketgoers purchased live animals. In Ghana, live animal purchasing was widespread among marketgoers (97.4%) predominantly domesticated birds and grazing mammals. Ghanaian markets were mainly outdoor (93.8%), Thai markets predominantly indoor (83.0%). Thai market attendance was more likely among females (OR 1.48 [95% CI 1.15-1.90]) and respondents with higher infection worry (1.64 [1.38-1.96]) and lower perceived control (0.78 [0.64-0.96]), whereas Ghanaian attendance aligned with lower infection worry (0.84 [0.76-0.94]), larger household size (1.03 [1.01-1.06]), and extensive home animal rearing (1.42 [1.16-1.75]). Masks and handwashing were more common in Thailand (94.0% vs 42.1 and 82.0% vs 26.4%), while species-specific inspection of animals before purchase was more prevalent in Ghana (51.3% vs 16.1%).

Conclusions: Wet markets represent heterogeneous human-animal interfaces shaped by market ecologies and behaviour. While attendance was higher in Thailand, Ghanaian marketgoers reported more frequent contact and engagement with live animals. Findings suggest zoonotic exposure is shaped by routine livestock contact across markets and households.

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Early detection of Influenza A(H3N2) subclade K in wastewater of Leuven, Belgium

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Introduction: Influenza remains a major cause of morbidity and mortality worldwide. Surveillance traditionally relies on clinical reporting and laboratory-confirmed cases, which may be influenced by healthcare-seeking behaviour, testing capacity, and reporting delays. Wastewater-based epidemiology (WBE) has emerged as a complementary approach for population-level monitoring of infectious diseases, independent of individual testing. This study aimed to evaluate the added value of WBE combined with viral genotyping for early detection of circulating influenza variants in Belgium.

Material & Methods: Wastewater samples were collected from the Leuven region as part of an ongoing WBE programme. Influenza A (IAV) RNA was detected using RT-qPCR. Samples showing elevated viral concentrations were selected for amplicon-based sequencing targeting the haemagglutinin (HA) gene. Sequencing was performed with Oxford Nanopore. Phylogenetic analysis was done with Nextclade to determine the circulating subtype and identify emerging variants.

Results: An increase in IAV concentrations was observed at the start of the influenza season and indicating a true rise in community-level virus circulation.

Sequencing analysis identified IAV (H3N2), specifically subclade K, a recently emerged variant associated with increased influenza activity internationally. To our knowledge, this represents the first detection of H3N2 subclade K in wastewater in Belgium. At the time of detection, no epidemiological data were yet available from the National Reference Centre for Influenza (Sciensano), highlighting the added value of wastewater-based surveillance for early variant detection.

Conclusions: These findings demonstrate that wastewater-based surveillance, when combined with viral genotyping, can provide early insights into circulating influenza variants at population level. WBE complements traditional clinical surveillance by capturing both symptomatic and asymptomatic infections and may serve as an early warning system to support timely public health responses and influenza preparedness strategies.

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The Hidden Complexity of RSV: Intra host viral diversity and distinct cytokine signatures during experimental infection in older adults

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Introduction: Respiratory Syncytial Virus (RSV) primarily infects ciliated epithelial cells in the upper respiratory tract. Though it maintains relatively stable antigenically, lifetime RSV re-infection is very common and can result in bronchiolitis and pneumonia in the immunocompromised as well as pediatric and geriatric populations. Older adults are among the most susceptible to severe RSV infection, yet the mechanism that dictates this susceptibility remains unclear. The growing reliance on monoclonal antibodies such as palivizumab and nirsevimab for treatment has allowed for further RSV intra-host genetic variation, possibly influencing viral gene expression and host susceptibility. Here, I aim to investigate how these viral genetic expression patterns and host inflammatory gene expression levels dictate disease severity in the older adults.

Methods: The RSV human challenge study "A Controlled Human Infection Study with RSV in older People" (CHIRP-01) inoculated 20 healthy older adults, aged 65–75 years, with 10⁶ plaque-forming units (PFUs) of RSV A/Memphis/37b intranasally. Study participants were quarantined for 10 days post inoculation (p.i.), and nasal and blood samples were taken from days -1 to 10, 14, and 28 p.i. Viral loads were quantified using RT-qPCR from nasal lavage and exhaled breath, and self-reported symptoms were collected daily. Immune-related cytokines and chemokines were quantified in blood and nasal mucosa throughout infection by Mesoscale Discovery. Transcriptomic analysis of RNAseq data identified differential expression of host factors and viral genes.

Results: There was no correlation between viral load and symptom severity in older adults, disregarding viral load as the main perpetrator for disease severity. Further results will correlate viral load quantification with cytokine/chemokine levels, uncovering drivers for disease susceptibility. Volcano plots will showcase differential viral gene expression levels in infected versus uninfected older adults.

Results will reveal viral and host genomic drivers of RSV infection in older adults.

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Molecular and phylogenetic characterization of predominant Influenza A(H3N2) in Croatia during the 2025/2026 season

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Introduction/Aim: Seasonal influenza surveillance enables monitoring of circulating viruses and detection of mutations associated with antigenic drift and vaccine effectiveness. This study aimed to characterize genetic diversity, mutation patterns, and phylogenetic relationships of influenza A viruses circulating in Croatia during the 2025/2026 season.

Materials and Methods: A total of 7,217 respiratory samples were tested, of which 940 were influenza-positive. Subtyping identified A(H3N2) (74.9%), A(H1N1)pdm09 (17.0%) and B/Victoria (0.4%); 49 samples were excluded and 16 remained untyped. Whole-genome sequencing was performed on 56 representative samples (45 A(H3N2), 11 A(H1N1)pdm09). Hemagglutinin (HA) gene mutation analysis was conducted using a closely related reference strain, and phylogenetic analysis included representative sequences and vaccine strains.

Results: All A(H3N2) sequences belonged to subclade K (3C.2a1b.2a.2a.3a.1) and showed a consistent mutation profile (S160N, N174D, I176K, Q189R, Y211F; 95.6–100%). Several substitutions were located within or near antigenic sites, indicating ongoing antigenic drift. Phylogenetic analysis demonstrated clustering of A(H3N2) sequences with limited genetic divergence. The sequences showed some genetic distance from earlier vaccine strains while remaining within the genetic context of recently recommended vaccine strains. A(H1N1)pdm09 (clade 6B.1A.5a.2a.1) was less frequent and showed a relatively conserved mutation profile (A156D, R130K; 90.9%).

Conclusion: During the 2025/2026 influenza season in Croatia, A(H3N2) was the predominant circulating subtype, mirroring broader European and Northern Hemisphere trends in which subclade K became dominant and exhibited a relatively stable evolutionary profile, characterized by antigenically relevant substitutions and limited sequence diversity. In contrast, A(H1N1)pdm09 circulated at a lower prevalence with a conserved genetic pattern. These findings highlight the value of genomic surveillance for monitoring influenza evolution and supporting vaccine strain selection.



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NGS-based HBV antiviral resistance testing: real-world experience from a referral centre in Portugal

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Hepatitis B virus (HBV) antiviral resistance is a clinical challenge, particularly in HIV co-infected patients exposed to lamivudine (3TC)/emtricitabine (FTC)-containing antiretroviral regimens. We report our experience with NGS-based HBV resistance testing in a Portuguese cohort.

NGS HBV resistance tests were performed in 44 samples from 38 patients between 2023 and 2026 at the Molecular Biology Laboratory, ULSLO. Patients in this study underwent testing due to virological failure or treatment complexity. Samples were sequenced using the NGS Vela Diagnostics System and interpreted with Vela software and/or with Geno2Pheno. Genotype, resistance-associated variants in the polymerase, surface, basal core promoter, precore and core regions, drug susceptibility, HIV co-infection status, and geographic origin were recorded.

42 tests were interpretable (7 positive from 6 patients, 16.7%). All patients with DRM were HIV co-infected with prior 3TC/FTC antiretroviral exposure. Genotype distribution: E (52.6%), D (26.3%), A (18.4%), F (2.6%); genotype E was predominantly found in patients of Lusophone African origin (17/20, 85%), consistent with its known restriction to West Africa. The dominant resistance pattern was M204V+L180M (5/6, 83%), conferring lamivudine/telbivudine resistance; additional substitutions (S202G, V173L, A181T) extended resistance to entecavir in 4/6 (67%). A181T (3/6) conferred adefovir resistance with intermediate tenofovir susceptibility. No full tenofovir resistance was detected. Surface variants within the HBsAg 'a' determinant were detected in multiple cases, illustrating the added clinical value of NGS beyond RT resistance testing.

Resistance was exclusively detected in HIV co-infected patients, consistent with selective pressure from prior 3TC/FTC regimens without adequate tenofovir coverage. The dominant lamivudine resistance pattern frequently extends to entecavir, while tenofovir remains universally active, supporting current guidelines. Genotype distribution reflects Portugal's epidemiological reality shaped by Lusophone African migration.

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A Spotlight on Rotavirus Genotypic Surveillance in Wales, UK 2023 - 2025

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Introduction: Rotavirus vaccination was introduced in the UK as part of the child immunisation programme in 2013. Despite dramatic reductions in cases, rotavirus remains a significant cause of acute gastroenteritis in Wales.

During 2025, there was an observed 76% increase in laboratory confirmed cases with higher genotypic diversity when compared to 2024 recorded in a Welsh health board. This prompted a review of rotavirus cases and genotypes across South Wales to understand if this increase was driven by specific genotypes or better case ascertainment.

Materials and Methods: Analysis of positive samples from 2023 – 2025 was performed across four health boards in South Wales. This included genotypic data, patient demographics and vaccine status. Faecal samples from symptomatic cases, were tested on different platforms including RT-PCR and ELISA, with genotype results from a UKHSA virus reference laboratory, London, UK.

Results: In total, 456 rotavirus positive cases were identified, of which 315 had genotypes assigned. Samples confirmed to be vaccine positive were excluded, leaving 273 in the final analysis. All cases were eligible for the rotavirus vaccine and 86% (N= 236) were fully vaccinated.

Over the time-period, 10 genotypes of rotavirus were detected with G3P8 and G2P8 accounting for 67% (N=184) and 18% (N=50) respectively. Of these, 195 were under 2 years old; 132 were infected with G3P8 and 34 with G2P8. The prevalence of G2P8 varied both in time and geography across 2024 and 2025.

Conclusion: G3P8 remains the most prevalent genotype in Wales with no clear change in epidemiology. This contrasts with G2P8 where there was an observed regional shift from west to east between 2024 - 2025.

This highlights the importance of systematic surveillance of rotavirus and its ongoing clinical impact and epidemiology in the post immunisation era.

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Evaluation of the INSaFLU Bioinformatics Platform for the Alignment and Mapping of Hepatitis B virus Next-Generation Sequencing Reads

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Introduction/Aim: Next-generation sequencing (NGS) for Hepatitis B virus (HBV) genotyping is a highly sensitive method, able to reliably detect minority viral variants. Other advantages of NGS include improved time efficiency and a reduction in cost. However, the transition to NGS may be challenging in many laboratories by the lack of advanced training in bioinformatics to deal with primary NGS data. Indeed, it often relies on the usage of commercial black-box bioinformatics pipelines. This study aimed to evaluate the performance of the INSaFLU open web-based platform in the alignment and mapping of HBV NGS reads.

Material and Methods: Raw NGS data in the FASTQ format generated by sequencing of the polymerase region from 44 HBV samples, were processed, aligned and reference mapped using the SmartVir bioinformatics pipeline integrated into the AD4SEQ HBV Solution (arrow Diagnostics), and the INSaFLU platform. The two resulting consensus sequences for each HBV sample, were aligned and compared to each other at the amino acid (AA) level.

Results: After processing and removal of low-quality reads, a median of 90.1% (range 35.3-97.4%) of input reads was used in downstream alignment, mapping and consensus sequence generation by the INSaFLU platform. It was found an overall mean concordance of 99.9% between the sequence generated by the SmartVir pipeline and the one resulting from the INSaFLU platform. Discrepancies were found in 11 samples (25.0%), presenting a median of 1 (range 1-3) discordant AA position. No discordances were found at drug-resistance positions.

Conclusions: Findings from this study showed that the INSaFLU platform is suitable and highly accurate for the alignment and mapping of HBV NGS reads. This platform enables users to perform advanced customization of the processing parameters in a user-friendly manner without previous training in bioinformatics.

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Genotypic characterisation of Respiratory Syncytial Virus circulating in Lombardy during the 2023–2024 and 2024–2025 seasons and variability in monoclonal antibody binding sites and small-molecule inhibitors

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Introduction/Aim: Respiratory syncytial virus (RSV) significantly affects infants and vulnerable populations. The objectives of our study was to investigate RSV genomic epidemiology and dynamics between 2023–2024 and 2024–2025 seasons

Methods and Methods: RSV-positive nasopharyngeal swabs were collected from symptomatic individuals at three clinical centers in Lombardy, Italy, where Nirsevimab was introduced during the 2024–2025 season. Whole genomes were obtained using an in-house amplicon protocol, sequenced on Illumina platforms, and assembled with vcf-consensus-builder. Maximum-likelihood trees (IQ-TREE2; GTR+F+I+G4) and Bayesian coalescent methods (GTR+F+I+G4; constant population and strict molecular clock) defined transmission clusters (TCs: ior probability=1, sequences>10). Amino acid mutations in Nirsevimab, Palivizumab, and Clesrovimab binding sites were assessed. Multivariate analysis compared 2023–2024 and 2024–2025 seasons.

Results: A total of 303 RSV sequences (130 from 2023–2024, 175 from 2024–2025), mostly from infants (<1 year, 58.0%) and males (57.1%), were collected; 92.3% of cases had lower-respiratory symptoms, 66.8% were hospitalized, 15.0% Intensive-Care-Unit (ICU)-admitted, and 17 were Nirsevimab breakthroughs. The most frequent clades were A.D.3 (27.1%) and B.D.E.1 (20.1%), followed by A.D.1.2 (17.5%) and A.D.5.2 (14.2%). Two TCs of 56 A.D.3 and 17 A.D.1.2 sequences were exclusively identified in 2024–2025. mAb-resistant mutations, two as minority variants (<20% abundance), were found in two 2023–2024 and one 2024–2025 samples (Palivizumab: K272E in A.D.5.3, N262Y in A.D.3; Nirsevimab: N208D in B.D.E.1). No Clesrovimab resistance was detected, though four minority mutations (three in 2024–2025 season) were found at its binding site (K433R in B.D.4.1.1; C439R in A.D.1.2; N437S in A.D.5.2; N446S in A.D.3).

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Conclusions: RSV genomic surveillance in Lombardy revealed emerging RSV clusters and F protein variability in 2024–2025, highlighting the need for continuous monitoring to guide prophylaxis.

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Rising Metapneumovirus Infections in 2026 Spring

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Introduction: Human metapneumovirus (HMPV) mainly affects young children, older adults, and immunocompromised individuals, causing respiratory illnesses ranging from mild cold-like symptoms to severe conditions such as bronchiolitis and pneumonia. Recent international surveillance data showed an increased circulation of HMPV following the influenza and respiratory syncytial virus (RSV) epidemics. In Portugal, HMPV circulation is monitored through the National Influenza and Other Respiratory Viruses Surveillance Program. The study aimed to evaluate the circulation of HMPV in Portugal during the 2025/2026 season by age and geographic region.

Material and Methods: From 2025-W40 to 2026-W15, 23 laboratories from the Portuguese Laboratory Network for the Diagnosis of Influenza and Other Respiratory Viruses tested respiratory samples by RT-PCR and weekly reported HMPV positive cases to the National Institute of Health (INSA) stratified by age groups (0-4; 5-15; 15-29; 30-64; 65-79 and ≥80).

Results: A total of 17,450 samples were tested for HMPV, with 842 (4.8%) confirmed cases. After a decline in influenza activity, from week 2026-W05 onward, HMPV positivity increased across Portugal mainland and Azores islands, peaking at 2026-W10 (17% positivity). The rise in positivity was first detected in children aged ≤4 years and subsequently among older adults. 57% of HMPV infections were confirmed in children ≤4 years, 11% among individuals aged 65–79 years and 15% among those aged ≥80 years.

Conclusions: HMPV demonstrated significant circulation during the spring of the 2025/2026 season, particularly affecting young children, and older adults. These results emphasize the importance of an integrated surveillance of respiratory viruses beyond influenza, SARS-CoV-2 and RSV. Given the absence of available vaccines or specific preventive measures for HMPV, continuous surveillance and improvement of genetic characterization remains essential to describe epidemiological trends and support public health preparedness.



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Age-specific circulation patterns of respiratory viruses in Portugal, 2024–2025

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Introduction/Aim: Respiratory viral infections are a major global health burden, causing significant morbidity. Clinical outcomes are influenced by age, particularly affecting children and older adults. Our study evaluates laboratory-confirmed respiratory virus (RV) infections by age-group to characterize their age distribution.

Material and Methods: During 2024-2025 season 29 hospital laboratories from the PLNDI reported positive cases for at least one RV [influenza, SARS-CoV-2, respiratory syncytial virus (RSV); parainfluenza virus (PIV); adenovirus (ADV); human coronavirus (HCoV); rhinovirus/enterovirus (HRV/HEV); Metapneumovirus (HMPV) and Bocavirus (BoV)]. Proportion of each RV was calculated by age group (0-4; 5-14; 15-29; 30-64; 65-79; ≥80).

Results: A total of 36,651 positive-cases for RV were reported [10,505 for 0-4; 4,636 for 5-14; 2,310 for 15-29; 5,873 for 30-64; 4,291 for 65-79 and 5,036 for ≥80 years old (yo)]. Influenza and RSV were the most frequently detected viruses, representing 55% and 18%, respectively. Among children ≤4yo, influenza and RSV accounted for more than 60% of the cases. In children 5-14yo, young adults (15-29yo) and adults between 30-64yo the influenza accounted for ≥70% of cases of respiratory infections. In these age groups HRV/HEV are the second most frequent virus (7-10%). Among the elderly, ≥65yo a decreased proportion of influenza and an increase of RSV and SARS-CoV-2 were detected. In individuals aged ≥80, influenza and RSV were detected in similar proportions (45% and 30%, respectively) followed by HRV/HEV (10%) and SARS-CoV-2 (9%).

Conclusion: Respiratory viruses showed distinct age-specific patterns. Children ≤4yo had the highest burden, mainly due to influenza and RSV. Influenza predominated in school-aged children and adults <30yo, while RSV and influenza contributed similarly in older adults, especially ≥80yo. SARS-CoV-2 mainly affected adults and the elderly. Despite differences in testing strategy, understanding these patterns may improve risk stratification, clinical management, and vaccination strategies.

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Construction and Application of an Effective In vivo Drug Efficacy Evaluation Model for Anti-HBV Compounds Using HBV-Producing Cells Transplanted into TK-NOG Mice

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Background: Animal models are indispensable for the development of hepatitis B virus (HBV) therapeutics. However, conventional humanized liver chimeric mice require complex procedures, incur high costs, and involve long durations to establish viremia. We aimed to develop a rapid, simple, and cost-effective in vivo HBV replication model to streamline the evaluation of anti-HBV drug candidates.

Methods: We established a liver chimeric model by transplanting Hep38.7-Tet cells (tetracycline-regulated HBV production) into the livers of immunodeficient TKNOG mice via splenic injection. Engraftment and viral production were monitored via serum human albumin (hAlb) and HBV DNA levels. For drug discovery, we utilized a Spike AD assay targeting HBc-HBc interactions to screen for candidates. This identified the seed compound CBT-G1, which was structurally optimized into CBT-G3. The pharmacokinetic (PK) profile of CBT-G3 was analyzed using ¹⁴C-labeled PET imaging and plasma concentration measurements in ICR mice. In vivo efficacy was evaluated by comparing CBT-G3, GLS-4 (core inhibitor), and entecavir (ETV) via oral administration.

Results: The Hep38.7-Tet transplantation model established viremia within 14 days, approximately one month faster than the conventional natural infection model. CBT-G3 demonstrated superior in vitro antiviral activity and metabolic stability. ADME analysis confirmed favorable bioavailability and sustained plasma concentrations. In the in vivo efficacy study, CBT-G3 significantly reduced serum HBV DNA by day 18 with potency comparable to ETV, strongly correlating with results from natural infection chimeric models.

Conclusion: This HBV-producing cell transplantation model provides a robust platform for the preliminary in vivo evaluation of HBV therapeutics. By bypassing the infection process, it allows for accelerated efficacy assessment. This system is particularly effective for compounds targeting the late stages of the HBV life cycle, offering a next-generation screening platform that significantly reduces time and economic barriers.

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Local interferon-mediated immune signatures in the Nasopharynx following SARS-CoV-2 infection and their association with clinical severity

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Introduction/Aim: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is characterised by disruption of interferon (IFN) signaling through suppression of both IFN production and interferon-stimulated gene (ISG) induction, which represent key mediators of the innate immune response. Adequate IFN- and ISG-mediated antiviral activity in the upper respiratory tract is crucial for limiting the replication and spread of respiratory viruses. In the present study, we investigated whether clinical outcomes of coronavirus disease 2019 (COVID-19) can be predicted from ISG expression profiles in nasopharyngeal (NP) samples from patients with mild and severe disease.

Material: Residual NP swab samples were obtained from individuals undergoing SARS-CoV-2 testing, including non-hospitalized patients with mild symptoms managed at home without follow-up, as well as hospitalized patients with severe disease, with a focus on the early stage of viral infection.

Methods: Multiplex real-time polymerase chain reaction assays were performed using gene-specific probes targeting the conserved N1 region of the SARS-CoV-2 nucleocapsid (N) gene, alongside the IFN-associated genes CXCL10 and MX1, to assess their relative expression levels within the interferon signaling pathway.

Results: MX1 relative expression was significantly elevated in patients with mild symptoms compared to those with severe disease ($p = 0.0496$), indicating a potential protective role. In contrast, CXCL10 expression levels did not differ significantly between groups.

Conclusions: Despite the modest sample size, our findings highlight upregulation of interferon-stimulated genes, including MX1 and CXCL10, in the nasopharynx during SARS-CoV-2 infection, with MX1 expression linking to milder disease outcomes and warranting further investigation.

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Construction of a Humanized Liver Mouse Model of Echovirus 11 Infection and Elucidation of Hepatocyte-Specific Pathogenesis

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Introduction: Echovirus 11 (E11) infection is usually self-limiting in adults but can cause severe hepatitis in neonates. Existing in vivo models rely on IFN-deficient mice expressing the human E11 receptor, limiting investigation of hepatocyte-specific disease mechanisms. This study established a humanized liver mouse model of E11 infection and characterized pathogenic responses in human hepatocytes in vivo.

Materials and Methods: Immunodeficient human liver chimeric mice (TK-NOG) were inoculated with E11. Viral RNA in serum, faeces, and tissues was quantified by qRT-PCR. Liver transcriptomes were analysed by RNA sequencing with strict filtering for human hepatocyte-derived transcripts.

Results: E11 infection increased circulating viral RNA, peaking at 10 days post-infection. Pegylated interferon (pIFN) treatment initiated at 24 days post-infection significantly reduced serum E11 RNA levels. Transcriptome analysis showed broad suppression of hepatocyte metabolic pathways, including mitochondrial and energy metabolism, together with activation of antiviral signalling. Despite activation of upstream sensing pathways, interferon-stimulated gene (ISG) responses remained incomplete, indicating insufficient endogenous IFN production. This impaired antiviral response was associated with metabolic and translational suppression, suggesting a cellular state that limits effective IFN induction. Exogenous pIFN restored robust ISG expression, indicating preserved IFN responsiveness despite defective endogenous activation.

Conclusions: We established the first humanized liver mouse model of E11 infection, recapitulating sustained viral replication in human hepatocytes. The model identifies insufficient endogenous IFN responses and metabolic reprogramming as key pathogenic features and provides a platform for investigating E11-associated liver disease and evaluating antiviral therapies.

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Laboratory performance of the molecular detection of influenza A and B viruses in the Portuguese National External Quality Assessment Program, 2011–2025

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Introduction/Aim: This study evaluates the performance of laboratories participating in the Portuguese National External Quality Assessment Program (PNAEQ) for molecular detection, typing, and subtyping of influenza A/B, in collaboration with the National Reference Laboratory for Influenza Virus and Respiratory Viruses (NRLIVRV).

The study aims to evaluate laboratory capacity to detect seasonal and potentially pandemic influenza viruses.

Materials and Methods: Participants were Portuguese laboratories from public hospitals, academic and outpatient institutions, and biotechnology companies.

Annual assessment included 5 control and 3 optional samples (until 2022), prepared by NRLIVRV; in 2025, an additional influenza A(H5N1) sample was added. Laboratories performed RT-PCR typing and subtyping, and qualitative results were compared with expected outcomes.

Descriptive and accuracy rate analyses were performed for 8 high-influenza years (2011–2019, excluding 2012) and across 3 periods: pre-pandemic (2017–2019), pandemic (2020–2022), and post-pandemic (2023–2025).

Results: Participation was high (97%), with a slight decrease during the pandemic (95%). Among 2060 typing results, accuracy was 97.4%, with low rates of incorrect (1.9%) and invalid (0.6%) results. Errors included false negatives (0.87%), typing errors (0.68%) and false positives (0.39%). Accuracy was higher for Influenza A (98.2%) than for Influenza B (94.4%). Subtyping performance was robust, with 97.9% accuracy for A(H1N1) pdm09 and 98.4% for A(H3N2). Overall performance was stable across periods (98.3–99.2%). For the influenza A(H5N1) all laboratories correctly identified influenza type and 3 laboratories performed H5 subtyping.

Conclusions: Results show sustained diagnostic reliability and consistency across laboratories over 15 years, with high performance even during periods of increased influenza activity and no significant impact from the COVID-19 pandemic. Although most laboratories successfully detected non-seasonal viruses, broader typing capacity is needed to identify emerging strains with pandemic potential.

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Herpes simplex virus reactivation after liver transplantation: a single-centre early post-transplant experience

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Introduction: Herpes simplex virus (HSV-1/2) reactivation may complicate the early post liver transplantation period, but its frequency and clinical significance remain uncertain. Despite this, there is a growing body of evidence to support the use of prophylactic antiviral therapy to prevent significant herpes virus infection in solid organ transplantation. We describe experience in a single-centre.

Method: We performed a retrospective review of all liver transplant recipients transplanted 2024 and 2025 inclusive.

Results: A total of 227 recipients were included. Recipient HSV serology was available in all cases; 169/227 (74%) were HSV-1/2 seropositive before transplantation. Donor HSV serology was unavailable as it was not routinely performed. Overall, 39/227 recipients (17%) underwent HSV PCR testing after transplantation. 8/39 had detectable HSV DNA, representing 4% of the total cohort. HSV viraemia was observed at a median of 16 days (IQR 7–20) post-transplant. All 8 recipients were HSV IgG-positive pre-transplantation; none were eligible for CMV prophylaxis with valganciclovir. Clinical presentations included HSV-1 lip ulceration (n=3), HSV-2-associated rash (n=1), and ICU respiratory presentations with HSV-1 in BAL confirmed, with or without viraemia (n=4). Aciclovir was commenced where indicated. Outcomes were favourable, except one fatality associated with HSV-1 pneumonitis.

Conclusions: In this single centre cohort, HSV reactivation was uncommon but clinically relevant in the early post-transplant period, strictly observed in HSV-seropositive liver recipients who were not eligible for CMV prophylaxis. These findings have supported a change in local practice to introduce aciclovir prophylaxis in the early transplantation period. Plans for donor/recipient HSV characterisation during transplant assessment are in inception.



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The 1st International Standard for anti-CHIKV antibodies correlate of immune protection in vivo

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Introduction: The development of Chikungunya virus (CHIKV) vaccines has encountered many challenges as the sporadic nature of CHIKV outbreaks make efficacy endpoint-driven clinical trials challenging. Currently, neutralisation potency data is used for regulatory approval of CHIKV vaccines. Establishing the anti-CHIKV antibody level needed for protection against CHIKV disease would support CHIKV vaccines through regulatory approval and provide guidance for immunisation regimes.

The 1st WHO International Standard for CHIKV neutralising IgG (1502/19) is endorsed for the quantification of neutralisation assays in International Units (IU). Here we investigated whether 1502/19 had capacity to confer protection against CHIKV challenge in vivo and define the threshold of protection, thus augmenting the value of 1502/19.

Methods: Cynomolgus macaques were administered neat or a dilution of 1502/19 and subsequently challenged, intra-dermally, with 10e5 infectious units of CHIKV isolate LR2006 – OPY1. Viremia was followed by RT-qPCR and de-novo synthesis of anti-CHIKV IgM and IgG quantified by binding assays. Furthermore, titres of CHIKV neutralising antibodies were quantified using a CHIKV glycoprotein pseudotyped virus assay and expressed in IU.

Results: All control animals became infected post-challenge and de-novo synthesis of anti-CHIKV IgM and IgG were detected in blood. Furthermore, all control animals generated a neutralising antibody response after infection. In contrast, animals administered with undiluted 1502/19 showed no CHIKV viremia and no anti-CHIKV de novo synthesis. Interestingly, animals administered with diluted 1502/19 (1:10 or 1:100), had titratable levels of protection.

Conclusion: These data provide the basis of a correlate of protection, this study demonstrates that animals with over 10 IU/mL of anti-CHIKV were protected and therefore facilitates a correlate of protection, to support vaccine development.

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Going further in HCV infection screening: real-life data of reflex HCV RNA testing at Bordeaux University Hospital

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Introduction: Hepatitis C virus (HCV) causes 240,000 deaths each year among 71 million infected people worldwide. More than 10 years have passed since the advent of very potent direct antiviral agents (DAA) for HCV eradication. Yet the French liver study society (AFEF) estimated that 79,000 individuals were still to-be-treated in 2023 in France. Several factors contribute to this glass ceiling, such as the lack of an efficient screening. Here we present real-life data of the implementation at Bordeaux University Hospital of national (ANRS-MIE, 2023) recommendation of a reflex HCV RNA testing for new HCV infection diagnoses.

Methods: From November 2025, each new diagnosis of HCV infection, defined as the first positive HCV antibody (Ab) assay (HCV-Ab; Architect platform, Abbott), triggered a reflex HCV RNA assay on leftover sera samples (cobas x800 platform, Roche). Results of these assays were retrospectively compiled.

Results: Since reflex HCV RNA testing was implemented (6-months period), 13,855 HCV-Ab assays were performed. Among 286 (2.1%) positive samples, 161 (56.3%) were not yet known for HCV infection, among which 107 (66.5%) had no concomitant HCV RNA prescription and were thus eligible for reflex HCV RNA. HCV RNA was positive in 18 of them (16.8%, median viral load 6,14 log₁₀ UI/mL [5.51–6.89]) and could not be performed in 31 (29%, mainly: insufficient sera sample).

Conclusions: Reflex HCV RNA testing helps to provide complete virological status in newly diagnosed patients. It allowed diagnosis of active HCV infection in 18 patients (16.8% of Reflex-eligible patients, 6-month period). Additional data will be prospectively collected to further explore the impact of this strategy, but it already appears as be a promising approach to extend populational screening and optimize access to DAA therapy to pursue HCV elimination.



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Broad-Spectrum Antiviral Activity of Red Algae Extracts Against Coxsackievirus A12 and a Lentiviral Vector

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The discovery and development of new antiviral drugs are urgently needed, mainly for the treatment of high mortality/morbidity viral diseases. In recent years there has been an increasing interest in natural substances, such as marine algae, as a potential source of antimicrobial agents because of their several benefits, namely, their presence in nature, their multiple targets, minor side effects, low potentials to cause resistance and low cost. We aimed to study the *in vitro* antiviral activity of extracts derived from two red algae species collected on the Portuguese coast: *Chondracanthus teedei* var. *lusitanicus* (tetrasporophyte and female gametophyte life stages) and *Osmundea pinnatifida* against Coxsackievirus A12 (CVA12) and a lentiviral vector (LV) model.

Extracts obtained from both red algae demonstrated significant *in vitro* antiviral activity at concentrations that did not induce cytotoxic effects. The tetrasporophyte-derived extract of *Chondracanthus teedei* showed virucidal activity against CVA12, with the data suggesting interference at multiple stages of the viral replication cycle, in addition to promoting an antiviral state in HEK-293T cells against LV infection. The female gametophyte extracts primarily targeted the early stages of CVA12 and LV infection and also exhibited a strong virucidal capacity. Among the tested samples, *Osmundea pinnatifida* displayed the most pronounced antiviral activity against both viral models. Collectively, these results underscore the therapeutic potential of red algal extracts as antiviral agents and support the need for further *in vivo* investigations.

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Priority Zoonotic and Vector-Borne Pathogens in Kosovo and Strengthening National Preparedness

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NIPH

Kosovo, despite being a small country in the Western Balkans, remains vulnerable to several emerging and re-emerging infectious diseases of major public health importance. Geographic position, climate, migration, human-animal interactions, and vector distribution contribute to the circulation and spread of zoonotic and vector-borne pathogens. Among the most significant pathogens identified in Kosovo are Crimean-Congo Hemorrhagic Fever, Hantavirus Infection, and West Nile Fever.

Kosovo is considered endemic for CCHF, particularly in central and southwestern regions, where several outbreaks have been reported since 1989. Hantavirus infections continue to occur sporadically, while the risk of West Nile virus transmission is increasing due to the presence of mosquito vectors and regional circulation in neighbouring countries. Other zoonotic diseases, such as Brucellosis, Leptospirosis, and Tularemia, also remain important public health concerns.

To strengthen preparedness and response capacities, Kosovo has developed several strategic and legal frameworks, including the Law on Prevention and Control of Communicable Diseases, the National Action Plan on Antimicrobial Resistance "One Health" 2025–2028, and the Strategic National Laboratory Plan 2024–2030. In addition, the establishment of multisectoral One Health coordination mechanisms has improved collaboration between the human health, veterinary, food safety, and environmental sectors for surveillance, early warning, and outbreak response.

Strengthening integrated surveillance systems, laboratory capacities, risk assessment, and intersectoral cooperation remains essential for improving the detection, prevention, and control of infectious disease threats in Kosovo.

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Engineering Broadly Neutralizing Nanobodies Against Dengue Virus: Cross-Serotype Potency at Subnanomolar Levels

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Introduction/Aim: Dengue virus (DENV) poses a major global health threat, with no specific antivirals and limited vaccination options, highlighting the need for effective broad-spectrum therapies. Nanobodies are small, stable, and easily engineered antibody fragments with strong antiviral potential and a reduced risk of antibody-dependent enhancement. This study aims the development of pan-serotype neutralizing nanobodies against the four DENV serotypes.

Material & Methods: Nanobodies were generated from immunized alpaca phage-display libraries, screened by phage ELISA, sequenced, expressed in *E. coli*, and isolated from periplasmic extracts. Neutralizing activity was assessed against a DENV-2 infectious clone and clinical isolates from all four DENV serotypes in Vero E6 and Huh 7.5 cells using immunofluorescence staining, while binding was characterized by bio-layer interferometry.

Results: Screening identified two monovalent nanobodies with potent inhibitory activity against DENV-2. Binding analyses confirmed high-affinity recognition of distinct epitopes on the viral envelope protein. We therefore engineered bivalent formats by pairing the two candidates and evaluated their neutralizing activity across DENV serotypes. The constructs demonstrated broad cross-serotype neutralization, reaching low-nanomolar to subnanomolar potency and showing substantially improved activity compared with the corresponding monovalent formats.

Conclusions: We generated, screened, and optimized a highly potent cross-neutralizing nanobody with broad activity against DENV, highlighting its promise as a next-generation antiviral platform for dengue virus therapeutics.

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Subtype-associated variability in HIV drug resistance EQA performance is evident in reverse transcriptase and integrase datasets

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Introduction/Aim: Accurate interpretation of HIV drug resistance (HIV-DR) is essential for guiding antiretroviral therapy. Beyond viral load, performance variability may be influenced by viral diversity, genomic region, and sequencing workflows. External quality assessment (EQA) schemes provide a framework to evaluate inter-laboratory consistency across protease/reverse transcriptase (PR/RT) and integrase (INT).

Materials and Methods: We analysed 2023-25 EQA datasets (n=1055) stratified by subtype (A/G, B, C and D; n=12, 10, 3 & 4, respectively), genomic region (PR/RT and INT), and sequencing methodology (Sanger vs NGS). Performance was assessed using participant scores for successful detection of drug resistance mutations. Max scores were 36, 32 and 25 for PR, RT and INT. 61% of participants used a Sanger technology while 39% used NGS workflows.

Results: Successful sequence rate exceeded 95% across all datasets. In PR/RT datasets, mean scores were comparable across subtype B and non-B viruses (PT 34.62, v 34.56; RT 31.46 v 30.67), although subtype C showed lower RT scores in NGS than Sanger datasets (29.06 v 30.71). In contrast, greater variability was observed in integrase datasets, where subtype C showed consistently lower mean scores than subtypes B, AG and D across both Sanger (23.90 v 24.52, 24.56, 24.73) and NGS workflows (23.60 v 24.32, 24.78, 24.74). Subtype-associated variability was observed in both RT and integrase datasets but was more consistent in integrase across sequencing workflows.

Conclusions: HIVDR EQA performance varied across sequencing methods and genomic regions. Subtype-associated variability was observed in both RT and integrase datasets, with lowest scores observed in subtype C. These findings highlight the importance of considering subtype diversity when evaluating HIV drug resistance testing performance.

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Long-read sequencing reveals extensive HBV-DNA integration capable to sustain HBsAg production and HDV persistence

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Background and Aim: HDV relies on HBsAg for its infectivity. HBsAg transcripts derive from cccDNA and from integrated double-stranded linear HBV-DNA. Here, we evaluated intrahepatic HBV/HDV reservoir and the contribution of HBV integration in sustaining HBsAg production and HDV activity.

Methods: 70 liver biopsies from eAg- patients (pts) were included: 35 with chronic hepatitis delta (CHD) and 35 with chronic hepatitis B (CHB). Droplet-digital PCR quantified intrahepatic HBV/HDV markers. In 19 CHD pts, long-read DNA sequencing by Nanopore assessed HBV integrations. RNA-Seq evaluated viral gene expression.

Results: In CHD, median(IQR) cccDNA, pgRNA and cccDNA-derived HBsAg transcripts were 1(0.02-12), 8(1-147) and 1(0.2-41)copies(-cps)/1000cells, all >1log lower than in CHB (p<0.001). HDV-RNA was 787cps/1000cells. cccDNA strongly correlated with HBV markers but not with HDV-RNA (p=0.6).

Despite limited intrahepatic HBV reservoir, >1 HBV-DNA integrant was detected in 14/19 of CHD pts. Notably, 10/14 CHD pts carried S-integrants. S-integrant carriers had high levels of integrated-derived HBsAg transcripts (median[IQR]: 32,410[16,733-53,180]cps/1000cells), absent in pts without HBV-integrants. S-integrant carriers had also higher serum HBsAg (4.3[4.0-4.4] vs 3.7[3.5-3.9]logIU/ml, p=0.04). By AUROC, HBsAg >4logIU/ml predicted the occurrence of S-integrants with the best accuracy (75% sensitivity; 75% specificity).

Finally, in 7/10 pts, S-integrants localised in 26 human genes regulating cell proliferation, most of which (N=21) known to dysregulate hepatocytes proliferation.

Conclusion: This study provides direct evidence that S-integrants sustain massive HBsAg production, supporting HDV persistence. High serum HBsAg (>4 logIU/ml) may reflect an enrichment of HBV-DNA integration. Localization of integrants in proliferation-related genes suggests a role in hepatocarcinogenesis.

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Genomic Epidemiology of Mpox in Norway, 2022–2025

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Introduction: The 2022 global mpox outbreak prompted sustained surveillance in Norway. Whole-genome sequencing (WGS) of mpox virus (MPXV) cases in Norway between June 2022 and December 2025 was performed to characterise MPXV genomic epidemiology.

Materials and Methods: WGS was performed on 106 of 132 MPXV cases collected between June 2022 and December 2025 using Nanopore and Illumina. Genomes with ≥70% coverage underwent clade/lineage assignment with Nextclade. Sequences were placed in a global clade IIb phylogeny using USHER. Maximum-likelihood phylogenies and APOBEC3 signatures were analysed with SQUIRREL, and multiple within-patient sequences were compared using SNP distances.

Results: High-quality genomes were obtained from 90 of 106 sequenced cases (85%), and all belonged to clade IIb. In 2022, B.1 (47%) and B.1.10 (16%) were most common. Norwegian B.1 genomes were highly similar to co-circulating international sequences, limiting discrimination between local transmission chains from repeated introductions; although clustered B.1 and B.1.10 genomes were consistent with sustained domestic spread. From 2023 onwards, lineage diversity increased, with E.1, E.2, and E.3 detected in 2023; E.1.1 accounting for 90% of cases in 2024; and E.3.1, F.4.1, and G.1 detected in 2025. At least 34 introductions were inferred, most without onward transmission. Within-host diversity was low in 22 of 23 patients with multiple samples (17 with 0 SNPs, 5 with 1–2 SNPs); one patient showed highly divergent lineages (49 SNPs) consistent with co-infection.

Conclusions: Genomic surveillance of mpox in Norway revealed evidence of domestic spread in 2022, followed by a transition to repeated introductions of increasingly diverse lineages. Within-host diversity was low in most patients, while one probable co-infection indicates that mixed infections can occur but are uncommon. These findings highlight the value of sustained WGS surveillance for tracking lineage diversity and informing public health response.

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Correlation of seroprevalence and molecular-genetic detection of hepatitis E virus in suspected patients

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Introduction/Aim: Viral hepatitis E presents a serious global health problem caused by the hepatitis E virus (HEV). Previously, HEV infection was localized in developing countries with low hygiene standards. However, viral hepatitis E has recently increased in industrialized countries related to HEV as foodborne pathogen. The aim of this study was to observe the correlation between anti-HEV antibodies and the occurrence of hepatitis E virus in hospitalized patients and also to search for the source of HEV.

Material: Biological samples (serum, stool) of human patients (n=99) were obtained from HEV suspected patients from Slovakian hospitals with informed consent of all patients and completed questionnaire about possible hepatitis E risk factors. Patients with different levels of anti-HEV antibodies were considered suspected.

Methods: Detection of anti-HEV total antibodies was done using ELISA kit (DRG Instruments GmbH). Total RNA was extracted by QIAamp Viral RNA Mini kit (Qiagen). HEV RNA was detected by real-time RT-PCR and nested RT-PCR. PCR products of partial ORF1 were sequenced by Sanger sequencing. MEGA X was used for phylogenetic analysis of HEV sequences.

Results: All hospitalized patients showed anti-HEV IgM antibodies in the range 1.1-23.6 IU/mL and anti-HEV IgG 1.8-25 IU/mL. HEV RNA was detected in 45/99 (45.5%) of stool and/or serum samples. Phylogenetic analysis of human HEV sequences based on partial ORF1 showed that all of them belonged to genotype HEV-3 mostly in genetic group 1. Previously our studies showed HEV sequences obtained from food-producing domestic and wild-living animals were grouped mostly in HEV-3 group 2.

Conclusions: Seroprevalence and molecular-genetic detection of HEV and observation of their correlation showed differences depending on the stage of viremia. The most human HEV sequences were clustered in different phylogenetic groups than HEV sequences from food-producing animals considered sources of HEV. The study was supported by the VEGA 1/0220/24.

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The level of Neutrophil Extracellular Traps and DNASE1 polymorphism in STEMI patients with previous SARS-CoV-2 infection

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Introduction: Previous studies of post COVID-19 disease show that neutrophil extracellular traps (NETs) have ability to persist after SARS-CoV-2 infection. NETs clearance is important for the balance between NETs formation and degradation and plays a crucial role in tissue homeostasis. Recent studies have shown that endonucleases known as DNases are particularly important for the regulation of NETs by degrading the extracellular DNA that forms the basis of this structure to prevent excessive inflammatory reactions. This study aimed to explore the association between DNASE1 polymorphism and NETs formation in STEMI patients with previous SARS-CoV-2 infection.

Material and Methods: Patients' blood and plasma samples were used in this study. Patients with acute MI (AMI) and with a history of COVID-19 disease were enrolled in the experimental group. The control group comprised patients who had previously tested negative for COVID-19 disease. Single-nucleotide polymorphism (SNP) of the DNASE1 (rs1053874) was evaluated using real-time PCR with TaqMan® SNP Genotyping Assay. Quantitative ELISA method and quantitative fluorescence spectroscopy method was used to measure the concentration of NET biomarkers. The difference was considered statistically significant at the value of $P < 0.05$.

Results: Slow metabolizing allele G of DNASE1 was more frequent ($P = 0.022$) in the patients with STEMI after COVID-19 compared with control group. Univariate logistic regression analysis revealed that platelets count ($P = 0.025$), dsDNA count ($P < 0.001$), GG genotype ($P < 0.004$), and high NETs level (vs lower) were significantly associated with higher odds of prior COVID-19 among STEMI patients, meanwhile MPO ($P = 0.025$) and GA or AA ($P = 0.025$) genotype were associated with decreased odds.

Conclusions: The DNASE1 and NETs level interaction analyses show that NETs burden may depend on DNASE1 genotype and could be associated with odds of previous SARS-CoV-2 infection among STEMI patients.

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HSV infection prevention practices in solid organ transplantation: A survey of francophone centres.

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Introduction/Aim: Herpes simplex virus (HSV) infections remain a concern in solid organ transplant (SOT) recipients and may lead to severe complications. In contrast to cytomegalovirus (CMV), for which prevention strategies are well established, HSV prevention recommendations vary across guidelines. We conducted a survey to describe current clinical practices regarding HSV prevention in SOT recipients across francophone transplant centres.

Material/Methods: A web survey was conducted among healthcare professionals involved in SOT management. The questionnaire (30 items) addressed respondent characteristics, CMV prevention strategies, HSV prophylaxis and monitoring. Responses were collected anonymously between 13 October 2025 and 13 February 2026 and analysed at the respondent level.

Results: A total of 103 responses (43 centres) from 7 francophone countries were included, mainly from France (n=92). HSV prophylaxis was reported by 41.7% (n=43) of respondents and varied by specialty (27/52 in nephrology, 4/30 in hepatology, 3/7 in cardiology). Indications for HSV prophylaxis varied and were most commonly based on the absence of CMV prophylaxis (17/43, 39.5%) or on donor/recipient pretransplant serological status (19/43, 44.2%). Donor HSV serology was rarely used, with 51.2% (22/43) of cases in which it was never requested. HSV prophylaxis also varied in terms of dosing regimen, timing of initiation and duration. Valaciclovir 500 mg twice daily was most common, typically initiated within the first week after transplantation for 1-3 months. Monitoring strategies were almost exclusively based on clinical assessment (n=100, 97.1%).

Conclusions: HSV prevention practices in SOT across francophone transplant centres are heterogeneous, reflecting the lack of consensus. In the era of letermovir-based CMV prophylaxis, which does not cover HSV, unlike valganciclovir, these findings highlight the need for dedicated guidelines on HSV prevention to harmonize clinical practices in SOT recipients.

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Assessing the impact of EBV latent protein variants on HLA-E-mediated NK cell regulation in Multiple Sclerosis

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Introduction/Aim: EBV infection is strongly associated with MS, but mechanisms linking EBV genetic variation to altered antiviral immune control remain unclear. We investigated whether sequence variation in EBV latent proteins affects HLA-E-mediated immune recognition and whether this differs between MS- and healthy control (HC)-associated EBV variants in a Cypriot cohort.

Methods: Based on previously identified MS- and HC-associated EBV variants, latent proteins LMP1, EBNA2, and EBNA3B were selected for in silico prediction of 9-mer peptide binding to HLA-E01:01 and HLA-E01:03 using NetMHCpan 4.2. Binding was validated by HLA-E stabilization assays in K562/HLA-E target cells, followed by assessment of NKG2A γ NK cell inhibition and CD8 γ T cell responses. The cohort was stratified by HLA-E genotyping and EBNA1-specific IgG responses.

Results: All tested EBV peptides significantly upregulated HLA-E surface expression on both HLA-E variant-expressing cell lines, confirming HLA-E stabilization. No significant differences in peptide-mediated HLA-E stabilization were observed between the HLA-E allelic variants. Functional NK cell assays showed that the tested peptides suppressed NKG2A γ NK cell degranulation, indicating that EBV-derived HLA-E ligands engage the inhibitory HLA-E/NKG2A axis irrespective of the target cell line. To date, HLA-E genotype frequencies did not differ significantly between MS and HCs, suggesting HLA-E genotype alone is unlikely to account for disease-associated differences in this setting.

Conclusions: EBV peptides function as HLA-E ligands capable of inhibiting NKG2A γ NK cell responses, supporting a potential role for the HLA-E/NKG2A pathway. Although no differences between HLA-E allelic variants have been observed so far, ongoing work will assess whether MS- versus HC-associated EBV variants affect NK cell inhibition or CD8 γ T cell recognition. Integration of EBNA1 IgG titres, HLA-E genotype, and functional immune readouts may clarify how viral and host factors shape EBV-specific immune responses relevant to MS susceptibility.



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Multi-Laboratory Evaluation of the HIV-1 International Standard (NIBSC 16/194) for NGS based resistance testing

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Introduction: Harmonisation of HIV molecular testing remains a critical challenge, particularly in the era of NGS, where assay variability can impact clinical interpretation. This study evaluates the WHO HIV-1 RNA International Standard (NIBSC 16/194) as a reference material to assess inter-laboratory NGS concordance across Europe. We aim to determine the impact of wet-lab and bioinformatic pipelines on HIV RNA resistance testing and minority variant detection to inform harmonisation efforts.

Methods: Seven European laboratories sequenced 16/194 twice using their clinical assay routines, providing assay details and sequencing files. To eliminate bioinformatics differences, raw sequence data was re-analysed using a centralised pipeline. Sequencing depth and variant frequencies were extracted. Wet-lab parameters were correlated with variant detection success.

Results: Reported DRMs include: D67N/L210W/T215D/T69D(NRTIs), K103E/V108I(NNRTIs) and V82A(PI). All 7 labs identified subtype B and high frequency presence of D67N and T69D. Four labs detected V108I, L210W, T215D (9-38% frequency). Results from the labs own bioinformatics pipelines and the standardised pipeline built for this study were highly concordant, therefore differences are primarily wet-lab processes. Analysis of the wet-lab methodologies identified a strong correlation between failure to detect minority variants and pre-extraction sample dilution. The 3 labs that did not detect the minority variants diluted the standard prior to extraction (range 1:3 - 1:10). We are continuing to interrogate the data to establish the exact mechanism of this dropout.

Conclusion: The WHO HIV-1 RNA International Standard (NIBSC 16/194) represents a valuable reference for comparison of HIV NGS assays. Differences between the performance of laboratories appear to be due to pre-extraction dilution.

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A novel Human papillomavirus typing assay utilising next-generation sequencing

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Introduction/Aim: Current clinical Human papillomavirus (HPV) assays provide limited subtype identification despite increasing interest in HPV epidemiology, vaccine-associated shifts in subtype prevalence, and the clinical significance of mixed HPV infections. The Roche Cobas® HPV assay individually identifies HPV16 and HPV18 but reports 12 additional high-risk HPV (HR-HPV) types collectively. This study aimed to provide proof of concept for a next-generation sequencing (NGS)-based HPV typing assay capable of identifying a broader range of HPV types.

Methods: Residual genital swab samples collected in PreservCyt® medium and previously tested using the Cobas® HPV assay were analysed. DNA extraction was performed using the QIAamp MinElute Virus Spin Kit (Qiagen). Broad range consensus HPV PCR amplification (PGMY09/PGMY11) was performed and amplicons underwent library preparation using the Nextera XT DNA Library Preparation Kit (Illumina) prior to sequencing on the Illumina MiSeq platform. Sequencing reads were analysed against reference HPV genomes for typing and compared with Cobas® results.

Results: A total of 13 patient samples were analysed. NGS successfully identified HPV genotypes in all samples and enabled specific typing of samples classified as "other HR-HPV positive" by Cobas®. Multiple HPV co-infections were frequently identified. In addition to HPV types detected by Cobas®, sequencing identified several additional HPV types not detected by routine screening. While most were low-risk HPV subtypes, HPV53 and HPV82, both classified as possible high-risk HPV types, were also detected.

Conclusions: NGS-based HPV typing demonstrated broader genotype detection capability than Cobas® testing and successfully identified mixed HPV infections. This approach may improve HPV epidemiological surveillance and enhance understanding of HPV co-infection patterns. Further validation using larger sample cohorts is required.



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Identification of Circulating Virome in Children with Severe Acute Hepatitis of Unknown Origin

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Aim: Severe acute hepatitis of unknown origin (SAHUO) in children is a major clinical concern with most cases explained by classical hepatotropic viruses, while some remain unexplained. This study aimed to characterize the circulating DNA virome in children with SAHUO from Southern India.

Methods: 26 children with acute hepatitis, negative for hepatitis A and E viruses and showing elevated AST and ALT levels, were enrolled and 43 subjects from Cebriá-Mendoza et al. (2021) study served as controls. Plasma DNA was extracted, amplified using multiple displacement amplification, and subjected to whole-metagenome shotgun sequencing using Illumina NovaSeq 6000. Viral sequence analysis was performed using sequential bioinformatic pipelines and R (v4.6.0). Statistical analyses were conducted in R.

Results: Viral reads constituted 2–4% of sequences in cases, whereas controls showed <0.01%. Cases harbored several pathogenic or potentially pathogenic DNA viruses, including CMV, HSV, Polyomavirus, Parvovirus, and Rubella virus. Additionally Orthohepadnavirus was detected in 2 cases, while Alphapolyomavirus, Gemykibivirus, Lipsivirus, and Rubivirus were identified in >5cases. In contrast, controls predominantly contained non-pathogenic virome components such as anelloviruses and bacteriophages. Only 4/43 controls showed pathogenic viruses, mainly Lipsivirus and Mimivirus. Alpha diversity analysis revealed greater viral diversity in cases, while beta diversity analysis demonstrated clear clustering differences between groups. LEFSe analysis identified pathogenic viral taxa as key discriminators. Correlation analyses also showed associations between bacterial taxa and pathogenic viral taxa, suggesting altered virome–microbiome interactions in affected children.

Conclusion: Metagenomic profiling revealed a distinct circulating virome in children with SAHUO.

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FluTyper: A genomic surveillance tool for avian influenza viruses

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Introduction: Global surveillance of zoonotic influenza is crucial for pandemic preparedness. Continuous monitoring of the genomic evolution of Influenza A viruses (IAV) in animal and human populations is essential to identify emerging threats. FluTyper, a bioinformatics pipeline for automated genotyping and mutational characterization of IAV genomic data within a One Health framework, addresses this need.

Methods: FluTyper uses Nextflow for scalability and portability. The workflow utilizes Seqkit and MAFFT for sequence processing, and Nextclade for genotyping. A key novelty is a standardized amino acid numbering system anchored to H5N1 references, allowing consistent mutational analysis across IAV subtypes. FluTyper automates segment extraction, subtyping, pathotype inference and translation. Mutations are phenotypically characterized using the curated FluMutDB repository.

Results: FluTyper generates protein-specific profiles and graphical reports detailing mutations, subtype distributions, and clade assignments. An interactive HTML table enables dynamic visualization of genetic markers, with interactive annotations to integrate coordinates, phenotypic effects, and supporting literature. The pipeline's performance was evaluated using IAV PCR-positive samples obtained through the avian influenza surveillance plan in wild birds in Catalonia (IRTA-CReSA). Validation with nf-test confirmed its reliability for effective genomic surveillance.

Conclusions: FluTyper provides a scalable framework for genomic surveillance of zoonotic influenza viruses. By integrating automated genotyping, standardized reporting, and interactive visualization, it facilitates rapid identification of emerging variants with potential public health impact. Within a One Health context, FluTyper strengthens our capacity to respond effectively to emerging threats.

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Impaired Interferon Pathway Activation in Patients with Obesity Hospitalized for COVID-19

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The COVID-19 pandemic has highlighted obesity as an important risk factor for increased clinical severity and poorer outcomes in patients infected with SARS-CoV-2. This increased risk is associated not only with the mechanical and metabolic alterations linked to obesity, but also with its capacity to negatively modulate immune responses, particularly interferon (IFN)-mediated antiviral pathways. However, studies investigating the local expression of IFN-axis genes and interferon-stimulated genes (ISGs) in the upper respiratory tract of patients with COVID-19 remain scarce, particularly when stratified by nutritional status and disease severity. This study investigated type I and type III IFN pathway responses in individuals with and without obesity hospitalized with COVID-19 and correlated these responses with viral load, clinical parameters, and hospital outcomes. This cross-sectional study was nested within a retrospective cohort including 56 individuals hospitalized with severe or critical COVID-19, with and without obesity, as well as uninfected controls. RT-qPCR was performed on nasopharyngeal samples to quantify the expression of IFNA2, IFNL2/3, IFNB, OAS, and IFI6, as well as viral load. IFNA2 and IFNL2/3 expression was not detected in the analyzed samples. IFNB and OAS expression levels were reduced in patients with obesity, regardless of clinical severity, whereas IFI6 was overexpressed in critically ill patients with obesity. Viral load did not differ between groups. Clinically, patients with obesity showed longer hospital stays, a higher frequency of oxygen desaturation, and SOFA scores ≥ 2 at admission. These findings suggest that obesity impairs the efficient activation of the type I IFN pathway during COVID-19, potentially contributing to an inadequate antiviral response and prolonged inflammation, thereby worsening disease progression. Taken together, these data reinforce the central role of IFN signaling in controlling SARS-CoV-2 infection and highlight its dysregulation in individuals with obesity.

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Deciphering antigenic determinants of humoral immune response upon vaccination against Turkey Meningoencephalitis Virus (TMEV)

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Turkey Meningoencephalitis Virus (TMEV), an avian flavivirus of Ntaya serocomplex, is endemic in Israel and imposes a significant burden on domesticated turkey farming, causing neuromuscular disease and high mortality rates. TMEV is closely related to Bagaza virus, a flavivirus occasionally causing encephalitis in humans. Routine vaccination with a live attenuated TMEV vaccine is crucial for controlling the disease, but the currently used vaccine does not provide robust and sufficient protection to prevent TMEV outbreaks. We focus on establishment of correlates of protection to instruct assessment of vaccine efficiency and facilitate design and development of novel vaccines. Using virus neutralization test and serological immune-assays using recombinant viral antigens we aim to establish reliable correlates of protection by laboratory serological tests. We assessed the correlation between results of traditional HI and neutralization assays, as well as with ELISA using inactivated virions and TMEV recombinant proteins. This work establishes a comprehensive and improved approach for serological surveillance and evaluation, offering significant advantages for both research and routine vaccination monitoring of TMEV in turkey flocks, potentially applicable to studying Bagaza virus immunity in humans.



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Oral Ribavirin for Respiratory Syncytial Virus Infection in a Neutropenic Patient with Acute Myeloid Leukaemia: A Case Report and Review of Viral Dynamics in Immunocompromised Hosts

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Respiratory syncytial virus (RSV) infection in immunocompromised adults is associated with prolonged viral replication, high viral loads, and an increased risk of progression to lower respiratory tract infection (LRTI). As an RNA virus, it can shed in the respiratory tract for prolonged periods of time in immunocompromised patients. Antiviral treatment with ribavirin is not widely practiced, with few consensus guidelines or protocols being available.

We present the case of a 52-year-old female with acute myeloid leukaemia (AML) who developed RSV infection during chemotherapy-induced neutropenia. What was of interest was that she experienced delayed clinical deterioration with respiratory compromise occurring 7 days after she developed sore throat and was found to have RSV-B RNA detected at significant level by RT-PCR [Crossing threshold value [Ct]= 23.96 cycles], which remained at high levels with Ct value consistently <25 cycles. 7 days later she developed fever and hypoxia requiring supplemental oxygen at which point the RSV-B RNA Ct value was 23.03.

Oral ribavirin therapy was initiated, resulting in rapid decline in viral load. In 48 hrs of ribavirin, the viral load fell as shown by CT value increase to 32.83, and no longer needing supplemental oxygen. Ribavirin was stopped after 7 days with RSV-B RNA staying undetectable even 14 days later. This case highlights altered viral kinetics in immunocompromised hosts and supports the rationale for ribavirin therapy on a case-by-case basis in these high-risk patients when they deteriorate after a period of clinical latency, even as there is a gap in consensus guidelines and criteria based approach for these sets of patients.

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Long-Read Nanopore Hybrid-Capture Workflow for Viral Whole-Genome Sequencing

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Background: Whole-genome sequencing (WGS) is essential for virus characterization, surveillance, and epidemiology. Nanopore sequencing (ONT) overcomes short-read limitations by capturing complex regions and structural variants while providing real-time data analysis. Its low equipment costs and portability further support flexible deployment in clinical and public health settings. However, most ONT workflows rely on amplicon-based methods, limiting their use for large or highly diverse viruses. Hybrid capture offers a flexible alternative, providing more uniform recovery across variable or complex regions. We developed a simplified, modular ONT long-read hybrid-capture WGS workflow that enables direct sequencing from clinical samples.

Methods: We evaluated this protocol using capture panels for human cytomegalovirus, Kaposi sarcoma-associated herpesvirus, and human adenovirus, and compared its performance with existing short-read workflows. The workflow was first validated using mock samples generated by spiking human DNA with reference material, then assessed across diverse clinical samples, including stool, blood, and respiratory samples.

Results: Our workflow generated viral genomes directly from clinical samples, achieving an N50 of ~1.5 kb. Consensus sequences showed high accuracy, differing by fewer than three nucleotide changes between replicates, a level comparable to short-read approaches. Long-read data also helped resolve repetitive and complex regions that short-read methods failed to reconstruct. The ONT workflow enabled genome recovery across a range of viral loads, including samples with Ct values up to 32. It is up to 60% cheaper per sample than short-read workflows, particularly for small sample numbers, and enables near real-time sequencing without batching.

Conclusion: This workflow supports the implementation of viral WGS in clinical settings by providing a rapid, simplified, cost-effective approach that enables timely analysis for surveillance, monitoring, and infection prevention.

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Epitope-based combinations of gH/gL antibodies reveal cooperative effects in HCMV neutralization

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Introduction/Aim: Human cytomegalovirus (HCMV) poses a major health challenge in immunocompromised individuals and following congenital transmission. As no licensed vaccine is available and treatment options remain limited, antibody-based therapeutics, especially targeting the gH/gL complex, might hold potential as safe and well-tolerated treatment. However, structural data and the understanding of epitope-dependent neutralization potency remain incomplete.

Materials and Methods: We previously isolated 136 unique monoclonal antibodies (mAbs) targeting HCMV gH/gL, including highly potent neutralizers (Immunity 2023). Comprehensive epitope mapping revealed eight clusters corresponding to novel target sites on the gH/gL surface. To investigate the mode of action and deduct potential therapeutic mAb combinations, cryo-EM analysis was performed, resolving eight non-overlapping mAbs simultaneously, to combine mAbs with potentially cooperative neutralization mechanisms.

Results: Cryo-EM resolution of selected mAbs provided detailed insights into binding modes, spatial orientations, and potential neutralization mechanisms for each novel antibody and epitope tested. Remarkably, epitope-guided combinations of non-competing antibodies showed enhanced neutralization activity compared to individual mAbs. Interestingly, the combinatorial effects followed epitope-specific patterns, suggesting functional cooperativity depending on spatial arrangement of the targeted sites. These effects were consistent across different cell types and virus strains.

Conclusion: This study defines vulnerable epitopes on gH/gL and links epitope specificity to neutralization capacity of respective mAbs. Subsequent synergism studies provide valuable insights for the rational design of HCMV-vaccines and antibody-based therapies.

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Patterns of spread of various respiratory viruses, comparing pre-pandemic, pandemic and post-pandemic phases

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Aim: The covid-19 pandemic led to the implementation of non-pharmaceutical interventions. The circulation of respiratory viruses such as influenza and respiratory syncytial virus (RSV) was disrupted. The strategy in Sweden was different and despite the absence of total lockdown, the circulation of these viruses was suppressed also in Sweden. The aim of this study was to compare the transmission dynamics of six respiratory viruses across three phases: pre-pandemic, intra-pandemic, and post-pandemic.

Methods: We analysed data from respiratory samples collected at outpatient and inpatient care units in the county of Skåne between 2014 and 2025. Samples were analysed at the regional laboratory of Clinical Microbiology using various nucleic acid detection methods. Over the study period diagnostic platforms varied to include both in-house assays and commercial high-throughput and rapid PCR systems. Number of samples, the positive detection rate and seasonal peaks were analysed and compared across the three phases.

Results: Our findings show a shift in the circulation for most respiratory viruses in the intra-pandemic phase. There was a drop to near absent levels for influenza, RSV, human metapneumovirus (hMPV), parainfluenzaviruses (hPIV), and other human coronaviruses (HCoV). In contrast, the non-enveloped rhino-enteroviruses and adenoviruses continued to be detected. In the post-pandemic phase, we saw an atypical resurgence of RSV and influenza. In contrast, this pattern was less pronounced for hPIV and HCoV. These viruses returned earlier than influenza, RSV and hMPV and their overall seasonal dynamics remained largely unaltered.

Conclusion: This study shows that the pandemic caused a disruption in the transmission of respiratory viruses. Highly seasonal viruses like influenza and RSV displayed out-of-season resurgences, whereas hPIV and HCoV demonstrated a rapid return to pre-pandemic levels. The non-enveloped viruses continued to be detected through the whole intra-pandemic phase. Understanding these transmission dynamics is important for predicting future outbreaks.



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In-House Metagenomics for Febrile Returning Travellers: A Faster and more Robust Nanopore-Based Workflow

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Metagenomics (mNGS) could improve the infection diagnosis in returning travellers by reducing turnaround times and reliance on reference laboratories. Our existing Oxford Nanopore Technologies ligation sequencing kit (LSK)-based workflow, adapted from a validated method, showed good sensitivity but was costly and labour-intensive. We aimed to improve performance by transitioning to the Rapid PCR Barcoding (RPB) method and optimising extraction, reverse transcription (RT), and library preparation. Workflow modifications were assessed sequentially using spiked controls (Jonesia denitrificans, phocine distemper virus, phocine herpes virus) and a Zepmetrix panel. Read counts and organism abundance were compared with modified RT (NEB vs LunaScript/Sequenase); extraction (spin-column vs magnetic bead automation) and library preparation methods (LSK vs RPB). Performance was further assessed using UK National Measurement Laboratory control materials (MCM2VY) (Butler et al., 2025), and re-analysis of 13 residual clinical extracts.

The RT methods were comparable (mean reads 893,458 vs 338,576; PDV 11% vs 14%). Spin-columns outperformed automated extraction, which was associated with reduced viral sensitivity. RPB produced higher read output than LSK (486,764 vs 221,341) and improved viral detection in control material, particularly for adenovirus 1 (35–42% vs <1%) and 3 (17–27% vs <0.5%).

The library preparation methods were applied to MCM controls and showed comparable detection of organisms. PCR cycle variation (20–25 cycles) produced consistent profiles. Concordance between RPB and LSK in clinical extracts was high (11/13), and included detection of Anelloviridae, hepatitis B virus, dengue virus and chikungunya virus. Discrepancies were limited to low-read, near-threshold detections.

The RPB workflow provides a faster, more robust, and user-friendly mNGS method than LSK, with reduced clean-up steps and overall greater suitability for diagnostic implementation. These features make it more suitable for diagnostic implementation and scalable staff training.

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Overcoming Technical Barriers in Hepatitis Delta Virus Genotyping: an Update on the Development of a Whole-Genome Sequencing Method

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Hepatitis delta virus (HDV) has a variable genome, eight genotypes (HDV-1-8), and complex secondary structure, presenting significant challenges for molecular detection, sequencing and genotyping. This includes under-quantitation by commercial assays. Genotypes differ by global region and there is evidence of a relationship with disease progression.

We aimed to develop, optimise and validate a method for HDV whole-genome sequencing and genotyping, and overcome technical challenges associated with the complex genome. The method was applied to a London-based cohort to characterise local genotype distribution.

RNA extraction was optimised with a spin column method, and whole genomes were amplified as two overlapping fragments using a modified published method. Sequencing was performed using Illumina technology and genome assembly was achieved using in-house bioinformatics. Genotypes were assigned using phylogenetic analysis. Demographic data, including country of origin and ethnicity, were collected and compared across genotypes.

Amplification of the HDV genome was highly challenging, with specific extraction, reverse-transcription and thermocycling parameters proving essential. Rotation of assembled genomes to the correct initial site was required due to inaccurate assembly in line with non-standardised reference sequences.

Whole-genome sequences were obtained from 58 samples. Most patients (25, 73.5%) had genotype 1d infection. Rarer genotypes (including HDV-5 and HDV-6) were detected in six patients. Country of origin ($p < 0.0001$) and ethnicity ($p < 0.0001$) were strongly associated with genotype. All patients in the genotype 5/6 group were of African origin.

We successfully overcame technical challenges and developed a HDV genotyping method suitable for diagnostic implementation. However, these challenges highlight the need for standardisation and collaboration in developing external quality assessment material. There is potential to assess association between genotype, clinical progression and response to therapy.



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When SARS-CoV-2 Receded: The Re-Emergence of Respiratory Pathogens in the Post-Pandemic Era

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Introduction and Aim: Respiratory tract infections are major causes of morbidity and mortality. Since the COVID-19 pandemic, changes have been observed in the epidemiology of respiratory pathogens. This study aimed to evaluate pathogen distribution and seasonal patterns across two consecutive post-pandemic seasons.

Materials and Methods: This study included 134,547 multiplex respiratory RT-qPCR tests performed at Ankara Bilkent City Hospital during 2023–24 and 2024–25. The panel included influenza A/B, RSV, SARS-CoV-2, adenovirus, Group A Streptococcus (GAS), rhinovirus, bocavirus, seasonal coronaviruses, parainfluenza viruses 1–4, metapneumovirus, B. pertussis and M. pneumoniae. Results were analyzed weekly.

Results: A total of 66,898 and 67,649 tests were performed in 2023–24 and 2024–25, with positivity rates of 24.7% and 27.9%, respectively. The most frequent pathogens were SARS-CoV-2 (26.5%), RSV (14.1%) and influenza A (13.4%) in 2023–24; and rhinovirus (20.5%), influenza A (17.6%) and SARS-CoV-2 (15.7%) in 2024–25. Among hospitalized patients, SARS-CoV-2 remained the leading pathogen in both seasons but decreased from 35.2% to 19.6%. Among in children aged 0–5 years, RSV was most frequent in 2023–24 (23%), whereas rhinovirus predominated in 2024–25 (23.7%). Among in the 6–18 age group, GAS was most frequent in both seasons (17.2% and 23.4%). SARS-CoV-2 was the leading pathogen in adults aged 19–60 and ≥60 years, but significantly decreased in 2024–25 (49.1% to 24.9% and 54.9% to 28.1%, respectively; $p < 0.05$). Influenza A/B and RSV were significantly more frequent in-season, whereas SARS-CoV-2, adenovirus and rhinovirus were more frequent off-season in both years ($p < 0.001$).

Conclusion:

SARS-CoV-2 maintained relative predominance in 2023–24 but declined in 2024–25 when other respiratory pathogens accounted for a larger proportion of detections. Its summer–autumn concentration may indicate a shift toward a seasonal-endemic pattern. Overall, 2024–25 pathogen patterns appeared closer to pre-pandemic seasonality.

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Maribavir for treatment of cytomegalovirus in different immunosuppressed populations

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Introduction: Maribavir (MBV) is an antiviral recently approved for treating refractory or resistant CMV disease in transplant recipients. This series describes the clinical course and outcomes for thirteen patients treated with MBV for clinically significant CMV infection.

Methods: Retrospective review of cases managed by clinical virology and relevant clinical teams. Viral load clearance was defined as two consecutive undetected CMV PCR results.

Results: Thirteen patients were included: renal solid-organ transplant ($n=6$), haematopoietic stem cell transplant ($n=4$), refractory multiple myeloma ($n=1$), inflammatory bowel disease ($n=1$) and CTLA-4 haploinsufficiency ($n=1$). Rationale for switch to MBV included ganciclovir resistance ($n=4$), refractory viraemia or end-organ CMV disease ($n=4$), cytopenia ($n=4$) and slow viral clearance on valganciclovir ($n=1$).

Following initiation of MBV, three patients achieved CMV viral load clearance; median time to clearance was 14 days. Two patients failed to suppress viraemia on MBV and were switched to another antiviral. Three patients developed MBV resistance requiring treatment switch. Three patients relapsed viraemia on completing MBV; median time to relapse was 21 days. One patient received a second course of MBV for relapse.

One patient died of coexisting COVID-19 and CMV pneumonitis following poor response to MBV and COVID-19 treatment. A second patient died of ganciclovir treatment-resistant CMV pneumonitis two days after switch to MBV. A third patient died of bacterial sepsis.

Conclusion: MBV cleared CMV viraemia in three patients. Development of on-treatment MBV resistance, failure to suppress CMV viral load and relapse following MBV cessation was also observed. The utility of MBV in real-world settings requires further evaluation.



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Beyond a Mild Virus: Human Metapneumovirus Burden and Severity in Adults (2022–2026)

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Introduction/Aim: Human metapneumovirus (hMPV) is an understudied cause of potentially severe respiratory infection, particularly among children under 5, older adults and immunocompromised individuals. This study investigated the burden and clinical severity of adult hMPV infection at “Evangelismos” Hospital, the largest tertiary hospital in Greece, between September 2022 and March 2026.

Materials/Methods: Syndromic molecular respiratory tests were conducted, followed by a retrospective analysis of epidemiological and clinical data of laboratory-confirmed hMPV cases.

Results: Among 4,620 molecular respiratory tests performed, 72 were positive for hMPV and 1,606 identified other respiratory pathogens. Overall, 42 hMPV-positive patients (58.3%), mean age=66.1±17.7 years/42.9% male, required hospitalization for a median (IQR) of 9.5 (6.0-13.5) days. Most patients (88.1%) had comorbidities, most commonly hypertension (59.5%), COPD (40.5%), and cardiovascular disease (31.0%), while 57.1% were smokers. Oxygen therapy and ICU admission was required in 64.3% and 16.7% of cases, respectively. Of the 42 hospitalized patients, 27 (64.3%) suffered from severe hMPV infection that was significantly associated with the presence of comorbidities (p=0.001), specifically COPD (p=0.008) and obesity (p=0.033). The overall mortality was 7.1%, though direct attribution to hMPV infection could not be definitively established in all cases.

Conclusion: hMPV is a significant respiratory pathogen in older adults, with a clear seasonal variation and a relatively high hospitalization rate. While severe disease is generally uncommon, a non-negligible mortality rate was observed, highlighting the potential clinical impact of hMPV, particularly in vulnerable populations.

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Extensive HCMV B cell library reveals novel neutralizing candidates against pre-fusion gB

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HCMV poses a major risk to immunocompromised patients and unborn children through congenital infection, highlighting the need to better understand protective humoral responses for improved treatment strategies. Given its role as an indispensable surface structure for viral fusion, antibody responses to the pre-fusion state of glycoprotein B (gB), promise great therapeutic potential. In this project, we identified a large panel of cross-reactive as well as pre- and post-fusion-specific monoclonal antibodies (mAbs) with different characteristics by utilizing two stabilized pre- and post-fusion proteins.

To this end, we implemented a novel labelling and gating strategy and chose thirty out of 106 pre-screened individuals, including six pre-selected “Elite donors”, to isolate 1,836 B cells via single cell sort. Sequence analyses based on this extensive B cell panel revealed clonal relationships, including 29 clonotypes shared by two or more donors, as well as V gene usage and CDRH3 features.

Out of 145 in vitro produced candidates derived from this library, 95 confirmed binding to gB via ELISA. Additionally, a novel assay using cells transduced with Fc-receptors, allowed us to identify four mAbs interacting only with the post-fusion, and twenty specifically with the pre-fusion variant. Pairing these results with our extensive sequence analyses revealed, that all mAbs with pre-fusion preference share VH1-69, suggesting a highly convergent response to the pre-fusion conformation. Comprehensive epitope mapping revealed that more than half of our panel, including all post-fusion and many pre-fusion targeting mAbs, does not share target sites with tested reference mAbs, leading us to define novel epitope clusters. Further characterization uncovered thirty strong neutralizers against different clinical strains. Among these are pre-fusion preferring ones, which reinforces the clinical importance of targeting anti-pre-fusion-gB-mAbs. The data from this project advances our understanding of HCMV’s immune response and offers the foundation for new therapeutic approaches.

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Unravelling similar evolutionary dynamics of emerging HIV-1 subtype A epidemic in Serbia and Montenegro

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Introduction: Since the beginning of the HIV epidemic, in the two neighbouring countries in the Balkans, Serbia and Montenegro, HIV-1 subtype B was found as the most prevalent subtype; however, the prevalence of subtype A has been increasingly recognised over the last few years. The true prevalence of HIV-1 subtype A, in both neighbouring countries, is still not well known and probably underestimated. This study aimed to reconstruct evolutionary dynamics and potential for further epidemic spread of HIV-1 subtype A, by analysing two datasets, from Serbia and Montenegro, encompassing 640 and 98 HIV-1 pol sequences, respectively, sampled from 2002 to 2024.

Material and Methods: The subtyping tools COMET and REGA 3.0 exhibited the best performance on the both datasets studied. Maximum Likelihood (ML) phylogenetic trees were constructed using IQ tree and MEGA software tools. Here we applied phylogenetic analysis and Bayesian coalescent-based methods to reconstruct the evolutionary patterns of HIV-1 subtype A, found as the most prevalent non-B subtype.

Results: Of 640 analysed Serbian HIV sequences, subtype B was found in 81.4% (521/640) of sequences. Among non-B subtypes, subtype A was identified as the most predominant one, in 10.6% (68/640), followed by small prevalence of other non B subtypes. Regarding dataset from Montenegro, the predominance of subtype B 82.3% (74/98) was revealed, with the highest prevalence of subtype A 17.3% (17/98). Phylodynamic analysis performed on all subtype A sequences revealed Re value of 1.4 in the latest 3 years, while for 17 subtype A sequences from Montenegro, Re value was slightly higher 1.7, with steep growth starting from 2022.

Conclusions: The current study provides insights into the similar patterns of the HIV subtype A evolutionary dynamics in Serbia and Montenegro. We found that the prevalence of non-B subtypes has increased over time in both countries, and that this significant trend can be mainly attributed to subtype A. Subtype A sub-epidemic showed high tendency for further epidemic spread in this part of the Balkans.

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Eukaryotic DNA virome in blood and stool distinguishes switched memory B-cell-deficient phenotypes in common variable immunodeficiency

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Aim: To characterize the eukaryotic DNA virome in plasma, whole blood and stool samples of patients with common variable immunodeficiency (CVID) and to assess their associations with clinical complications.

Materials and Methods: Plasma, whole blood and stool samples were collected from 48 CVID patients with chronic gastrointestinal symptoms and 20 healthy volunteers. Patients were stratified into class-switched memory B-cell-deficient (SmB-; n=28) and SmB+ (n=20) CVID phenotypes according to the frequency of CD19+CD27+IgD-IgM- B cells (<2%, EUROclass classification). Viral loads and genome sequences of 41 persistent DNA viruses were assessed using qPCRs and viral target-enriched Illumina NGS. The virological findings were correlated with clinical, endoscopic and immunological data.

Results: We detected atypical blood or stool virome, defined as viruses absent from healthy controls, in 64% of SmB- patients (18/28) compared with 10% of SmB+ (2/20) cases (p<0.001). Cytomegalovirus (CMV) was the most frequent atypical finding, followed by polyomaviruses BK, JC and HPyV6, as well as varicella zoster virus, herpes simplex virus-1 and cutavirus. Anelloviruses were detected in all three groups but were most prevalent in SmB- plasma (25/28; 89%), with 12-fold and 90-fold higher viral loads compared with SmB+ patients and controls, respectively (p<0.05). Within the SmB- group, viral DNA detection was associated with older age and recent thrombocytopenia (p<0.01). Stool viral species richness correlated with peak fecal calprotectin levels (p<0.001). Age and plasma CMV positivity were associated with mortality during 13-year follow-up; however, neither remained significant when jointly modeled.

Conclusions: The atypical eukaryotic DNA virome in CVID SmB- patients suggests impaired control of persistent viruses consistent with greater immune dysfunction. Frequent occurrence of CMV in elderly SmB- individuals with gastrointestinal symptoms may represent an underestimated contributor to morbidity.

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Seasonal Dynamics and Type-Specific Circulation of Respiratory Syncytial Virus Across Two Consecutive Seasons (2024–2026) in Croatia

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Introduction: Respiratory syncytial virus (RSV) is a major cause of respiratory tract infections (RTIs) worldwide, affecting all age groups. In temperate climates, RSV exhibits a seasonal pattern, typically occurring from winter to early spring. We analyzed the epidemiological characteristics of RSV infections over two consecutive seasons (2024–2026).

Materials and Methods: Laboratory-confirmed RSV infections were identified in patients tested at the National Influenza Laboratory, Croatian Institute of Public Health, during the 2024/2025 and 2025/2026 seasons. Patients were stratified according to sex, age, and clinical characteristics. RSV testing was performed on all samples, while RSV typing was conducted on a subset of positive specimens.

Results: RSV was detected in 815 patients (7.7%) in the 2024/2025 season and 807 patients (10.7%) in the 2025/2026 season. Peak activity occurred in February in both seasons (epidemiological week 7 in 2024/2025 and week 9 in 2025/2026). Both RSV types circulated in each season; however, RSV B predominated in 2024/2025 (67.4%), while a more balanced distribution was observed in 2025/2026 (46.2% RSV A vs. 41.9% RSV B). Children aged 0–4 years were the most affected population in both seasons.

Conclusions: Our findings confirm typical seasonal RSV circulation in the Zagreb area, with co-circulation of both RSV types and variability in their relative predominance between seasons. Continuous respiratory virus surveillance, including selective viral typing, remains essential for the timely identification of epidemiological shifts and for guiding public health interventions.

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Discriminating True HIV Infection from Indeterminate Assay Reactivity in Emergency Department Opt-Out Screening

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Introduction/Aim: Emergency Department (ED) opt-out HIV screening improves case detection but may generate indeterminate reactive results that delay interpretation and create uncertainty. We evaluated the frequency, characteristics, and clinical significance of indeterminate HIV results from the ED opt-out screening programme at Norfolk and Norwich University Hospital, and assessed the value of HIV-1 RNA testing in resolving reactive results.

Methods: We audited reactive fourth-generation HIV-1/2 screening results on serum samples collected from ED between July 2025 and March 2026. Indeterminate results represented weak reactivity in screening tests, not confirmed by second test or antibody differentiation assays. HIV-1 RNA PCR was performed on indeterminate samples.

Results: Of 25,897 samples, 43 were reactive (0.17%): 19 confirmed infections, 21 indeterminate results, and 3 insufficient samples. Indeterminate results accounted for 0.08% of all tests and had low signal-to-cut-off (S/CO) values with undetectable HIV-1 RNA. Confirmed infections showed much higher S/CO values, often above 600, with detectable or suppressed viraemia. Indeterminate results were more common in older adults and women, and a likely cause of non-specific reactivity was suggested in 76% of cases, most often autoimmune disease, previous pregnancy, or malignancy. No indeterminate cases were associated with HIV-related morbidity, mortality, or immunosuppression.

Conclusions: Indeterminate HIV results in ED opt-out screening mainly reflect non-specific assay reactivity rather than true infection. HIV-1 RNA testing enables rapid clarification and reduces unnecessary follow-up and patient anxiety.

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RdRp Inhibitors for Respiratory Virus Infections in the Immuno-suppressed: current evidence and future perspectives

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Community acquired respiratory viruses (CARVs) are a significant cause of morbidity and mortality in immunocompromised patients which include patients with haematological malignancies (HM), recipients of haematopoietic stem cell transplantation (HSCT), B-cell targeting therapies, immunomodulatory monoclonal antibodies, chimeric antigen receptor T cell therapies (CAR-T), and solid organ transplantation (SOT). (Ison and Hirsch 2019; Von Lilienfeld-Toal et al. 2026; Rubin and Hirsch 2008)

While several antiviral drugs are licensed for treatment of SARS-CoV-2 and Influenza, we focus here on treatment of non-SARS-CoV-2 and non-Influenza RNA respiratory viruses, including Respiratory Syncytial virus (RSV), Rhinovirus (RV), seasonal human coronaviruses (sCoVs) (OC43, HKU1, 229E, NL63), Parainfluenza virus (PIV), and human Metapneumovirus (hMPV).

We review the evidence supporting current guidelines for treating these viruses in immunocompromised populations and review the data on in-vitro and in-vivo efficacy of repurposed and other drugs reported to be active. Given the success with combination antivirals for treatment of HIV, and hepatitis C we address the potential for this strategy in the management of severe respiratory infections.

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Seroprevalence against selected Orthoflaviviruses and Alphaviruses in children from Dengue-endemic and non-endemic regions of Costa Rica

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Arboviral diseases represent a critical public health challenge throughout the tropical Americas. While transmission dynamics in Costa Rican adults are partially characterized, epidemiological data for pediatric populations remain notably scarce. This study evaluated the prevalence of neutralizing IgG against selected Orthoflaviviruses and Alphaviruses in children sampled from both endemic (92 samples) and non-endemic (87 samples) dengue regions. We characterized the serological response to circulating viruses across these two groups using Plaque Reduction Neutralization Test (PRNT). Initial screening PRNT50 at a 1:10 serum dilution yielded seropositivity rates for Orthoflaviviruses and Alphaviruses of 31.5% and 9.78% in non-endemic samples, and 66.7% and 23.0% in endemic samples, respectively. Confirmatory titration detected predominant Orthoflavivirus seropositivity, reaching 46.0% in endemic areas vs 3.4% in non-endemic settings. Conversely, Alphavirus seroprevalence was at 5.7% in endemic samples vs 1.1%, in non-endemic samples. In the endemic group, the highest seropositivity (42.5%) was detected against Dengue virus (DENV); in contrast the non-endemic group presented an equal prevalence of 2.2% for both DENV and Zika virus (ZIKV). The PRNT against Alphaviruses showed a positivity of 5% Eastern Equine Encephalitis virus (EEEV) and Venezuelan Equine Encephalitis virus (VEEV) only in the endemic pediatric population. Thus, confirming the circulation of both viruses as we have previously found in adult samples. Our findings demonstrate a significantly higher serological burden in rural pediatric populations compared to their urban counterparts. These results underscore the necessity for intensified active surveillance, integrated vector management and diagnosis in Dengue endemic areas in Costa Rica, where pediatric Orthoflavivirus prevalence exceeds 40%.

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Etiological Diversity of Viral Enteric Infections Diagnosed by Multiplex PCR

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Objective: The aim of this study was to determine the distribution of viral enteric pathogens and to describe the epidemiological and microbiological characteristics of enteric infections diagnosed by multiplex PCR.

Materials and Methods: A retrospective descriptive study was conducted (May 2017-May 2026) at the Central Military Hospital of Algiers. A total of 645 stool samples from patients (11 days-89 years) presenting with acute diarrhea were analyzed using a gastrointestinal multiplex PCR panel (FA-GI). The study population included 394 children (293 under 5 years of age) and 251 adults. The male-to-female ratio was 1.51.

Results: Overall, 78% of samples (504/645) tested positive for at least one pathogen. Positivity rates and co-infections were more frequent in children (84%) than in adults (69%). All targeted pathogens were detected except *Cyclospora cayetanensis* and *Entamoeba histolytica*. Twenty etiological agents were identified, including 551 bacterial, 396 viral, and 28 parasitic detections. Rotavirus was the most frequently detected pathogen (28.68%), followed by *Campylobacter* spp. (23.72%), EAEC (17.89%), norovirus (12.56%), and astrovirus (9.46%).

In children, bacterial and viral etiologies were similarly distributed (50% and 48%, respectively), while viral infections were particularly frequent in children under 5 years old (95%). In adults, bacterial etiologies predominated (77%) compared with viral etiologies (22%). The prevalence of pathogens differed between children and adults. In children, the most prevalent pathogens were rotavirus (46%), *Campylobacter* spp. (30%), EAEC (23%), followed by astrovirus and norovirus (17%). In adults, the main etiologies were *Campylobacter* spp. (19%), *Clostridioides difficile* (14%), EAEC (13%), followed by rotavirus and norovirus (9%).

Rotavirus was the predominant viral pathogen in both age groups. A seasonal increase in viral infections was observed during the summer.

Conclusion: Multiplex PCR is a rapid and sensitive diagnostic tool that improves pathogen detection

Keywords: viral enteric infections; multiplex PCR

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Prevalence and Genotype Distribution of High-Risk Human Papillomavirus Diagnosed by Real-Time PCR

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Introduction and Objective: Human papillomaviruses (HPV) are the second leading infectious agents associated with cancer worldwide. Cervical cancer is almost entirely attributable to high-risk oncogenic HPV (HR-HPV). According to the International Agency for Research on Cancer (IARC), 13 HR-HPV genotypes have been identified: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The aim of this study was to determine the overall prevalence and genotype distribution of HR-HPV diagnosed in the microbiology laboratory of the Central Army Hospital of Algiers.

Materials and Methods: A retrospective descriptive study was conducted from January 25, 2017 to April 15, 2026, in the microbiology laboratory of the Central Army Hospital (HCA), Algiers. Cervico-uterine samples were collected and analyzed for HR-HPV detection and genotyping using real-time PCR. Three diagnostic systems were used: Cobas 4800 (Roche®), Sacace Biotechnologies®, and GeneXpert (Cepheid®).

Results: During the study period, 806 samples were analyzed. The overall prevalence of HR-HPV infection was 4.71% (n = 38). The genotype distribution was as follows: HPV-16 in 15 cases (39.47%), HPV-18 in 2 cases (5.27%), and other HR-HPV genotypes (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) in 21 cases (55.26%). Co-infection with HPV-16 and another HR-HPV genotype was observed in three patients.

The mean age of HR-HPV-positive patients was 51 years (range: 30–71 years). Two-thirds of patients infected with HPV-16 (66.66%) were over 50 years of age.

Conclusion: The prevalence of high-risk human papillomavirus infection remains low in our population. HPV-16 and HPV-18 together accounted for more than 40% of circulating HR-HPV genotypes. Continuous molecular surveillance is essential to improve prevention and screening strategies for cervical cancer.

Keywords: HR-HPV; HPV-16; HPV-18; real-time PCR; cervical cancer; genotyping.



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The effects of older siblings in near term infant with RSV infections

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Introduction: The association between early-life respiratory syncytial virus (RSV) infections and respiratory morbidity is well established. Preterm babies, CV diseases and siblings are the most important risk factors of severe RSV infections.

Purposes: Our study was performed to investigate of the effect of older siblings on risk of near term infant with acute RSV infections.

Methods: We reviewed medical records of infants less than 36 months old with RSV infections who was born from 34+0 weeks to 36+6 weeks gestational ages who visited Dongtan Sacred Heart Hospital, Gyeonggi-do, South Korea. We divided patients into two groups. One is infants with no sibling, and the other group is infants with siblings. Infants with only fever and respiratory symptoms are included in mild groups and infants with respiratory difficulty needed high flow oxygen or ventilator care are included in moderate to severe groups. Risk factors such as birth weight, gestational age, sex, having siblings, artificial/mix feeding, and having CHD were evaluated.

Results: 426 patients were admitted and 1235 patient visited to out patient clinics from Jan. 2015 to Dec. 2024 at Dongtan Sacred Heart Hospital, Gyeonggi-do, South Korea. Among the infants visited to out patients clinics, 735(59.5%) infants had siblings and 500(40.5%) had no siblings. Among the infants admitted groups, 275(64.5%) had no sibling and 151(35.4%) had siblings. Among the infants who were admitted, 15(5.5%) were moderate to severe group with no siblings 8(5.3%) were moderate to severe group with siblings. 1

Conclusion: Infants with siblings had RSV infections more frequently and milder than no sibling. That means recurrent infection makes adoptive immunity. Future RSV vaccination program must protect children throughout early childhood. Vaccination and/or interventions to reduce transmission by older siblings could substantially reduce RSV hospital admissions.

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hnRNPA1-mediated nuclear retention of HIV-1 RNA limits latency reversal in ART-suppressed individuals

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Introduction: Despite effective ART, HIV persists in long-lived latent reservoirs, resulting in rapid viral rebound upon treatment cessation. Current shock-and-kill strategies aim to transcriptionally reactivate latent cells; however, clinical & ex-vivo studies show that increased HIV transcription rarely translates to productive viral reactivation. Since export of HIV-RNA is essential for proper infection, host factors regulating this step may contribute to reservoir persistence. This study aimed to investigate the role of host RNA-binding protein hnRNPA1 in HIV-RNA export to evaluate its relevance in latency reversal.

Methods: hnRNPA1 was identified by targeted screening of various hnRNPs in primary CD4+ T-cells affecting HIV-RNA export. Functional analyses were performed in CD4+ T-cells from PWH, including ART-suppressed individuals. hnRNPA1 knockdown and overexpression studies, RNA export assays, FISH, IP, EMSA, and latency reversal experiments using the FDA-approved-drug SAHA in combination with another FDA-approved drug were conducted to study clinical relevance

Results: Inhibiting hnRNPA1 significantly enhanced cytoplasmic export of HIV-RNA in primary CD4+ T-cells, whereas hnRNPA1 overexpression reduced p24 expression and release across multiple HIV-1 subtypes, indicating broad antiviral relevance. RNA-FISH showed hnRNPA1 to retain HIV-RNA in nuclear stress granules. RNA-IP/EMSA confirmed direct interaction of hnRNPA1 within HIV-RNA at RRE, mediated by its RRM1-domain. Importantly, hnRNPA1 expression was elevated in PBMCs from ART-suppressed individuals, supporting its potential role in reservoir persistence. In PBMCs from 35 ART-individuals, hnRNPA1 inhibition increased HIV-1 RNA export and enhanced SAHA-mediated viral reactivation, highlighting its translational relevance for latency reversal strategies

Conclusion: hnRNPA1 acts as a post-transcriptional checkpoint restricting HIV-RNA export and limiting latency reversal. Our study provides clinically relevant insight into HIV reservoir during ART and highlights RNA retention as a potential target to improve HIV cure.



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Evaluation of UBM™ Transport Medium Stability Across Multiple Molecular Diagnostic Platforms for Respiratory Pathogens

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Introduction/Aim: As molecular diagnostics form the cornerstone for the diagnosis of respiratory infections, transport media that preserve the quality of nucleic acids from specimen collection to analysis are needed. UBM™ (Copan) is a new inactivating medium that is non-guanidine based. We compared its performances to a reference, non-inactivating medium used in clinical settings, Deltaswab™ (Deltalab).

Material/Methods: Two pools containing different respiratory viruses and bacteria were created: Influenza A (H3N2, H1N1) and B, Coronaviruses (SARS-CoV-2, OC43, HKU1), Pneumoviruses (hMPV, RSV), parainfluenza 3, and Mycoplasma pneumoniae. Performances were evaluated on replicates of a 5-point, 10-fold dilution range. Stability was evaluated by repeating assays at various time points (day 0, day 4 and day 28), after incubating media at different temperatures (4°C, 20°C and 37°C). Five analytical platforms were used: Cobas 6800 (Cobas SARS-CoV-2 & Influenza A/B), Panther Fusion (SARS-CoV-2/Flu A/B/RSV), FilmArray (Biofire Respiratory 2.1 Plus Panel), QIAgen (QIAstat-Dx Respiratory Panel Plus), and Seegene (Allplex Respiratory Panel).

Results: At day 0, while performances vary greatly between platforms for a given pathogen, performances were similar between UBM and Deltaswab on the same platform. When differences were observed, they were limited (-1.07 to +0.56 Ct).

Cycle thresholds remained stable over 28 days in UBM (-0.05 to +0.63 Ct), regardless of the storage temperature, while they decreased significantly in the reference media (+0.49 to +1.66 Ct at day 4 and +2.32 to +5.75 Ct at day 28).

Conclusions: This study highlights that, although the media has a limited impact on fresh samples, the choice of the transport media is important for laboratories receiving samples that have been stored for longer periods of time (e.g., self-sampling, nursing homes).

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Rapid detection of human bocavirus 1 by a CRISPR-Cas12a-based diagnostic assay

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Human bocavirus 1 (HBoV1), a parvovirus first identified in 2005 in pediatric airway samples, is a common agent of pediatric respiratory tract infections (RTI) globally, with genoprevalences of 2-20% among pediatric RTIs. Although qPCR, mRNA-RT-PCR, and serological assays are commonly used for HBoV1 detection in research, these methods require specialized equipment and trained personnel, limiting their suitability for rapid routine point-of-care testing. To address this challenge, we developed a rapid HBoV1-diagnostic method based on the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins) system.

A total of 98 nasopharyngeal swabs (NPS), 44 aspirates (NPA), and 14 serum samples from 156 children aged 0.5 months to 12 years were employed for pre-amplification of HBoV1 DNA by a 20-min isothermal recombinase-polymerase pre-amplification (RPA), followed by CRISPR-Cas12a detection. The results were analyzed by both fluorescence intensity and by lateral-flow assay on paper strips. Both readouts were obtained within 30 minutes. qPCR was performed in parallel to evaluate and compare the detection sensitivity of the CRISPR-Cas12a assay in clinical samples. Among the 142 airway samples and the 14 sera, we found a 100% HBoV1-DNA correlation between the RPA-CRISPR/Cas-assay and qPCR results. RPA pre-amplification provided a sensitivity comparable to that of qPCR, but offering a faster and easier alternative. Furthermore, in 82 NPS samples, direct detection of HBoV1 DNA without extraction, showed 100% concordance with our in-house qPCR on extracted DNA. Lateral flow analysis of the RPA-CRISPR-Cas12a-detected genomes in NPA and NPS samples mirrored the fluorescence results, with high-copy samples producing stronger visual bands than low-copy samples.

Conclusion: We have developed a rapid, sensitive, and cost-effective CRISPR-Cas12a-based platform for HBoV1-DNA detection, supporting both fluorescence and lateral flow readouts.

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HCV RNA Kinetics in Routine Laboratory Practice: Longitudinal Analysis of Viral Load Dynamics

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Introduction/Aim: Hepatitis C virus (HCV) RNA kinetics are typically described as a predictable decline in viral load leading to clearance under controlled clinical conditions. However, both biological variability and analytical limitations of PCR, particularly near the limit of detection, may result in low-level fluctuations, requiring careful laboratory interpretation to avoid misdiagnosis of treatment failure or relapse. This study aimed to analyze longitudinal HCV RNA viral load dynamics in patients with documented viremia, with a focus on viral load patterns relevant to routine laboratory interpretation.

Material: We retrospectively analyzed 246 patients with at least one positive HCV RNA result and repeated viral load measurements obtained between 2022 and 2025.

Methods: Viral load values (IU/ml) were extracted from the laboratory information system; HCV RNA quantification was performed using the Alinity m system (Abbott), and data were evaluated longitudinally. The lower limit of detection/quantification of the assay was 12 IU/ml.

Results: The majority of patients demonstrated a decline in viral load leading to negativization. However, a clinically relevant subset exhibited non-linear trajectories, including persistent viremia, rebound, and fluctuating low-level positivity. Time to negativity varied widely, and transient shifts between detectable and undetectable viral load were observed. Persistent viremia was observed in approximately 21% of patients, while low-level fluctuating positivity and rebound viremia occurred in 14% and 4% of cases, respectively.

Conclusions: These findings indicate that, in contrast to simplified models derived from clinical studies, HCV RNA dynamics in routine laboratory practice are often irregular and influenced by both biological and analytical factors. Such variability may complicate interpretation based on single time-point results and highlights the importance of longitudinal assessment in clinical and laboratory decision-making.

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Investigation of nucleic acid sequences associated with antiretroviral resistance in whole blood, leukocyte and plasma samples from HIV-1 positive patients by deep sequencing

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Introduction/Aim: Plasma HIV-1 RNA genotyping is standard for resistance testing, but may miss low-abundance variants or mutations archived in cellular compartments. We compared plasma, whole-blood and leukocyte HIV-1 sequences to assess compartment-specific resistance and intrapatient heterogeneity.

Material and Methods: Viral RNA was extracted from plasma; total nucleic acid was extracted from whole blood and leukocyte samples from 16 patients. The HIV-1 pol gene region was amplified by RT-PCR followed by nested PCR and sequenced on the Illumina MiniSeq platform. Consensus-level resistance mutations were interpreted using Sierralocal/Stanford HIVdb. Low-frequency variants were analyzed with LoFreq using a minimum coverage threshold of 100× and a variant frequency range of 2%–20%. APOBEC-associated G-to-A hypermutation was screened using Hypermut 3.0. Haplotype reconstruction was performed with CliqueSNV. Compartmentalization was evaluated using TN93-based FST analysis, the Slatkin-Maddison test and the Snn test.

Results: Among 48 compartment samples, three failed amplification and one plasma sample was excluded because of a low mapping rate (0.39%) and insufficient target-region coverage (23.18%). On average, 99.5% of the target region was covered; mean sequencing depth was 6,441×. Few consensus-level resistance mutations were detected whereas low-frequency variants were identified in 10/16 patients (62.5%). Overall, 22/25 resistance-associated mutations (88%) were discordant across compartments, mainly in leukocyte-derived sequences. APOBEC screening was positive in 2/93 tests. Haplotype reconstruction identified 515 haplotypes across 44 samples. The highest haplotype counts were observed in P01, P03 and P08, whereas the single P09 haplotype clustered within the P08 clade.

Conclusions: Plasma consensus genotyping alone may miss low-frequency and compartment-restricted HIV-1 resistance variants. Cellular DNA and haplotype-based analyses provide complementary information when APOBEC artifacts, preanalytical variability and treatment history are considered.

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Evaluation of Humoral and Fc-Mediated Immune Responses Against VZV Glycoprotein E Following Herpes Zoster and Recombinant Zoster Vaccination

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Introduction: VZV glycoprotein E (gE) is a key immunodominant antigen. While humoral response to recombinant zoster vaccine (RZV) are well characterized, Fc-mediated responses such as antibody-dependent cellular cytotoxicity (ADCC) remain poorly understood. This study compared humoral and ADCC responses between herpes zoster patients and RZV-vaccinated individuals.

Material and Methods: Serial blood samples were collected from herpes zoster patients (N=7) at symptom onset and at 1 and 3 months, and from RZV-vaccinated individuals (N=19) before the first dose and at 1 and 3 months after two-dose series completion (2-month interval). Anti-VZV gE IgG was measured by ELISA; ADCC was assessed by a gE-based reporter assay.

Results: In the herpes zoster group, anti-gE IgG peaked at 1 month and then declined to near-baseline levels by 3 months (7.02 $\pm$ 3.52 $\pm$ 8.62 AU/mL; p<0.05). ADCC activity also increased more than four-fold at 1 month and decreased by 3 months; however, it remained approximately 1.5-fold higher than baseline levels (5.37 $\pm$ 5.83 $\pm$ 8.03; p<0.01), indicating greater durability of Fc-mediated effector function than humoral immunity. In the RZV-vaccinated group, both IgG and ADCC increased markedly and were well maintained at 3 months after vaccination completion (IgG: 1.71 $\pm$ 7.18 $\pm$ 23.21 AU/mL; ADCC: 1.88 $\pm$ 4.84 $\pm$ 18.82; both p<0.0001), with ADCC activity sustained at 76% of peak levels.

Conclusions: Both herpes zoster and RZV vaccination induced robust anti-gE IgG and ADCC responses. ADCC demonstrated greater durability than IgG, particularly following herpes zoster, where IgG waned to near-baseline levels while ADCC remained significantly elevated at 3 months. RZV vaccination more effectively sustained both responses, highlighting ADCC as a key and durable component of protective immunity against VZV.

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Genomic diversity of norovirus from hospitalised and foodborne outbreak-related cases in Catalonia (Spain)

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Introduction: Norovirus is the leading cause of gastroenteritis in Europe. It belongs to the Caliciviridae family, that comprises single-stranded RNA viruses. Norovirus classification is based on VP1-coding gene (G group) and RNA polymerase-coding gene (P group). Noronet surveillance network, involving 23 countries, monitors global trends in genotype distribution and supports vaccine development.

This study aimed to perform genomic characterisation of noroviruses detected in hospitalized patients and foodborne outbreaks in Catalonia (Spain).

Materials and Methods: Stool samples were collected from inpatients at Vall d'Hebron University Hospital and cases associated with foodborne outbreaks in Catalonia (October 2025 and February 2026). Gastrointestinal viruses were identified using Allplex™GI-Virus Assay (Seegene). Norovirus-positive samples were further characterized by Sanger sequencing targeting the region between the 5'end of ORF1 and ORF2.

Results: A total of 44 stool samples were positive for genogroup I (GI) and 571 for genogroup II (GII). Most samples (105 sequences, 83%) were from hospitalized patients. Sex distribution was equal (male: 51%), and 33% of patients were under 5 years of age. Due to methodological challenges, 3 GI (30%) and 113 GII (20%) viruses were sequenced, prioritizing pediatric samples.

In adults and children over 5 years, the most prevalent genotypes were GI.1.P1 and GI.1.P5 in equal proportion (3%) and GII.17.P17 (42%). Among children under 5 years, GII.4.P16 predominated (59%), whereas GI was not detected.

Ten foodborne outbreaks were analyzed (21 sequences, 17%); 3 were caused by GI.1.P1 and 3 by GII.4.P16.

Conclusions: Identification of circulating norovirus genotypes is essential to understand gastroenteritis epidemiology, both in children and in adults, among whom differences in circulating viruses have been reported. Notably, the frequency of genotype GII.4.P16 in children under 5 years of age, supports its relevance for vaccine development strategies.

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Genomic diversity of rotavirus from hospitalised and primary care-related cases in Catalonia (Spain)

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Introduction: Rotavirus is the leading cause of acute gastroenteritis in children under 5 years of age. Its genome comprises 11 double-stranded RNA segments and classification is based on structural VP4 (P group) and VP7 (G group) proteins. The epidemiology of rotavirus is changing following vaccine introduction. In Catalonia (Spain), Rotarix vaccine has been included in the infant vaccination schedule since January 2025 at 2 months of age, underscoring the importance of surveillance for emerging genotypes. This study aimed to perform the genomic characterisation of rotavirus detected in hospitalised and primary care paediatric patients in Barcelona.

Materials and Methods: Stool samples were collected from Vall d'Hebron University Hospital and primary care centres of Barcelona (October 2025 and February 2026). Gastrointestinal viruses were detected using AllplexTMGI-Virus Assay (Seegene). Rotavirus-positive samples were further characterized by Sanger sequencing targeting VP4 and VP7 encoding regions.

Results: Among 280 positive stool samples, 121 (43%) viral genomes were selected for sequencing, mainly from pediatric patients. Of these, 71 (59%) were successfully classified, including 21 (17%) partially sequenced genomes (VP4 or VP7 only). Most samples (94%) were from hospitalized patients, with equal sex distribution (female: 50%). Median age was 2.5 years (IQR, 1–34 years), and 43 sequences (62%) were from children aged 0–5 years.

The predominant G group was G3 (79%), the predominant P group was P[8] (99%), and the most frequent combination was G3.P[8] (89%). Two sequences matched the Rotarix vaccine strain (G1.P[8]) and were confirmed as vaccine-derived by phylogenetic analysis and patient history review.

Conclusions: Identification of circulating rotavirus genotypes is crucial for understanding epidemiology and viral dynamics. Genomic surveillance emphasizes the importance of continued monitoring to update vaccine compositions and improve public health strategies.

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Enhancing the Reconstruction, Analysis, and Classification of Persistent Human Viral Sequences

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Introduction: The increasing availability of human sequencing data has created new opportunities for detecting and characterising viral sequences. However, accurate reconstruction and classification remain challenging, particularly in clinical and forensic contexts, where data may have low coverage, high mutation rates, fragmentation, and limited reference information. This highlights the need for specialised tools to improve reconstruction and interpretation.

Methods: We conducted a systematic review of open-source tools for reconstructing human viral genomes, evaluating their characteristics, methodologies, and performance across different scenarios. The analysis showed no single tool outperformed all others, suggesting that cooperative strategies could improve reconstruction accuracy. We therefore developed CoopPipe, a cooperative pipeline that integrates existing reconstruction tools and leverages their complementary strengths. In parallel, we developed Virumble, a reference-free tool that computes k-mer frequencies, iteratively extends reads, and merges overlapping sequences. Finally, we developed ViromeClass, an alignment-free tool that extracts intrinsic genomic features from viral sequences and trains machine learning models for taxonomic classification.

Conclusion: This work offers an integrated computational approach to reconstruct, analyse, and classify persistent human viral sequences. The review identified the strengths and limitations of existing tools, guiding the development of CoopPipe and Virumble. ViromeClass extends this framework through alignment-free taxonomic classification based on genome features. Together, these tools support more accurate viral sequence analysis for diagnostics, forensic investigation, and personalised clinical decision-making. All tools are available at <https://github.com/viromelab>.

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Diagnostic Performance of a SARS-CoV-2/Influenza Fluorescence Immunoassay and Its Correlation With RT-PCR Ct Values

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Introduction: SARS-CoV-2 and influenza viruses remain major causes of acute respiratory tract infections worldwide. Rapid and accurate diagnosis is essential for early treatment initiation, infection control, and appropriate patient management. This study aimed to evaluate the diagnostic performance of a fluorescence immunoassay (FIA)-based rapid antigen test for the detection of SARS-CoV-2, Influenza A virus (IAV), and Influenza B virus (IBV) compared with reverse transcription polymerase chain reaction (RT-PCR), and to investigate the relationship between FIA signal intensity and viral load.

Materials and Methods: In this study, combined nasopharyngeal/oropharyngeal swab specimens collected from 216 patients between November 2025 and January 2026 were included. The study population consisted of 90 SARS-CoV-2-positive, 60 IAV-positive, and 46 IBV-positive patients confirmed by RT-PCR, together with 20 respiratory pathogen-negative controls. Specimens were analyzed using the Bio-Speedy Respiratory-ID-2 Panel (Bioeksan, Türkiye). Cycle threshold (Ct) values were recorded for all PCR-positive samples. Residual specimens were subsequently tested using Standard F COVID/Flu Ag Combo FIA assay (SD Biosensor Inc., South Korea), and cut-off index (COI) values were obtained. Correlation analysis between Ct and COI values was performed.

Results: A total of 216 patients aged 0–88 years (median age: 7 years); 58.3% were female, 41.7% were male. In samples with Ct values <20, assay sensitivities were 100%, 100%, and 84% for SARS-CoV-2, IAV, and IBV, respectively. In samples with Ct values >20, sensitivities decreased to 40%, 79%, and 23.8%, respectively. Overall specificity was 100%. A statistically significant moderate negative correlation was observed between COI and Ct values ($r = -0.569$, $p < 0.001$).

Conclusions: FIA-based rapid antigen testing demonstrated high diagnostic performance in samples with high viral burden. These assays may contribute to rapid diagnosis and timely clinical decision-making, particularly in high-transmission settings.

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Replication and compartmentalization of Dobrava-Belgrade hantavirus in the central nervous system

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A heart-transplant recipient developed a fatal neurological disease of initially unknown etiology. Retrospective high-throughput sequencing (HTS) identified Dobrava-Belgrade virus (DOBV) in cerebrospinal fluid (CSF), respiratory samples, and serum. The highest viral RNA concentration was detected in the first available CSF sample, and DOBV RNA remained detectable for 36 days in CSF and 46 days in serum, consistent with persistent infection. Viral kinetics showed an initial decline followed by a secondary increase in CSF and serum, suggesting impaired viral clearance in the setting of immunosuppression. Genomic analyses revealed distinct DOBV populations in serum, CSF, and brain tissue, supporting compartmentalized and likely independent viral replication within the central nervous system. The viral strain was closely related to DOBV strains previously identified in rodents from the patient's region of origin in north-eastern Germany. To our knowledge, this represents the first well-documented case of DOBV persistence and compartmentalized replication in the CNS. This case highlights the atypical presentation and prolonged course of viral infection in immunosuppressed patients and underscores the diagnostic value of HTS.



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HCMV Glycoprotein Genotyping Using Nanopore Technology

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Background: HCMV encodes 9 major envelope glycoproteins, several of which are highly polymorphic. Their genetic diversity has been associated with disease severity in congenital infection and immunocompromised patients. Few, if any, have considered the association between genotypes within the complexes.

Objectives: To develop and validate a reproducible sequencing protocol for simultaneous genotyping of nine polymorphic glycoprotein loci (gB, gN, gH, gL, gO, gM, and UL128/UL130/UL131A).

Methods: Multiplex PCR products targeting nine loci were sequenced using Oxford Nanopore Technologies (ONT) R10.4.1 flow cells. Five well-characterised HCMV laboratory strains (AD169, Merlin, Towne, Toledo, and Davis) were used for pipeline validation. Genotype assignment required concordance across five evidence tiers: (1) pairwise nucleotide identity, (2) diagnostic SNP identification, (3) amino acid profiling, (4) maximum-likelihood phylogenetic analysis, and (5) read-level variant analysis to detect mixed-strain co-infections.

Results: All five strains were correctly assigned across all nine loci with 100% concordance between phylogenetic clustering and pairwise identity. Sequence variance analysis identified gO as the most variable locus, with gM and gL being most highly conserved, aligning with published HCMV biology. Read-level analysis confirmed single-strain populations, with no evidence of mixed strains.

Conclusions: The five-tier ONT analysis pipeline provides a robust, bias-minimised methodology for simultaneous genotyping of 9 HCMV surface glycoproteins. The ability to genotype glycoproteins within the three surface complexes may allow better determination of whether glycoprotein polymorphisms provide biomarkers for predicting CMV disease severity to better guide clinical management.

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Viral Replication and Immune Modulation in Respiratory Coinfections

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Introduction/Aim: Respiratory virus replication and pathogenesis are key to understanding disease severity. Here, viral dynamics and inflammatory responses induced by Human Adenovirus (HAdV) and Respiratory Syncytial Virus (RSV) were investigated in single and coinfection models.

Material: Infection was conducted using the A549 human lung epithelial cell line, able to support both HAdV and RSV infection. Cell culture medium and infected cells were collected at 24, 48, and 72 hours post-infection to evaluate viral replication, apoptosis, and inflammatory mediators release.

Methods: Viral replication kinetics were quantified through virus-specific real-time PCR on culture medium. Apoptosis was assessed by flow cytometry. Cytokine profiling was performed using ELISA immunoassays on culture medium to measure temporal dynamics of IL-6, IL-1 γ TNF γ and CCL2 in response to single and coinfections.

Results: Both viruses exhibited progressive increases in viral genome copies, confirming efficient replication in A549 cells, showing an approximately 1 log increase between 24 and 72h post-infection for RSV as single infection and HAdV as coinfection. Apoptosis remained low (<10%) across all conditions. RSV induced a significant, time-dependent increase in IL-6, while HAdV alone elicited minimal cytokine responses comparable to controls. CCL2 increased during RSV single infection but progressively declined in coinfecting cells, indicating modulation of monocyte-recruitment pathways.

Conclusions: These findings reveal distinct inflammatory signatures and complex interactions during respiratory viral coinfections. The modulation of cytokine responses—particularly IL-6 and CCL2—highlights mechanisms potentially contributing to clinical variability and underscores the importance of integrated analyses of viral dynamics and host immunity.



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Whole-genome sequencing for RSV genomic surveillance in Luxembourg over three consecutive epidemic seasons

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LNS

As part of the PANDOMIC project, we implemented and optimized an amplicon-based Whole-Genome Sequencing (WGS) workflow for Respiratory Syncytial Virus (RSV) to support routine genomic surveillance in Luxembourg. RSV positive nasopharyngeal samples collected through the national sentinel surveillance network were analyzed over three consecutive epidemic seasons (2022-2025). Several Illumina-based sequencing strategies were compared and optimized to improve genome recovery, especially in low viral load samples. A protocol based on multiple short amplicons showed the best overall performance and was selected for routine implementation. In parallel, a dedicated bioinformatics workflow was established for quality control, consensus sequence generation, clade assignment and phylogenetic analysis. Across the three seasons, more than 600 RSV-positive samples were sequenced, yielding over 500 genomes with sufficient coverage for downstream analysis. This dataset revealed marked year-to-year changes in circulating viruses, with alternating dominance of RSV-A and RSV-B and a rapid turnover of clades between seasons. These findings highlight the dynamic nature of RSV circulation and the value of sustained multi-season genomic surveillance. Beyond tracking viral diversity, WGS also enables the monitoring of mutations in key antigenic regions, particularly in the F gene, which may affect susceptibility to preventive monoclonal antibodies such as Palivizumab and Nirsevimab. This aspect is becoming increasingly important in the context of expanding RSV prevention strategies, including infant immunoprophylaxis and maternal vaccination, where genomic surveillance can help detect potential immune escape variants at an early stage. Overall, this work demonstrates that integrating WGS into routine RSV surveillance strengthens variant tracking, resistance monitoring and preparedness for future respiratory virus outbreaks.

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Investigation of the mechanism for Adenovirus 5 Inactivation by 222 nm Far-UVC

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Background: 222 nm Far-UVC exposure inactivates a range of different viruses and is increasingly proposed for antiviral disinfection. The mechanism(s) underlying viral inactivation is not fully understood. We investigated inactivation of Adenovirus 5 (Ad5) by 222 nm Far-UVC considering: dose response, damage to genome and capsid proteins, and the modifying role of the suspending medium.

Methods: Ad5 infectivity was quantified by TCID₅₀ after direct irradiation or exposure to pre-irradiated media. Direct irradiation at 371 mJ/cm² produced matrix-dependent reductions: approximately 3.0 log₁₀ in 10% FBS/EMEM, 4.7 log₁₀ in 2% FBS/EMEM, 4.9 log₁₀ in 0% FBS/EMEM and 5.5 log₁₀ in PBS.

Results: Viral genome damage was assessed using T4 Endonuclease V-based analysis of cyclobutane pyrimidine dimer (CPD)-associated lesions. Virus was exposed to Far-UVC doses at 0, 37, 186, 371 and 742 mJ/cm². Post-exposure genome analysis showed evidence of CPD formation. Pyrimidine-related compounds, particularly cytidine and uridine/uracil-related structures, showed greater dose-associated absorbance loss than purine-related compounds. Protein effects following the same dosages of UV were analysed by SDS-PAGE and Western blotting and showed loss or alteration of viral proteins, notably the AdV5 hexon protein.

Conclusions: These findings indicate that 222 nm Far-UVC inactivates viruses through more than one mechanism. These include CPD-associated genome damage and capsid protein alteration. In addition we demonstrate that the surrounding medium strongly modifies antiviral efficacy by absorbing radiation and limiting photon delivery to the virus. This is important for the correct and safe application of Far-UVC as an antiviral tool in real-world biological environments.

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Performance Study of the Hologic Panther Fusion® EBV Assay on Whole Blood Samples

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Introduction: Epstein Barr virus (EBV) viral load (VL) monitoring is used for the follow up of immunocompromised patients, especially allogeneic hematopoietic stem cell or solid-organ transplant recipients. We aimed at evaluating the performance of a new Panther Fusion® assay (Hologic) for EBV DNA quantification using whole blood (WB) samples.

Methods: Prospective (n=243) and retrospective (n=49) WB samples were analysed using Panther Fusion® EBV Quant assay (QA) compared with our EBV routine assay (RA) combining FLOW extraction/amplification systems (MagnaPure96/LC480 II platforms, Roche Diagnostics) using EBV R-GENE® reagents (bioMérieux). VL are expressed in log₁₀ IU/mL; limits of quantification (LoQ) are 2.54 and 2.7 log₁₀ IU/mL for QA and RA, respectively.

Results: A total of 292 samples with a median VL of 3.32 log₁₀ IU/mL (range [0.0-6.59]) determined by RA were concomitantly analysed by QA. Among the 33 samples with undetectable EBV DNA by RA, 25 remained undetectable, 7 were detected but not quantified and 1 showed a VL of 3.06 log₁₀ IU/mL with EBV QA. Among the 59 samples with EBV DNA detection above LoQ by RA, QA reported 13 as undetectable, 34 as detected but not quantified, and 12 as quantifiable (median VL = 2.89 log₁₀ IU/mL; [2.59-3.9]). Among the 200 samples with quantifiable VL by RA, QA reported 7 as undetectable, 26 as detected below LoQ, and 167 remained positive. The comparison of VL between these 167 samples showed good correlation between assays with $r^2 = 0.84$ (slope = 0.94; $p < 0.001$) with a mean difference of -0.005 (standard deviation = 0,36) log₁₀ IU/mL.

Conclusions: Panther Fusion® EBV QA provided a easy-to-use, fully automated system for EBV DNA quantification with a good performance using whole blood samples.

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Emergence of reduced susceptibility to oseltamivir in influenza A(H1N1)pdm09 due to S247N+I223V mutations in the neuraminidase, 2023-2026, Portugal

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Introduction: Monitoring antiviral susceptibility of influenza is a public health priority. Neuraminidase (NA) mutations can reduce oseltamivir and zanamivir susceptibility. In addition to H275Y substitution, S247N and I223V were unusually detected since 2023. This study aims to evaluate the emergence of S247N+I223V substitutions in the NA of sequenced influenza A(H1N1)pdm09, from 2023 to 2026, in Portugal.

Methods: Influenza-positive nasopharyngeal swabs (PCR Ct≤28), collected through sentinel and hospitals networks, between 2023/24 to 2025/26, were selected for whole-genome sequencing using amplicon-based NGS and analysed with INSAFLU-TELEVIR. NA mutations annotation was done according to 2024 WHO guidelines. MUNANA neuraminidase inhibition assays were performed on viruses with the S247N, I223V and S247N+I223V substitutions.

Results: A total of 1025 NA sequences were analysed (374 in 2023/24; 281 in 2024/25 and 370 in 2025/26). H275Y was detected in two viruses, one of them in combination with S247N. S247N showed a marked increase over time, rising from 0.5% (2/374) in 2023/24 to 5.3% (15/281) in 2024/25 and 41.6% (154/370) in 2025/26. I223V was detected in 1.6% (6/374) of the viruses in 2023/24. S247N+I223V substitutions emerged only in 2025/26 in 4.6% (17/370) viruses. MUNANA assays showed increased IC₅₀ for oseltamivir and zanamivir in viruses with S247N+I223V, although the majority had an increase <10-fold (normal susceptibility). Three viruses showed >10-fold increases, consistent with reduced inhibition to oseltamivir. Viruses carrying S247N alone exhibited slight IC₅₀ increases (<10-fold) for zanamivir and oseltamivir, maintaining normal susceptibility.

Conclusion: The study highlights the emergence of A(H1N1)pdm09 with reduced oseltamivir susceptibility associated with S247N+I223V substitutions. The rapid spread of these dual mutants across multiple continents underscores the need for continuous monitoring of their evolution and transmission.



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Evaluation of the SD Biosensor M10 OPX/MPX rapid RT-PCR Test

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Introduction: The 2022 emergence of MPox (formerly Monkeypox), and the resulting need for rapid diagnosis of suspected cases lead to the development of rapid cartridge-based RT-PCR Nucleic Acid Tests (NATs) by several manufacturers. As the strains responsible for the 2022 outbreak mostly belonged to Clade II (formerly West-African clade), these tests were geared towards detecting isolates of this clade. However, in late 2025/early 2026 infections with the other dominant MPox clade, Clade I (Formerly Congo basin clade) began to appear outside of Africa. The OPX/MPX-cartridge developed by SD Biosensor for their M10 instrument is currently the only rapid NAT test on the market that is able to specifically detect this clade.

Aim: To perform an evaluation of the SD Biosensor M10 OPX/MPX rapid RT-PCR test in a retrospective study.

Materials and Methods: Anonymized leftover clinical samples from the 2022 outbreak, from 2023-2025, and from a small cluster that recently appeared in the Zurich region were selected. These samples had all been identified as MPX-positive and been assigned to clade Ib (n = 4) or II (n = 3) using laboratory derived test (LDT)s for routine diagnostics. Additionally, four quality control specimens obtained from QCMD, including two samples containing non-MPX Orthopoxviruses, and 2 negative samples were tested.

The OPX/MPX-cartridges were prepared and run according to the manufacturer's instructions.

Results: The results obtained with the SD Biosensor M10 OPX/MPX cartridge were 100 % concordant with the results obtained with previous LDT analyses for both clinical and quality control samples, both for MPX positivity and clade assignment.

Conclusion: The SD Biosensor M10 OPX/MPX cartridge test is a useful tool for the rapid diagnostic analysis of suspected MPX infections of both Clades I and II.

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Genomic Diversity and F Protein Substitutions Associated with Monoclonal Antibody Susceptibility in Respiratory Syncytial Virus Circulating in Portugal, 2025/2026 season

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Introduction: Respiratory syncytial virus (RSV) is a major cause of acute lower respiratory tract infections in infants, young children, and older adults, associated with significant morbidity and mortality. The fusion (F) protein is a key target for vaccines and monoclonal antibodies such as palivizumab and nirsevimab. Portugal implemented nirsevimab immunization for newborns and infants in the 2023/2024 season, with nationwide rollout in 2024/2025. This study aims to characterize the genetic diversity of RSV circulating in Portugal and monitor F protein substitutions potentially associated with monoclonal antibody resistance during the 2025/2026 season.

Material and Methods: RSV from children and adults with respiratory infections during the 2025/2026 season were sequenced. Whole-genome sequencing was performed using the Illumina platform. Lineage assignment, F gene translation and alignment were performed using Nextclade.

Results: We analyzed 123 RSV strains (82 RSV A, 41 RSV B) collected from weeks 44/2025 to 05/2026. Eleven RSV A and five RSV B lineages were identified, with A.D.3.11 (34.1%, 28/82) and B.D.E.1 (61.0%, 25/41) being predominant. Among RSV A strains, two isolates (2.4%) carried the N63S substitution in antigenic site Ø of F protein, without impact on nirsevimab sensitivity. All RSV B sequences had S211N, and 97.6% also had Q209R. Both are known polymorphisms in antigenic site Ø not associated with reduced nirsevimab susceptibility.

Conclusions: RSV strains circulating in Portugal showed high genetic diversity during the 2025/2026 season. No F protein substitutions previously associated with monoclonal antibody resistance were detected. Integration of genomic and epidemiological data improves understanding of RSV evolution and supports global efforts to prevent severe RSV infection specially in high-risk groups



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Epidemiological trends of viral respiratory infections during and after the COVID-19 pandemic in an Algerian hospital

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Introduction and purpose: COVID-19 pandemic significantly modified the epidemiology of respiratory viral infections; measuring the impact of these changes is important to know how to face this health problem.

The objective of our study is to compare the epidemiological and virological characteristics of respiratory viral infections during and after the COVID-19 pandemic using multiplex PCR.

Methods and materials: This is a retrospective comparative study was conducted in Central Military Hospital in Algeria, including patients investigated for respiratory viral infections during two periods: the COVID-19 period (from 1st June 2020 to 31 December 2022) and the post pandemic period (from 1st January 2023 to 30 April 2026). Respiratory samples were analysed using multiplex PCR assays using QIAstat -Dx Respiratory panel and Biofire FilmArray RP 2.1 plus respiratory panel.

Results: A total of 1251 nasopharyngeal swabs were included with 386 positive PCR from 1005 test (38,41% of positivity rate) for the pandemic period and 160 positive PCR from 246 test (65,04% of positivity rate) for post pandemic period. The mean age of patients between was 41,31 years and 44,07 years for the first and the second period respectively. During the pandemic Sars-Cov2 Predominated with a rate of 42,48% followed by Rhinovirus/Enterovirus 34,41% however Influenza and RSV represented just 5,01 % and 2,98 % respectively of positive samples. After pandemic analysed samples showed Predominance of Rhinovirus/Enterovirus infections (25,62%) followed by Influenza viruses, Sars-Cov2 and RSV virus with 19,37% ,12,50% and 11,25% respectively.

Discussion and Conclusion: The epidemiology of respiratory viral infections changed considerably after COVID-19 pandemic. Sars-Cov2 took an important place between viral respiratory agents whereas the pandemic impact on Influenza viruses and RSV was significant which would likely from the prevention measures implemented against Sars-Cov2.

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Investigating the role of two-pore channels in SARS-CoV-2 infections

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Introduction: From the start of the SARS CoV-2 pandemic, throughout different waves and later, the virus has been continuously mutating until today. Early variants of SARS-CoV-2 such as EU.1, Alpha, and Delta use the membrane fusion and endosomal pathway equally to enter host cells. However, the Omicron variant relies more on the endosomal pathway. In the endosome-mediated pathway, acidic conditions of endosomes and lysosomes activate proteases that cleave viral envelopes. This cleavage releases viral genomes to enter the nucleus for replication. In the endo-lysosome compartment, ion channels regulate release of the virus from the late endosomes/endolysosomes into the cytosol. One of the major ion channels is the Two-Pore Channel (TPC). TPCs are of three types: TPC1, TPC2, and TPC3. Only TPC1 and TPC2 are found in humans. TPC1 is primarily located in early endosomes and lysosomes. TPC2 is found in late endosomes and lysosomes.

Objectives:

- To study SARS-CoV-2 host cell entry pathway.
- To assess the role of TPC in SARS-CoV-2 infection and pathogenicity.

Material and methods: For this study, TPC1, TPC2, and TPC1+TPC2 double knockout cell lines in A549-ACE2 human lung cells using the CRISPR/Cas9 knockout system. We performed viability assay and confocal microscopy to study virus cell entry and infectivity in A549-ACE2 cells.

Results: Viability assays suggested that knocking out TPCs help prevent Omicron XBB.1.5 infection more effectively than the Delta and EU.1, early variant. Confocal microscopy revealed Omicron infected cells contains significantly higher early and late endosomes compared to early variants.

Conclusion: The data supports that Omicron virus infection relies more on the endosomal pathway than the Delta virus.

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Comparative analytical sensitivity of antigen rapid tests and PCR assays for detection of Monkeypox virus across subclades

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The co-circulation of multiple monkeypox virus (MPXV) clades, including the emerging clade Ib, highlights the need for diagnostic tools with consistent performance across clades. Comparative evaluations remain limited as few laboratories have access to isolates representing all four clades (Ia, Ib, IIa, IIb). We conducted an analytical benchmarking of eight antigen- and PCR-based assays across all clades.

Analytical sensitivity was assessed for three antigen rapid diagnostic tests (Ag-RDTs) and two near point-of-care (POC) molecular assays using serial dilutions of cultured MPXV isolates of all clades, tested in triplicate. Additionally, five manual PCR kits were evaluated using serial dilutions of extracted MPXV DNA in five replicates. Limits of detection (LOD) were defined as the lowest concentration detected in all replicates.

All molecular assays (POC and manual PCR kits) showed high sensitivity with an LOD of ~10–100 copies/mL, with no major differences between clades. However, clade-dependent limitations were observed for multiplex assays: the Xpert assay failed to detect clade Ib via its MPXV target; the TIB Molbiol assay detected clades Ia and Ib via its clade II target; and the iSolutions kit failed to detect clade Ib via its clade I target. All Ag-RDTs showed a much lower sensitivity, with LODs of 10y- 10ycopies/mL.

This study provides a comprehensive analytical comparison of mpox diagnostic assays across all four MPXV clades. While molecular assays show high sensitivity, target design may influence clade-specific detection. The utility of simple and affordable tests such as Ag-RDTs is limited by their low sensitivity, whereas highly sensitive POC molecular platforms remain costly, and manual molecular assays require specialized laboratories and personnel, limiting their access. These findings highlight the importance of multi-clade validation and the need for continued development of accurate and affordable MPXV diagnostics. Currently, the lack of such tools, limits diagnostic capacity for outbreak response and our understanding of mpox epidemiology.

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The Overlooked Threat in Chronic Hepatitis B Management: Analysis of Delta Antibody Request Rates in HBsAg-Positive Patients and the Need for Reflex Testing

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EGE ÜNİVERSİTESİ

Background & Aim: HDV is a severe infection increasing cirrhosis and cancer risks in Hepatitis B patients. International guidelines strongly recommend Anti-HDV screening at least once in HBsAg-positive individuals. This study aims to demonstrate the necessity of an automated reflex testing model through retrospective analysis of HDV screening rates at a tertiary center.

Methods: HBsAg, Anti-HDV, and HDV RNA results processed via LIS between 01.01.2025 and 14.05.2026 were analyzed. Duplicates were eliminated to evaluate unique patient data. Patients with no prior test history in the system were defined as “newly diagnosed HBsAg-positive cases.”

Results: Over the 1.5-year period, 2,828 unique HBsAg-positive patients were identified. Anti-HDV was requested for 420 (14.9%) of the general group; 47 (11.2%) tested positive. Among 669 newly diagnosed cases, Anti-HDV was requested for only 187 (27.9%), and HDV RNA for 63 (9.4%). Overall, among 98 Anti-HDV positive patients, HDV RNA was requested for 68 (69.4%), with viremia detected in 21 (30.9%). Concurrent HBsAg positivity was absent in the current cross-section for 78.3% (n=1,515) of 1,935 patients with Anti-HDV requests.

Conclusion: While general screening rates may be influenced by past testing, our study clearly shows that Anti-HDV screening is missed at diagnosis in 72% of newly diagnosed cases. Furthermore, failing to request HDV RNA for 30% of confirmed Anti-HDV positive patients highlights a need for standardization. These real-world data prove that implementing automated “Reflex Anti-HDV” and subsequent “Reflex HDV RNA” algorithms via LIS is essential to close diagnostic gaps, prevent missed screenings, and standardize clinical care.

Keywords: HBsAg, HDV, Anti-HDV, Reflex Testing, HDV RNA.



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Analytical Sensitivity of Influenza Antigen Rapid Tests for Detection of Avian A(H5N1) and Seasonal Influenza Viruses

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Introduction/Aim: The performance of influenza antigen rapid diagnostic tests (Ag-RDTs), originally developed for diagnosing seasonal influenza in humans, remains poorly characterized for the detection of avian influenza A(H5N1) in humans. We evaluated the analytical sensitivity of commercially available influenza Ag-RDTs for A(H5N1) compared to seasonal influenza A viruses.

Materials and Methods: Four commercially available lateral flow Ag-RDTs were assessed using quantified serial dilutions of cultured influenza A viruses, including A(H1N1)pdm09, A(H3N2), and a recent A(H5N1) strain. Viral loads were determined by quantitative RT-PCR and infectious titers (TCID₅₀). Each dilution was tested in duplicate and RDT results were interpreted by two independent readers. Limits of detection (LoD) were defined as the lowest concentration consistently detected.

Results: All assays detected influenza A viruses at high concentrations with no observable differences between A(H5N1) and seasonal strains. Detection thresholds were similar across subtypes, with LoDs ranging from approximately 5.7×10^2 to 2.0×10^3 TCID₅₀/mL ($\approx 10^4$ – 10^5 RNA copies/mL), depending on the assay. Only one test showed slightly higher sensitivity at lower concentrations. At 2×10^2 TCID₅₀/mL or 7.5×10^5 RNA copies/mL, all assays lost their detection capacity.

Conclusion: Influenza Ag-RDTs show similar analytical sensitivity for avian A(H5N1) and seasonal influenza A viruses but their overall sensitivity remains low. Detection thresholds overlap with the range if viral loads reported in human A(H5N1) infections, indicating that a substantial proportion of cases may be missed. As analytical sensitivity was assessed using cultured virus under controlled conditions, results may not reflect the complexity of clinical specimens. In addition, differences in viral tropism—upper respiratory tract for seasonal influenza versus primarily lower respiratory tract involvement for A(H5N1)—may further reduce sensitivity in routine sampling. Clinical studies are needed to confirm diagnostic performance in real-world settings.

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Community-Acquired Viral Meningitis and Meningoencephalitis: A 10-Year Multiplex PCR Analysis

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Objective: To analyze the epidemiological and microbiological characteristics of community-acquired viral meningitis and meningoencephalitis.

Materials and Methods: A retrospective descriptive study was conducted in the microbiology laboratory of the Central Military Hospital of Algiers between November 2016 and May 2026. A total of 373 cerebrospinal fluid (CSF) samples were included (male-to-female ratio: 2.66; 193 children and 180 adults). CSF samples were analyzed using the FilmArray Meningitis/Encephalitis Panel (FMEP).

Results: Among the 373 CSF samples analyzed, 130 (35%) tested positive by multiplex PCR. Viral etiologies accounted for 55% of positive cases, while bacterial etiologies represented 34%. Approximately 65% of positive samples originated from the emergency department. Multiplex PCR identified 13 different pathogens: 6 viral, 6 bacterial, and 1 *C. neoformans*. Enterovirus (n=49) was the most frequently detected pathogen, followed by *S. pneumoniae* (n=19). Eleven cases (7 children) of co-infection were identified (9%).

Viral etiologies were detected in 61 children (prevalence: 31.61%) and 11 adults. Enterovirus accounted for 68% of viral cases (prevalence: 38%; 47 cases in children and 2 in adults), followed by HHV-6 (11%; prevalence: 6%; 8 cases in children) and HSV-1 (11%; prevalence: 6%; 4 children and 4 adults). VZV represented 6% (1 child and 3 adults), while CMV accounted for 4% (1 child and 2 adults).

Enterovirus infections were mainly observed in children aged 0–3 months (n=17), 4 months–2 years (n=7), 3–5 years (n=7), and 6–15 years (n=16).

A seasonal increase in viral meningitis cases was observed during summer (32%) and spring (31%). Most viral meningitis cases presented with clear CSF (91.49%), with pleocytosis ranging from 10 to 500 cells/mm³ (74.42%), including 48% lymphocytic predominance. CSF biochemistry was variable.

Conclusion: Viral etiologies represent a significant proportion of community-acquired meningitis and meningoencephalitis, with enteroviruses being the predominant pathogens in children.

Keywords

Meningitis; multiplex PCR; viral etiologies

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Contribution of Viral Etiologies in Community-Acquired Meningitis and Meningoencephalitis Diagnosed by Multiplex PCR

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Objective: To evaluate the contribution of viral etiologies in community-acquired meningitis and meningoencephalitis diagnosed by multiplex PCR.

Materials and Methods: A retrospective descriptive study was conducted in the microbiology laboratory of the Central Military Hospital of Algiers between November 2016 and May 2026. A total of 373 cerebrospinal fluid (CSF) samples collected from patients with suspected community-acquired meningitis or meningoencephalitis were included. The study population comprised 193 children and 180 adults, with a male-to-female ratio of 2.66. The mean age of patients was 16.2 years (range: 1 day–81 years). CSF samples were analyzed using the FilmArray Meningitis/Encephalitis Panel (FMEP), a real-time multiplex PCR assay.

Results: Among the 373 CSF samples analyzed, 130 (35%) tested positive by multiplex PCR. Viral etiologies accounted for 55% of positive cases (61/72 cases in children), whereas bacterial etiologies represented 34% of cases (25/44 cases in adults). Multiplex PCR enabled the identification of 13 different etiologies: 6 viral, 6 bacterial, and 1 *C. neoformans*. Enterovirus (n=49; 38%) was the most frequently identified pathogen, followed by *S. pneumoniae* (n=19; 15%). Eleven cases of co-infection (9%) were detected.

Positive CSF samples were obtained from 87 children (prevalence: 45%) and 43 adults (prevalence: 24%). Among viral etiologies, enterovirus accounted for 68% of cases, followed by HHV-6 and HSV-1 (11% each, n=8), VZV (6%, n=4), and CMV (4%, n=3).

Enterovirus infections occurred predominantly in children: 0–3 months (n=17), 4 months–2 years (n=7), 3–5 years (n=7), 6–15 years (n=16), and >15 years (n=2). A seasonal increase in viral meningitis cases was observed during summer (32%) and spring (31%).

Conclusion: Viruses represent a major cause of community-acquired meningitis and meningoencephalitis, particularly in children. Multiplex PCR significantly reduced diagnostic turnaround time and improved therapeutic management.

Keywords: Meningoencephalitis; multiplex PCR; viral etiologies; cerebrospinal fluid.

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Genomic surveillance of monkeypox virus clade Ib in Portugal reveals multiple introductions and potential sustained transmission during 2026

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Introduction/Aim: Portugal was one of the first countries to report monkeypox virus (MPXV) cases during the 2022 multinational outbreak. Since the onset of the outbreak, only MPXV clade Ib lineages had been identified in Portugal. Following the spread of MPXV clade Ib across Europe, the first Portuguese clade Ib case was detected in late 2025, with an increase in confirmed cases during 2026. This study aimed to characterize the genomic diversity of MPXV clade Ib circulating in Portugal and investigate introduction events and transmission patterns.

Materials and Methods: Clinical samples from MPXV clade Ib-confirmed cases identified through routine molecular diagnosis between October 2025 and May 2026 were subjected to whole genome sequencing and analysed with the INSaFLU-TELEVIR bioinformatics platform.

Results: Phylogenetic analysis of MPXV clade Ib cases identified three genomic groups. One sequence detected in late 2025 clustered with African MPXV clade Ib strains, representing the first introduction of clade Ib in Portugal, for which no transmission has been identified to date. In contrast, all sequences in 2026 grouped within the circulating European/global clade Ib lineage, including a second group of two identical sequences in February 2026 in northern Portugal and a third group of seven sequences in the Lisbon Metropolitan Area during March and April 2026. The third group was closely related to the German-associated cluster circulating in Europe, suggesting sustained transmission linked to the broader European outbreak network. As of May 2026, 15 confirmed clade Ib cases have been identified nationally and sequencing analysis of the remaining confirmed cases is ongoing.

Conclusions: Our study identified independent introductions and ongoing circulation of MPXV clade Ib in Portugal. The identification of sequences linked to the European clade Ib cluster highlights the critical role of continuous genomic surveillance to monitor viral dissemination, identify transmission chains, and support timely public health interventions.

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Hepatitis B virus (HBV) next generation sequencing (NGS)- 7 years of experience at Barts Health NHS Trust

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Introduction: Barts Health NHS Trust introduced Illumina NGS for HBV in 2019, achieving UKAS accreditation in 2025. The Trust has a large population of people with chronic HBV patients: ~6000 HBsAg-positive people detected via testing (>2% of tested).

Aim: To review seven years of HBV sequencing data to identify novel antiviral resistance mutations, maintain reporting standards, and assess clinical impact.

Methods: HBV nucleic acid was extracted from plasma (VL ≥195 IU/mL). Whole-genome and targeted polymerase/sAg PCR amplicons were sequenced on Illumina MiSeq/Miniseq platforms following Nextera XT library preparation. In-house bioinformatics used a 15% variant threshold to generate consensus sequences analysed using HBV-GRADE, Geno2Pheno, and Stanford HBVseq.

Results: Overall, 298 samples from 261 patients were analysed and genotypes D (32.2%), E (30.2%), A (15.7%), C (12.2%), B (9.1%), and F (0.4%) identified. Lamivudine/telbivudine resistance mutations L180M+M204V were detected across major genotypes, particularly A and C. Entecavir-associated S202G mutations were observed, mainly in genotype C. A tenofovir-associated A194T mutation was detected in 2 treatment-naïve patients, suggesting possible transmitted resistance. Among 22 patients with longitudinal samples, resistance emerged in 5 receiving entecavir or lamivudine. Minority variant surveillance identified significant resistance mutations in 1 patient. Two patients required treatment modification following sequencing review.

Conclusions: HBV genotyping results were available 94% of cases. Significant genotype diversity supports continued genotype-informed resistance surveillance in our large multi-ethnic HBV cohort. Antiviral resistance mutations were identified across multiple HBV genotypes, including possible transmitted tenofovir resistance. Longitudinal NGS detected emerging minority resistance variants before they became clinically dominant, albeit patient numbers analysed are small. Further analysis of pre-core and core promoter mutations may improve understanding of treatment response and disease progression.

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Standardization of RSV-FRNT and Insights into Maternal–Neonatal Transplacental Antibody Transfer

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Introduction: Respiratory Syncytial Virus (RSV) is a major cause of lower respiratory tract infections in infants. This study standardized RSV fluorescence reduction neutralization test (FRNT), assessed in maternal and cord blood neutralizing antibody (Ab) titers (ND50), evaluated factors affecting transplacental Ab transfer and three months follow up was done

Material and methods: A total of 240 paired maternal and cord blood samples were collected during delivery at JIPMER (2024–2026). RSV-G-FP neutralization assay in Vero cells was standardized using the NIBSC reference standard (16/284) by assessing LOD, GSD, GCV, repeatability, and reproducibility. Viral foci were quantified by EVOS M7000 Imaging System. Neutralizing Ab titers (GMT) and factors affecting maternal–cord Ab transfer were analysed.

Results: The RSV-FRNT showed a strong linear relationship ($R^2=0.98$), indicating high sensitivity and reliable quantification of neutralizing Abs. Both intra- and inter-assay evaluations showed acceptable precision and accuracy. The mean log₂ RSV antibody titres were 9.7 in maternal and 9.8 in neonates, while the GMT was 630.73 IU/mL and 696.77 IU/mL, respectively. The cord-to-maternal Ab (CMR) ratio was 1.03, indicating efficient transplacental Ab transfer, which was statistically significant ($p<0.001$). Among the 240 mother–neonate pairs, 68% showed higher transfer efficiency, whereas 32% demonstrated lower transfer. Regression and multivariable analyses identified maternal Ab titre as the primary determinant of transplacental Ab transfer efficiency, whereas other demographic, obstetric, and clinical factors, showed no statistical significance. In neonates, no hospitalization was noted during the follow up period.

Conclusion: Standardization of RSV-FRNT assay for reliable quantification of neutralizing Abs was achieved successfully. Efficient transplacental transfer of RSV neutralizing antibodies was demonstrated, with maternal antibody titre identified as the key determinant of transfer efficiency which helps early protection against RSV infection.



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Surveillance and molecular characterization of Tick-borne encephalitis cases in north-eastern Italy

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Tick-borne encephalitis (TBE) is one of the most important diseases in Europe. The Tick-borne encephalitis virus (TBEV), usually transmitted by tick bites can cause bi-phasic fever and severe neurological complications¹. In north-eastern Italy, Verona province, TBEV is expanding its geographical range. For this reason, since 2023 our hospital is collecting ticks and blood samples from bitten subjects and performing species identification, human serology, molecular testing in both ticks and humans plus molecular characterization and phylogeny of positive cases.

RNA was extracted from human whole blood with the EZ1 DSP Virus kit (Qiagen) and from tick via RNAGEM Kit (MicroGEM). TBEV molecular detection was performed via alphaCube TBE (Mikrogen Diagnostik). Sanger and NGS characterization were performed via Genetic Analyzer 3500 (ThermoFisher) and NextSeq1k (Illumina) respectively. Public sequences were downloaded from NCBI virus and phylogenetic trees were built via iqtree.

At the end of 2025, thirteen subjects were tested positive for IgM and IgGs. Of these, three resulted positive to molecular assay. For one of the subjects, it was possible to analyse the associated tick, which tested positive for TBEV. Interestingly, this tick and its human host were coming from a reportedly TBE-free area of north-east Italy. Due to Ct constraints, only two TBEV of the three molecular-positive subjects and the one from the tick were characterized. Phylogenetic analysis showed that these three sequences diverged accordingly to their geographical separation. The geographical spread and presence of phylogenetically different viruses confirm the importance of TBEV genomic surveillance.

1. Pulkkinen LIA, Barrass SV, Domanska A, Överby AK, Anastasina M, Butcher SJ. Molecular Organisation of Tick-Borne Encephalitis Virus. *Viruses*. 2022 Apr 11;14(4):792. doi: 10.3390/v14040792. PMID: 35458522; PMCID: PMC9027435.

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Human-Derived Japanese Encephalitis Virus Genotype V Isolates in Korea Reveal Distinct Domestic Clades and Unique Molecular Signatures

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Introduction/Aim: Japanese encephalitis virus (JEV) genotype V (GV) is the predominant circulating genotype in Korea. However, only one human-derived GV isolate from Korea had previously been characterized. This study investigated additional human-derived GV isolates collected between 2018 and 2023.

Material: Cerebrospinal fluid samples from unvaccinated Japanese encephalitis patients positive for JEV RNA by real-time RT-PCR were used for virus isolation. Three human-derived GV isolates (K18P80, K23P84, K23P88) were obtained using LLC-MK2 cells.

Methods: Complete open reading frame sequences (~10.3 kb) were analyzed. Phylogenetic and amino acid analyses were performed using representative JEV strains, including domestic mosquito-derived isolates.

Results: The three isolates formed a lineage distinct from the prototype Muar strain and reference sequences. Korean human-derived GV isolates were divided into two domestic clades, with the 2015 isolate clustering separately from the 2018 and 2023 isolates despite association with the same mosquito species, *Culex bitaeniorhynchus*. Unique envelope protein substitutions (Q52E, S156T, D292E) and additional substitutions in NS3 and NS5 were identified. Similar patterns were also observed in domestic mosquito-derived isolates. Compared with genotype III vaccine strains, the isolates showed relatively low sequence similarity (79% nucleotide and 91.1% amino acid identity), suggesting regional molecular diversity among Korean GV isolates.

Conclusions: These findings expand the limited genomic data of human-derived JEV GV and demonstrate distinct domestic clades and characteristic molecular signatures among Korean isolates, supporting continued genotype-based molecular surveillance.



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Influenza A and B seroprevalence and cross-protection against the new A(H3N2) subclade K: a cross-sectional survey, Portugal, 2025

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Introduction: The 2025/26 season was dominated by influenza A subtypes and the emergence of the new influenza A(H3N2) subclade K, genetically and antigenically distinct from the previous circulating viruses. Our aim was to estimate the prevalence of protective antibodies against influenza A subtypes, including the new subclade K, and influenza B by age group and previous season vaccination status, in Portugal, during the summer 2025.

Methods: An annual cross-sectional seroepidemiological survey was conducted in August 2025. 33 laboratories across the country selected residual sera by age group (0–4, 5–14, 15–44, 45–64 and ≥65 years). Sera were tested by haemagglutination-inhibition (HAI) assay (HAI titre ≥40 was seroprotective). We estimated the proportion of population with protective antibodies and geometric mean titer (GMT), with 95% confidence intervals (95%CI), against vaccine (A/Victoria/4897/2022, A/DO.Columbia/27/2023, B/Austria/1359417/2021) and circulating (A/Missouri/11/2025, A/Norway/8765/2025_subclade K) strains.

Results: A total of 882 sera were selected from all age groups. Vaccination status was recorded for 156 sera, 61.5% were vaccinated (people aged ≥45 years). The highest seroprevalence was against A(H3N2) A/DO.Columbia/27/2023 (61.8%; 95%CI: 56.2–67.7) followed by A(H1N1) pdm09 A/Missouri/11/2025 (28.1%; 95%CI: 24.4–32.0). Cross-reactive antibodies against the emerged A(H3N2) subclade K, A/Norway/8765/2025, was found in 14.8% (95%CI: 12.2–17.8). Seroprevalence was highest in children aged 5–14 (26.7%; 95%CI: 20.2–34.1) and lowest in those aged ≥65 (11.2%; 95%CI: 7.0–16.8). GMT followed the same pattern. Seroprevalence overall against B/Austria/1359417/2021 was 23.6% (95%CI: 20.4–27.1). Vaccinated individuals had higher seroprevalence and GMT vs. non-vaccinated.

Conclusions: Higher seroprevalence was observed against influenza A(H3N2) and in children aged 5–14 years. Vaccination appeared to induce broader antibody responses against circulating and emerging influenza strains, crucial for guiding vaccination strategies and public health interventions.

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Missed Early, Diagnosed Later: A 10-Year Observational Study of Retrospective Congenital CMV Diagnosis Using Newborn Dried Blood Spots

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Introduction/Aim: Congenital cytomegalovirus (cCMV) infection is one of the most common congenital infections worldwide and a major cause of long-term morbidity, including sensorineural hearing loss (SNHL) and neurodevelopmental impairment. Definitive diagnosis requires CMV DNA detection in saliva or urine within the first three weeks of life; however, many cases are missed during this period. This study presents a 10-year observational analysis using archived dried blood spots (DBS), collected in the first week of life, enabling retrospective cCMV diagnosis beyond the standard diagnostic period.

Material/Methods: Between 2016 and 2025, 1,268 archived DBS samples were tested for CMV DNA at paediatricians' request, based on clinical suspicion of cCMV. The study population included children aged 4 weeks to 10 years (708 males; 560 females). Viral DNA was extracted using a validated DBS-specific commercial purification method and detected by a CMV-targeted real-time PCR commercial assay.

Results: CMV DNA was detected in 58/1,268 children (4.6%), with equal sex distribution. Most positive cases (40; 69.0%) were referred within the first year of life (23 males; 17 females). The most frequent clinical indication was SNHL (23/58), followed by microcephaly, psychomotor delay, and lenticulostriate vasculopathy (14/58). Further indications included positive viruria (9/58) and suspected cCMV without additional detail (8/58); data were unavailable for 4 cases.

Conclusions: CMV DNA remains detectable in archived neonatal DBS for years after birth, providing a highly specific and clinically useful tool for retrospective cCMV diagnosis when the standard diagnostic window has been missed. Improvements in methodology have increased sensitivity, reinforcing the role of DBS-based testing in enabling timely intervention for children with SNHL or neurodevelopmental concerns. By facilitating diagnostic confirmation beyond the neonatal period, this approach aids clinical decision-making and may improve long-term outcomes. Also supports DBS-based testing as a relevant cCMV screening option.

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Assembly of Viral Genomes from Long-Read Metagenomic Sequencing of Febrile Returning Travelers

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Introduction/Aim: Febrile illness in returning travellers is associated with a wide range of potential pathogens. Clinical metagenomic sequencing provides an untargeted approach for pathogen detection directly from patient samples, while long-read sequencing technologies offer additional advantages for genome assembly and rapid analysis. We aimed to evaluate the use of Oxford Nanopore long-read metagenomic sequencing for the recovery and assembly of viral genomes from clinical samples collected from returning travellers presenting with fever.

Method: Plasma samples underwent nucleic acid extraction followed by metagenomic sequencing using an ONT MinION sequencing device. Bioinformatic analysis incorporated host-read depletion, taxonomic classification, reference-based mapping, and de novo assembly approaches to recover viral genomes from metagenomic data. Genome assemblies were assessed for coverage breadth, depth, and consensus accuracy. Phylogenetic analysis was used to identify viral subtypes where possible with comparison to global reference sequences.

Results: Between February 2025 and February 2026 there were 96 plasma samples from febrile returning travellers that underwent long-read metagenomic sequencing, of which nine were positive for viral DNA or RNA. This approach successfully generated partial to near-complete viral genomes of Dengue (n=3) and Chikungunya (n=2) viruses.

Conclusions: Long-read clinical metagenomics is a helpful diagnostic tool for febrile returning travellers. These results demonstrate the potential for simultaneous viral genome characterisation directly from clinical samples that could support genotyping and outbreak investigation if integrated into routine workflows.

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Molecular Detection of Imported and Probable Autochthonous Oz Virus Infection in South Korea

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Background: Oz virus (OZV), a tick-borne thogotovirus belonging to the family Orthomyxoviridae, has recently emerged as a potential zoonotic pathogen in East Asia. Following the first fatal human case reported in Japan in 2023, concerns regarding undetected circulation in neighboring countries have increased. In South Korea, molecular diagnostic assays for OZV were proactively established to investigate unexplained tick-borne febrile illnesses.

Method: Residual serum specimens collected from patients with suspected tick-borne infections who tested negative for Severe Fever with Thrombocytopenia Syndrome virus (SFTSV) were retrospectively screened for OZV RNA. A total of 1,017 SFTSV-negative specimens collected in 2023 and 1,853 specimens collected in 2025 were analyzed using in-house real-time RT-PCR assays targeting the PB2 and M segments of the OZV genome. Positive samples underwent sequencing analysis and epidemiological investigation.

Results: Among the 2023 specimens, one OZV-positive case was identified in a 56-year-old woman with recent travel history to Japan and documented tick exposure. Sequencing analysis showed 96.7% nucleotide similarity to the Japanese OZV EH8 strain. Among the 2025 specimens, one additional OZV-positive case was identified in an 85-year-old woman without international travel history, suggesting probable autochthonous infection. The patient had a history of outdoor agricultural activity and presented with fever, chills, and myalgia. Sequence analysis demonstrated complete identity to the Japanese EH8 strain within the amplified region. Both cases were initially investigated as suspected SFTS cases due to overlapping clinical manifestations.

Conclusion: This study provides the first molecular evidence of imported and probable domestically acquired Oz virus infection in South Korea. The findings suggest possible undetected circulation of OZV in local ecological cycles involving ticks and animal reservoirs. Enhanced surveillance for unexplained tick-borne febrile illness is warranted.

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Human Papillomavirus Detection in Laryngeal Papillomatosis

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Introduction/Aim: Human papillomavirus (HPV) infection in the head and neck region constitutes a significant public health concern due to its association with malignancy. Squamous cell carcinoma accounts for approximately 90% of head and neck cancers (HNSCC). Although tobacco and alcohol consumption remain the predominant risk factors, the aetiological landscape of HNSCC is shifting, with increasing evidence supporting a growing role of HPV infection in tumour development.

The objective of this study was to analyze the HPV infection in biopsies from the larynx and the oral cavity with suspected papillomatosis.

Methods: A total of 58 fresh biopsies were analysed between 2019 and 2026. HPV detection and genotyping were performed using the AllplexTM HPV28 Detection assay (Seegene Inc., Korea). Additionally, all samples were screened using conventional PCR with MY09/11 primers to detect possible infections by other HPV types, and positive samples were sequenced.

Results: This study included 58 samples (33 males; 25 females; mean age 39.3 years) with lesions suggestive of papillomatosis. HPV infection was detected in 34.5% (20/58) of samples. Among positive infections, HPV 6 was the most prevalent with 90% (18/20), followed by HPV 16 and HPV 32, both with 5% (1/20) each. The HPV 32 was detected in the sample suspected of Heck's disease.

Conclusions: The predominance of HPV 6 is consistent with established laryngeal papillomatosis associated with low-risk genotypes, namely HPV 6 and HPV 11. The identification of the low-risk genotype HPV 32 in a lesion suggestive of Heck's disease further supports its recognized association with benign oral cavity lesions.

In contrast, the detection of HPV 16, a high-risk genotype, is well established in the literature as being associated with HNSCC, especially in the oropharynx.

Its presence in cases diagnosed as laryngeal papillomatosis may represent a potential risk factor for disease progression and malignant transformation, warranting careful clinical monitoring.

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Genomic surveillance of influenza A(H1N1)pdm09 and A(H3N2) viruses during the 2025/2026 influenza season in Portugal

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Introduction: The 2025/26 season was marked by early detection of A(H1N1)pdm09 alongside the emergence and global spread of A(H3N2) subclade K, which was genetically distinct from the Northern Hemisphere (NH) vaccine strain. This study overviews the genetic diversity of influenza viruses circulating in Portugal during the 2025/2026 season.

Methods: Nasopharyngeal swabs, from hospitals and sentinel networks, influenza-positive with PCR Ct ≤28 were selected for whole-genome sequencing, followed by bioinformatics analysis with INSaFLU-TELEVIR. Clade/subclade classification and phylogenetic analyses were conducted using Nextclade/Nextstrain. Antigenic characterization was performed by hemagglutination inhibition assay.

Results: Influenza A subtypes co-circulated during the 2025/26 season in Portugal. A(H1N1)pdm09 were genetically related to A/Missouri/11/2025 (2026/27, NH vaccine strain). Most sequences belonged to subclade D.3.1.1 (n=360, 97%), with 54.2% clustering within a subgroup characterized by the HA1 substitution D139N. Antigenically 20 A(H1N1)pdm09 viruses were well inhibited by A/Missouri/11/2025 and A/Victoria/4897/2023.

A(H3N2) (n=274) were predominantly assigned to subclade K within the A/Norway/8765/2025 clade (n=250, 91.2%). Within K subclade, a marked genetic diversification was identified, with clusters characterized by distinct mutations in HA (HA1:A328T, A272T, D122E, and S96R; HA2:K143R). A subgroup highlighted the HA1:S96R mutation accounted for 24.4% of the sequences and showed a progressive increase over time. Antigenic characterization showed that 25 viruses were well inhibited by the A/Darwin/1415/2025 and poorly recognized by A/D.O.Columbia/27/2023 anti-sera.

Conclusion: The 2025/26 influenza season in Portugal was marked by the predominance and diversification of A(H1N1)pdm09 D.3.1.1 and the rapid expansion of A(H3N2) subclade K, highlighting the ongoing evolution of influenza viruses and the importance of continuous molecular surveillance.



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Longitudinal characterisation of cell-mediated immune responses following influenza A(H5N8) vaccination

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In 2023, Finland experienced a major outbreak of highly pathogenic avian influenza caused by the clade 2.3.4.4b influenza A(H5N1) virus, with transmission from wild birds to fur farms. In June 2024, Finland became the first country to vaccinate occupational risk groups using the MF-59-adjuvanted influenza A(H5N8) vaccine (A/Astrakhan/3212/2020, Seqirus). We aimed to characterise the quality and durability of vaccine-induced cellular immune responses induced by this novel vaccine. Peripheral blood mononuclear cells were collected from individuals before vaccination and at 3 weeks, 6 months and 12 months after the second vaccine dose. Antigen-specific T-cell responses were analysed using activation-induced marker assay and flow cytometry following stimulation with overlapping H1, H5 and N8 peptide pools. Vaccine-induced B-cell responses were evaluated using ELISpot assay and flow cytometric detection of H5-specific memory B cells. Cytokine responses from stimulated PBMC supernatants were analysed using fluorescent multiplex immunoassay. This work demonstrates robust vaccine-induced H5/N8-specific CD4⁺T-cell and relatively weak CD8⁺T-cell responses after vaccination. Vaccine-induced cellular responses remained detectable 12 months after vaccination. The analyses also indicate induction of H5-specific memory B cells and influenza-specific antibody-secreting cells, with declining kinetics during longitudinal follow-up. The MF59-adjuvanted influenza A(H5N8) vaccine induces detectable cellular immune responses in individuals at occupational risk of avian influenza exposure. This longitudinal analysis provides important information on the durability of influenza A(H5N8) vaccine-induced immunity, which supports future pandemic preparedness and avian influenza vaccine development.

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Mapping the HIV Resistance Landscape in Crete (2023–2025): A Comparative Study of High- and Low-Abundance Mutations.

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Introduction-Aim: Understanding HIV drug resistance under the evolutionary pressure of antiretroviral therapy (ART) is critical for effective clinical management. Next-Generation Sequencing (NGS) has uncovered a complex ecosystem of low-abundance (LA) resistance mutations, though their long-term clinical impact remains under investigation. This study characterizes and compares high-abundance (HA) and LA mutations among ART-naïve and ART-experienced HIV carriers in Crete, Greece.

Materials and Methods: We analysed 74 HIV-positive samples collected between 2023 and 2025 from patients at the University Hospital of Heraklion. NGS was performed using an Illumina platform targeting the Protease (PR), Reverse Transcriptase (RT), Integrase (IN), and gp120 V3 loop regions. Mutations were identified via SMARTVIR v2.0 and the Stanford HIVDB algorithm, utilizing variant-calling thresholds of 20% for HA and 5% for LA.

Results: The predominant viral subtypes were A1 (45%) and B (26%), followed by F1 (9%), G (7%), C (3%), and several circulating recombinant forms. Analysis revealed 74 resistance-associated events. Codon 74 of the Integrase gene displayed the highest frequency among ART-naïve donors (18%), followed by codon 461 of Protease (1%) and RT 184 (7%). Among ART-experienced donors the highest frequency was found in RT 106 (4%), Protease 33 (1%) and Integrase 74 (9%). ART-naïve patients demonstrated cumulatively similar resistance HIVDB scores to PIs, NRTIs, and NNRTIs, although INSTIs indicated increased resistance mutations to ART-experienced individuals. Finally, predicted CCR5-antagonist sensitivity was highly diverse, showing no significant differences between the two groups.

Conclusions: This study provides a vital genomic map of the HIV subtype landscape in Crete, revealing HA and LA transmitted drug resistance in ART-naïve individuals. The persistence of specific mutations without direct drug pressure suggests potential viral evolutionary advantages, warranting deeper investigation into their impact on disease progression and future treatment efficacy.



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Analytical Performance Evaluation of Seegene Allplex RP-Virus (II) Assay for the Diagnosis of Viral Pathogens Using Pediatric Respiratory Tract Infection (RTI) samples

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Introduction/Aim: The increasing number of respiratory viral infections are a major public concern over the last years. Our goal was to run a single-center retrospective comparative study to evaluate the efficacy of the Seegene Allplex RP-Virus (II) Assay in pediatric samples derived from respiratory tracts.

Material/methods: In our analysis 87 patients under 18 years of age with suspected upper respiratory tract infections between January 2018 and March 2026, whose nasopharyngeal swabs collected in saline were previously tested for respiratory pathogens by BioFire Respiratory Panel 2.1 plus and Panel 2 (Reference) respectively, were enrolled. All the 87 patients had at least 500 µL sample as remnant stored at -24 °C so we could perform Seegene Allplex RP-Virus (II) assay (Test) on our collection. Then our results were compared to the results of the BioFire. Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OPA) were calculated.

Results: Based on Reference assays, of the 87 clinical specimens 50 was negative for the pathogens tested, 33 was positive for 1 viral pathogen and 4 for 2 viral pathogens. 26 Rhino/Enteroviruses, 9 Parainfluenza viruses (PIVs) (eight PIV3 and one PIV2), 3 Adenoviruses and 3 Metapneumoviruses cases were detected. Test assay detected 39 positive specimens, although dismissed detecting some of the pathogens in co-infections. PPA was 91,9%, NPA was 93,9% and OPA was 93,0%.

Conclusions: Overall, the study showed a high level of agreement between the Test assay and the Reference assays. However, as the number of positive for 1 or 2 viral pathogens were low, further studies with larger sample sets would be more advantageous to achieve a more definitive conclusion, especially regarding the detection of co-infection.

While Seegene Allplex RP-Virus(II) provides a more feasible, cost-effective, high-throughput performance to BioFire Respiratory Panel, the latter functioning as a POCT-like method provides an extremely short turn-around-time and has a unique agreement for co-infections based on our research.

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From neglected cold to SARI: Genomic characterization of human rhinovirus in Brazil

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Human Rhinovirus (HRV) is a major driver of respiratory morbidity, causing significant economic burden and severe illness even in immunocompetent individuals. Since Brazil implemented systematic testing in 2023, HRV has emerged as a leading cause of Influenza-like Illness (ILI) and Severe Acute Respiratory Syndrome (SARI), with impact comparable to influenza and RSV. However, its vast genomic diversity hinders vaccine and antiviral development.

We characterized HRV infections in Brazil using samples from 2023–2025. Genomic analysis involved capture-enrichment or VP1/VP4 sequencing. Reads were processed using SPAdes and ViralFlow 1.0, with phylogenetic reconstruction via iQTree 2.4.0.

National SIVEP-Gripe data identified >55,000 HRV-associated SARI cases, primarily affecting children under 14 (83%), with a 3% fatality rate. In our laboratory, 891 HRV-positive samples were recovered; 746 were eligible (398 ILI, 348 SARI, 4 fatalities). Among participants, 404 (54%) were male and 263 (35%) were under 14 years-old. From 127 sequenced samples (63% success), 80 complete genotypes were identified across 62 distinct strains (41 HRV-A, 14 HRV-B, 7 HRV-C). Genotypes were distributed across SARI (58%) and ILI (61.2%) as follows: for ILI 21 HRV-A, 10 HRV-B, 7 HRV-C; and for SARI, 25 HRV-A, 8 HRV-B, 3 HRV-C), revealing a complex mosaic of continuous co-circulation without a dominant strain. While concentrated in the Southeast, high diversity was observed nationwide. Three pediatric fatalities were linked to HRV-A12, -A19, and -B70. Additionally, a case of prolonged shedding (44 days) revealed four nucleotide substitutions, offering insights into intra-host evolution.

No genotype-severity correlations were established due to extensive genetic variety. These findings provide a robust genomic snapshot of HRV in Brazil, demonstrating that HRV is a significant pathogen that warrants integration into routine molecular surveillance and prioritized therapeutic development.



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Diagnostic Value of Multi-Viral Intrathecal Antibody Synthesis in Neurological Disorders

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Introduction/Aim:

To date, French guidelines limit intrathecal antiviral IgG synthesis (ITS) testing to suspected Varicella-Zoster virus (VZV) encephalitis with a negative PCR. This study evaluates the clinical relevance of testing for anti-virus IgG in the cerebrospinal fluid (CSF) in various central and peripheral neurological manifestations.

Materials and Methods: A prospective monocentric study (2018–2026) included patients with neurological symptoms, paired serum and CSF samples collected within 24 hours. Specific antiviral IgG assays were performed on DiaSorin® Liaison XL, total IgG and CSF was measured on Roche® Cobas (serum) and Siemens® BNII (CSF). The viral antibody index (AI) was calculated as (specific IgG CSF/serum)/(total IgG CSF/serum), with positivity defined as AI ≥ 1.5. In total, 306 patients were included who had at least two viral AIs assessed among Herpes simplex virus (HSV), VZV, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), measles virus (MeV), and rubella virus (RuV).

Results: Isolated VZV ITS was identified in 36/277 patients (median AI 5.2 [1.7–46]), mainly associated with meningoradiculitis (n=13), meningitis (n=4) meningoencephalitis (n=4) and myelitis (n=4). Isolated CMV AI (median 15.1 [2.5–28.6]) was observed in 4/63 patients, all presenting with Guillain–Barré syndrome. HSV AI was isolated in 4/181 cases (median 4.5 [2.3–7.1]) associated with motor impairment and facial paralysis. Single positive RuV AI was detected in 17/90 cases (median 2.1 [1.5–4.8]), associated with multiple sclerosis, myelitis, and meningoencephalitis. Positive MeV AI was isolated in 5/269 cases (median 4.8 [1.7–10]), including two cases of myelitis. EBV ITS was detected in 2/127 cases.

Conclusions: Intrathecal antiviral IgG synthesis may be a relevant diagnostic marker in various neurological disorders, notably VZV radiculoneuritis and CMV Guillain–Barré syndrome. Developing guidelines to standardize AI interpretation and positivity thresholds is essential, particularly to assess the specificity of multiplex viral testing in inflammatory CNS disorders.

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Whole-Genome and Recombination Analysis of a Neurotropic Enterovirus A71 C1-like Variant

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Introduction/Aim: Enterovirus A71 (EV-A71) is a major etiological agent of hand, foot and mouth disease, with recognized neuroinvasive potential leading to severe neurological complications. In Europe, C1-like strains have been recently associated with these outcomes. This study aims to molecularly characterize an EV-A71 strain obtained from a pediatric case presenting with encephalomyelitis and acute flaccid paralysis, and to investigate its phylogenetic placement and recombination profile.

Material and Methods: Cerebrospinal fluid (CSF), nasal swab, and fecal samples were collected from the patient. Viral RNA was extracted and subjected to sequence-independent single-primer amplification (SISPA), followed by whole-genome sequencing using Oxford Nanopore technology. Genome assembly was performed, and phylogenetic analysis was conducted using representative reference EV-A71 sequences. Recombination detection analysis was applied to identify potential recombination events.

Results: A near-complete EV-A71 genome was successfully recovered. Phylogenetic analysis placed the strain within a cluster adjacent to established C1-like lineages, consistent with recent European isolates linked to severe neurological disease. Recombination analysis identified a single recombination event, with C1-like strains as the major parental lineage and the recently described C6 strain as the minor parent. The recombination breakpoint was located within the 3D polymerase coding region, suggesting potential functional implications.

Conclusions: This study describes a recombinant EV-A71 strain associated with severe neurological manifestations, highlighting the ongoing evolution of C1-like viruses in Europe. The detection of a C6-derived genomic fragment within the polymerase region raises concerns regarding the emergence of variants with altered neurotropism. These findings underscore the importance of continuous genomic surveillance to monitor viral evolution and its potential impact on public health.

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Detection of equine parapoxvirus in the stable environment during pastern dermatitis outbreak in Finland

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Introduction/Aim: Equine parapoxvirus (EqPPV) has emerged as a cause of recurring pastern dermatitis outbreaks in horses in Finland. First detected in 2013, EqPPV has since been associated with potentially hundreds of clinical cases and suspected zoonotic transmission. Following an outbreak in 2021-2022, suspected EqPPV infections in Finnish trotters have been monitored through PCR diagnostics of swab samples submitted by veterinarians and stable staff. This study investigated the environmental presence and potential indirect transmission routes of EqPPV to improve preparedness for future outbreaks and support targeted hygiene recommendations.

Material: Environmental samples (n=161) were collected from eight Finnish horse stables during spring 2025, including six EqPPV case stables and two control stables. Additional samples included horse swabs, wild small mammals, birds, faeces, and other environmental materials.

Methods: EqPPV DNA was detected using probe-based real-time PCR targeting the viral myristylated protein gene. Borderline samples were confirmed using SYBR Green PCR and nested PCR. DNA was extracted from swabs, environmental materials, and animal samples using commercial extraction kits.

Results: After no detected EqPPV cases between April 2022 and January 2025, a new outbreak emerged in early 2025. Between February and May 2025, 8/10 Finnish trotting stables submitting diagnostic samples tested positive for EqPPV DNA. Environmental sampling showed how widely EqPPV DNA had spread in infected stables, including grooming tools, stall infrastructure, bedding, shared equipment, and hands of humans. Overall, 83.3% (5/6) of case stables and 17.4% (24/138) of environmental samples tested positive.

Conclusions: EqPPV DNA was widely distributed in clinically affected stables, supporting the possibility of indirect transmission through contaminated environments, shared equipment, and personnel. These findings highlight the potential for recurring outbreaks and emphasize the importance of surveillance and early preventative practices to minimize future outbreaks.

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Antiviral Resistance and Genetic Diversity of Cytomegalovirus in Portugal: A Comprehensive NGS-based Surveillance of High-Risk Populations

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CMV remains a major complication in transplant recipients and neonates. Although resistance classically involves UL97 and UL54, newer antivirals such as maribavir and letermovir have expanded relevant targets to UL27 and the terminase complex. This study uses NGS to assess CMV resistance-associated mutations (RAM) in Portugal, helping distinguish genetic resistance from host-related factors and optimize individualized salvage therapy.

From March 2023 to January 2026, 67 CMV resistance tests were performed on 45 patients with persistent viremia or suspected resistance. The cohort comprised 31 transplant recipients, 12 neonates with congenital infection, and 2 HIV-1 co-infected patients. NGS (Vela Diagnostics Sento-sa) was used to sequence the UL27, UL54, UL97 genes and the terminase complex.

Our cohort reflects a broad geographical diversity (Portugal (n=35), Lusophone Africa (n=7), South America (n=2), and Germany (n=1)). RAMs were identified in 12 tests (26.7%), mainly in transplant recipients (11/12). High-frequency UL27 variants (V310A, n=15; D351N, n=13; P307L, n=12) appear to be geographical polymorphisms found in treatment-naïve individuals. Despite in vitro associations with decreased maribavir susceptibility, their clinical relevance remains to be established. Clinically significant RAMs were detected in UL97 in 15.6% of patients (n=7), mainly ganciclovir-resistance mutations M460V/I, A594V/T, and E596G; one patient showed a multidrug-resistance profile with T409M/C480F, associated with maribavir and ganciclovir resistance. UL54 RAMs K513N and T503I were found in 6.7% (n=3), linked to ganciclovir, foscarnet, and cidofovir resistance, while UL56 R369S was identified in one case, conferring letermovir resistance.

In conclusion, resistance mutations were detected in 26.7% of cases, mainly affecting ganciclovir, reflecting both the refractory cohort and the higher sensitivity of NGS for low-frequency variants. Overall, NGS supports exclusion of genetic resistance, individualized therapy, and epidemiological surveillance.

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Analytical Evaluation of Digital PCR for Arboviruses Detection and Quantification

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Introduction/Aim: Molecular diagnosis of arboviral infections may be challenging when viral loads are close to the limit of detection. This occurs in blood donors identified through highly sensitive nucleic acid testing and in selected patients with strong clinical, epidemiological and serological evidence of arboviral infection despite negative conventional real-time RT-PCR results. We evaluated the analytical performance of digital PCR (dPCR) for the detection and quantification of clinically relevant arboviruses.

Material and Methods: Commercial dPCR assays on the QIAGEN QIAcuity One for West Nile virus, Dengue virus and Chikungunya virus were compared with routine CE-IVD Real-Time PCR assays (Altona Diagnostics RealStar® RT-PCR Kits). Viral cultures were spiked into negative plasma and tested after ten-fold serial dilutions. RNA was extracted using the AltoStar® AM16 System, and the same eluate was analysed by both methods. Six serial dilutions were tested in triplicate to assess linearity, followed by six replicates of three low-concentration dilutions for each target (54 replicates per method). Each target was tested in dPCR singleplex and triplex formats. Real-Time PCR used 10 µL RNA input and dPCR 2.5 µL.

Results: All dPCR assays showed linear detection across serial dilutions and successful triplex amplification. In the low-concentration range, Real-Time PCR detected 25/54 replicates (46.3%), whereas dPCR detected 8/54 (14.8%) in singleplex and 6/54 (11.1%) in triplex format. dPCR also provided absolute quantification using a four-fold lower RNA input volume.

Conclusions: The commercial dPCR assays tested did not match the sensitivity of established Real-Time PCR assays at the lowest target concentrations. However, they showed good linearity, reliable triplex amplification and absolute quantification. In their current configuration, these assays do not support immediate implementation as a second-line confirmatory approach, but represent a promising platform for further optimization.

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Genomic surveillance of respiratory syncytial virus (RSV) in Catalonia, Spain: Impact of nirsevimab immunization (2025–2026)

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Introduction/Aim: Respiratory syncytial virus (RSV) remains a major pediatric threat. After three seasons of widespread nirsevimab (NIR) use, monitoring mutations in antigenic site Ø is crucial for assessing sustained efficacy. This study evaluates the emergence of RSV escape variants in Catalonia over the last season (2025–26).

Material/Methods: RSV samples were collected through the surveillance network (SIVIC) in Catalonia. All infants ≤12 months of age who tested positive for RSV during the 2025–2026 season were included. RSV-positive infants ≤12 months were assigned to the NIR cohort, given the 93% immunization coverage, whereas RSV-positive children >1 year and adults comprised the non-NIR cohort. Whole-genome (WG) sequencing was performed by next generation sequencing; sequences with >95% F-gene coverage were included in the analysis. Lineages were assigned using Nextclade v3.21.2, focusing mutational analysis on epitope Ø.

Results: A total of 245 WG and 382 F genes (F) were sequenced. HR-SV-A included 103 NIR samples (71 WG) vs. 149 (97 WG) non-NIR; HRSV-B included 37 NIR cases (21 WG) vs. 93 (56 WG) non-NIR. Both NIR and non-NIR genomes were predominantly A.D.3-like (83% vs 86%) and B.D.E.1-like (96% vs. 100%). Regarding F-protein mutations in antigenic site Ø, RSV-A sequences showed K209E (1/103 NIR, intermediate resistance) and N63S (1/149 non-NIR). In RSV-B, NIR cases harbored N208D (1/37, full resistance), R209Q (6/37), and S211N (37/37), the latest two also observed in non-NIR (R209Q (4/93) and S211N (93/93)). Potential emergent minority variants were identified.

Conclusions: The emergence of resistance associated mutations (RSV-A: K209E, RSV-B: N208D) suggests immune-driven viral selection under nirsevimab pressure. Continued genomic surveillance is vital to track the long-term efficacy of RSV monoclonal antibodies.



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Whole-Genome Sequencing and Molecular Characterization of Respiratory Syncytial Virus in the 2025–2026 Epidemic Season Using Oxford Nanopore Technology in Ankara, Türkiye

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Introduction: Respiratory syncytial virus (RSV) is a major cause of acute lower respiratory tract infections worldwide, particularly affecting young children, older adults, and immunocompromised individuals. With the increasing clinical use of RSV-specific monoclonal antibodies and vaccines, local genomic surveillance has become essential. This study aimed to characterize circulating RSV strains during the 2025–2026 epidemic season using amplicon-based whole-genome sequencing (WGS) on the Oxford Nanopore Technology platform.

Material: A total of 172 RSV-positive respiratory specimens, including nasopharyngeal and throat swabs, were collected from Bilkent City Hospital, Ankara, Türkiye, during the 2025–2026 season. WGS amplification was performed using the 10-amplicon one-step RT-PCR scheme described by Wang et al. Amplicons were quantified by Qubit fluorometry, normalized equimolarly, and pooled separately for RSV-A and RSV-B. Libraries were prepared using the Rapid Barcoding Kit (SQK-RBK114-96), followed by sequencing on PromethION R10.4.1 flow cells with Super Accuracy base-calling using Dorado v0.7+.

Methods: Bioinformatic analysis was performed using a custom Snake-make v9.20.0 pipeline. Alignments were generated with MAFFT, and phylogenetic trees were inferred using IQ-TREE 2 with the GTR+G model and 1,000 UFBoot replicates. One sample produced no sequencing reads, and 10 samples were excluded due to poor genome quality, leaving 162 samples for final analysis.

Results: Among 162 analyzed samples, 122 were RSV-A (75.3%) and 40 were RSV-B (24.7%). Mean sequencing depth was 13,585x, ranging from 268x to 92,954x. Overall, 160 samples (98.8%) achieved ≥95% genome coverage at ≥20x depth. Nextclade assigned all RSV-A sequences to clade A and all RSV-B sequences to clade B.2. Most patients were pediatric, with 76.7% under 5 years of age.

Conclusion: Amplicon-based WGS enabled high-depth, near-complete RSV genome recovery. RSV-A clade A predominated, while RSV-B clade B.2 represented approximately one-quarter of cases.

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Challenges and opportunities of viral respiratory infections surveillance in the era of syndromic and near-patient testing at National Influenza Centre in Slovenia

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Introduction/Aim: Introduction of diagnostic approaches like syndromic and near-patient testing and clinical metagenomics, impact the capacity of acute respiratory infections (ARI) surveillance. At National Influenza Centre Slovenia we analysed primary and secondary healthcare data, integrated into ARI surveillance in the past 10 seasons (from 2016/17 to 2025/26), to investigate their contribution to public health management.

Material and Methods: In Slovenia national surveillance of ARI is based on a sentinel of 60 primary healthcare clinics (PHCs), representing 5% of the population. In 10 seasons totally 9428 samples were tested for respiratory viruses (RVs).

Additionally, weekly cumulative data of total number of samples tested and positive for RVs from all laboratories testing for RVs in Slovenia are collected. These samples mostly originate from hospitals (Hs). In 10 seasons totally 308032 samples were tested for RVs.

Data were analysed to investigate their contribution to ARI surveillance. Weekly rates (%) of samples positive for influenza(INF), respiratory syncytial virus(RSV), metapneumovirus(HMPV), parainfluenza(PIV), bocavirus(HBoV), enterovirus(EV), seasonal coronavirus(HCoV), SARS-CoV-2 were analysed.

Results: Comparisons showed that sentinel PHCs captured first signals of seasonal upsurges earlier than Hs for INF, RSV, hCoV, SARS-CoV-2, enabling timely interventions. Seasonal upsurges occurred simultaneously in PHCs and Hs for HMPV, PIV, HBoV, while EV did not show a pattern. Peak positive rates (%) mostly coincided in PHCs and Hs but were delayed in Hs for INF, RSV. Peak positive rates (%) were higher in Hs than PHCs for RSV, HMPV, HBoV, suggesting a higher burden of these RVs in severe ARI in comparison to RVs with peak positive rates (%) higher in PHCs than Hs (INF, PIV, hCoV, SARS-CoV-2).

Conclusions: Our results emphasize the importance of integration and correct interpretation of various segments of RV testing for comprehensive ARI surveillance. Guidelines should be continuously updated to enable informative integrated ARI surveillance globally.



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Saliva and Plasma Viral MiRNA-based Detection of Cytomegalovirus Reactivation

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Reactivation of human cytomegalovirus (CMV) in immunocompromised patients post-cell therapy treatment is a clinically significant infectious complication that is associated with increased morbidity and mortality. CMV reactivation frequently occurs in at-risk patients in the absence of prophylaxis, and late onset CMV reactivation occurs in 12-50% of patients following cessation of Letermovir prophylaxis, underscoring the importance of long-term monitoring and early detection. However, current CMV DNA qPCR tests are limited by the need for frequent blood draws, the lack of universal thresholds and clinical ambiguity in cases of low level DNAemia, highlighting the need for alternative virus testing modalities. Virus-encoded miRNAs have emerged as informative biomarkers of viral reactivation. They are detectable in body fluids, remain abundant during viral latency and change in abundance during viral reactivation. This prospective cohort study therefore sought to identify candidate saliva and plasma CMV miRNA biomarkers of CMV reactivation in 20 patients with haematological malignancies. Patients were recruited at the time of CMV reactivation diagnosis and followed up until resolution of viremia at the Singapore General Hospital. Paired saliva and plasma samples were profiled for a panel of 10 CMV miRNAs using the gold standard target-specific reverse transcription coupled with hydrolysis probe-based quantitative PCR. We achieved fast (< 4 hours) and sensitive (attomolar) quantification of CMV miRNAs from saliva and plasma. Our findings on candidate reactivation-associated CMV miRNA signatures and correlations with the respective clinical data will be discussed. The findings of this pilot study would thus create opportunities for saliva-based point-of-care monitoring and early detection of CMV reactivation in the outpatient setting.

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Reliability of used facial tissues to monitor respiratory viral infections in primary care, France, 2024-2026

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Introduction/Aim: Virological surveillance of acute respiratory infections in primary care in France relies, in part, on respiratory specimens (nasopharyngeal (NP) or saliva (SAL) swabs) collected by a small number of volunteer general practitioners and pediatricians (GP) during the winter season. This study aimed to compare the performance of used tissues (UFT) with that of swabs (S) for the surveillance of these infections.

Material and Methods: In a prospective study, volunteer GPs from two French regions sent a swab and a tissue—both collected simultaneously from the same individuals—to 2 independent virology laboratories. These samples were incorporated into each laboratory's daily testing workflow. The testing targeted the following viruses: influenza A and B, RSV, SARS-CoV-2, metapneumovirus, parainfluenza, rhinovirus, bocavirus, and seasonal coronaviruses.

Results: A total of 476 S and UFT samples were analyzed, and a virus was detected in 75% of cases (n=357). The positive rate was significantly higher for S samples (65.5%) than for UFT samples (64.5%, p<0.001). The positive agreement rate was 85.9%, with a satisfactory Kappa coefficient of 0.617. Positive concordance was comparable regardless of the virus, laboratory, or sample type (SAL/NP swab) studied. Of the 318 positive S samples, 46 UFT samples (14.4%) were negative, and of the 311 positive UFT samples, 39 S samples (12.5%) were negative. The median CT values obtained on UFT were lower (-1.85) than those obtained on SAL but higher than those obtained on SNP (+4; p<0.001) with no significant difference regardless of the virus detected.

Conclusions: As UFT are better tolerated and cheaper than swabs and that the two sample types show a high level of positive agreement, using UFTs to monitor respiratory infections seems entirely appropriate. The ability to collect UFTs independently would be a real advantage in the event of an unusual virus outbreak.



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NA:S247N substitution in influenza A(H1N1) pdm09 viruses: from residual to mainstream

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Aim: While the prevalence of antiviral-resistant influenza viruses remains very low in Europe, continuous genetic drift could trigger the emergence of resistant variants even without direct selective pressure. This study aims to genotypically and phenotypically characterise low oseltamivir-susceptibility influenza A(H1N1)pdm09 viruses in Catalonia, as well as to assess the global dissemination of the NA:S247N substitution during the 2025–2026 season.

Methods: Respiratory samples included in the present study were collected through the surveillance system for acute respiratory infections in Catalonia (Sept 2025–March 2026). Genotypic characterization was performed with WGS (Illumina) and MinMutfinder to identify WHO-listed NA1 resistance markers. Phenotypic susceptibility was assessed using MD-CK-SIAT1 cultures and a MUNANA-based NA activity assay. Regional data were compared against GISAID sequences from Europe, Asia, the Americas, and Oceania (including April–Sept 2025 for the Southern Hemisphere) using Nextclade.

Results: Of 221 A(H1N1)pdm09 viruses sequenced in Catalonia, 67.9% (150/221) harboured the NA:S247N substitution. One virus carried NA:I223V+S247N, and another harboured NA:H275Y. Phenotypic assays confirmed that isolates with IC50 values >0.3 nM correlated with the NA:S247N substitution.

Globally, NA:S247N prevalence remained high in early-detection countries (Spain 76%, Norway 71%, Netherlands 63%) and surged by season-end in others (Romania 77%, Germany 66%, UK 57%). Prevalence was lower but increasing in the Northern Hemisphere outside Europe (USA 37%, Japan 30%, China 12%) and remained significantly lower in the Southern Hemisphere (South Africa 7%, Australia 2%, Chile 0.3%). Compared to the previous season, NA:S247N prevalence showed a 6- to 51-fold increase worldwide.

Conclusions: The 2025-26 season confirmed the spread of NA:S247N-bearing A(H1N1)pdm09 in the Northern Hemisphere. The rapid global increase underscores the need for strengthened surveillance to monitor the evolution of these substitutions and their impact on clinical antiviral efficacy.

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Transmission dynamics of an occult Hepatitis B infection in an inpatient and outpatient haemodialysis setting

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Historically Hepatitis B virus (HBV) infections in Haemodialysis (HD) patients have been a significant cause of outbreaks, morbidity, and mortality (Rosenheim Report, 1972). Gradually, HBV outbreaks in HD settings have become rare, due to the introduction of modern HD machines, bloodborne virus screening, vaccination and infection prevention and control (IPC) measures. However, diagnostic challenges remain and knowledge of transmission dynamics with newer HD machines is limited.

We present a case of occult HBV in a patient on HD in an acute UK hospital and a linked HD unit over a 10-month period. The patient was identified as Hepatitis B surface antigen (HBsAg) positive, Hepatitis B core antibody (HBcAb) positive, Hepatitis B 'e' antigen (HBeAg) positive, and Hepatitis B surface antibody (aHBs) <10 mIU/mL with a HBV DNA level of log 6.87 IU/mL.

At baseline, the patient had detectable post-vaccine aHBs (90.24 mIU/mL) and was HBsAg negative with further screens also negative. Retrospective testing of stored samples showed initial HBcAb positivity and HBV DNA log 1.59 IU/mL. HBV DNA had increased to log 2.0 IU/mL (month 3), and log 3.0 IU/mL (month 8) at the start of their hospital admission. DNA peaked in month 10, at time of identification.

The patient was isolated on HD, started on Tenofovir, and an incident management team convened. Contact tracing found 604 possible contacts (143 inpatients, 461 outpatients). All outpatient contacts had baseline HBV bloods and repeats one month later. Inpatient contacts were risk-assessed by contact type and aHBs level. Of the inpatients, 36 had shared a HD machine and 68 were on the same ward. Appropriate IPC actions were taken (notification, HBIg, vaccination, isolate HD machines, and enhanced surveillance).

Reassuringly, to date, no new linked cases of HBV have been found, 51 patients remain in follow-up and 49 patients RIP, with cause of death pending review.

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Emergence of human herpesvirus (HHV) drug-resistance (drug-R) in immunocompromised patients with multiple DNA virus infections: RegaVir data from a 16-years surveillance study

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Background: HHVs and adenoviruses (AdVs) frequently cause infections in IC patients. Multiple viral (co-)infections increase the risk for life-threatening complications and mortality.

Methodology: We analyzed drug-R prevalence among samples received by RegaVir (NRC for typing drug-R among DNA viruses) between 01/2009 and 03/2026 from cases presenting multiple HHVs and/or AdVs infections. 411 samples from 84 IC individuals presenting concomitant and/or consecutive multiple DNA virus infections were selected. The samples were prospectively genotyped for identifying mutations in viral genes involved in drug-R. HSV phenotyping was performed to determine the impact of known drug-R mutations and novel changes.

Results: Our cohort included 41 males and 43 females, median age 44.7 years-old. For 13/84 patients, no data on transplantation was available, being the remaining 71 patients all transplant recipients. The most prevalent HHVs was CMV (79/84 patients), followed by HSV-1 (61/84 patients). The most frequent type of multiple infection was due to CMV and HSV-1 (55/84 patients), being the majority of them consecutive infections (39 patients). Other multiple infections were found in (≤ 4 patients. Infections with viruses other than CMV and HSV-1 were found in 29 patients.

More than half of the patients developed drug-R to one or more viruses during their follow-up (48/84, 57.1%). Among the 55 patients with CMV/HSV-1 infections, 34 developed confirmed drug-R to one or both viruses. CMV drug-R was identified in 23/79 CMV-positive patients, and in 32/62 HSV-1-positive patients. Among patients presenting infections with viruses other than CMV and HSV-1, drug-R was found in 14/29 patients. In patients having infections with ≥3 viruses, 4/5 had known drug-R mutations.

Conclusions: Management of multiple infections and emergence of drug-R is highly challenging. A better understanding of multiple infections dynamics as well as research into characterizing novel HHV mutations are warranted.

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HRSV genomic surveillance in Belgium during the 2025-2026 respiratory season.

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Prophylactics for prevention of severe human respiratory syncytial virus (HRSV) infection target the viral fusion (F) glycoprotein or a specific antigenic site thereof. As national reference center for respiratory pathogens in Belgium, we monitored the HRSV genetic diversity and possible selection of escape variants.

HRSV subgroups were determined by qPCR and the complete HRSV genomic sequence was determined by amplicon-based Nanopore sequencing. Two-hundred respiratory samples were received for HRSV typing, of which 42 were from SARI cases (30 HRSVA, 11 HRSVB and 1 HRSVA+B dual infection), 17 were from ILI cases (7 HRSVA and 10 HRSVB) and for the remaining 141 samples, the case definition was unknown or unreported (70 HRSVA, 64 HRSVB and 7 with negative subtyping result). Information on RSV vaccination was reported for 50 of the patients (9 vaccinated, 41 not vaccinated). In 53 cases, information on administration of monoclonal antibodies was provided (14 treated patients, 39 untreated).

Both HRSV subgroups co-circulated during the 2025-2026 epidemic season, with 108 HRSVA positive samples and 86 HRSVB positive samples. So far, 152 samples were genomically characterized. Within HRSVA, 10 distinct lineages were detected: A.D.1.4, A.D.1.5, A.D.1.10, A.D.1.11, A.D.3, A.D.3.7, A.D.3.8, A.D.3.11, A.D.3.12 and A.D.5.2. For HRSVB, 6 lineages were found: B.D.4.1.1, B.D.E.1, B.D.E.1.1, B.D.E.1.2, B.D.E.1.8 and B.D.E.6. Mutations with reported relevance for strain sensitivity to the monoclonal antibody Nirsevimab were present in 2 HRSVB sequences: F:K65E, reported to be associated with possible decreased sensitivity to Nirsevimab, detected in a sample from a 57 year old patient and F:K68E, reported to be associated with decreased sensitivity to Nirsevimab, detected in a sample from a 1 year old infant. No information on case definition, vaccination status or treatment was received for these two patients.

Our results indicate co-circulation of many distinct HRSV lineages in Belgium, with the vast majority of circulating strains remaining sensitive to the monoclonals in use.

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Norovirus takes the lead: six years of enteric virus surveillance by multiplex PCR in a tertiary-care hospital

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Introduction/Aim: Enteric viruses are leading causes of acute gastroenteritis, but their relative burden is shifting in the post-rotavirus vaccine era. Local hospital-based data are needed to guide diagnostic stewardship and outbreak detection.

Methods: Retrospective observational study of all stool samples tested at a single tertiary-care hospital between 2020 and early 2026 using a commercial multiplex-PCR panel targeting rotavirus, norovirus, astrovirus, adenovirus and sapovirus. Age was categorised into seven groups. Norovirus outbreak-compatible months were defined as months exceeding the within-year median plus two standard deviations.

Results: A total of 1,681 samples were analysed. Testing volume rose more than three-fold, from 114 samples in 2020 to 347 in 2024. Overall, 311 samples (18.5%) were positive for at least one virus. Norovirus was the dominant pathogen (171/1681; 10.2% of tests; 55.0% of positives), with outbreak-compatible peaks in February 2020, October 2021, July 2022, January 2023 and April 2024. Sapovirus ranked second (60/1681; 3.6%), followed by rotavirus (41/1681; 2.4%), astrovirus (30/1681; 1.8%) and adenovirus (26/1681; 1.5%). The median patient age was 5 years (IQR 1–25); approximately half of tests were requested in children under 5. Adults aged ≥40 years accounted for 20.4% of tests, with 37.6% of this group having documented comorbidities or immunosuppression (malignancy, transplantation, HIV, chronic kidney disease, neutropenia).

Conclusions: Norovirus stands out as the most frequently detected enteric virus via hospital-based multiplex-PCR, associated with recurrent seasonal peaks, while rotavirus contributes only minimally, consistent with the sustained impact of vaccination. The tested population reflected a dual case mix of paediatric and high-risk adult population, underscoring the value of continuous laboratory surveillance in tertiary-care settings.

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Single RealTime-PCR protocol and reagents set for simultaneous multi-virus detection

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Introduction/Aim: Viral infections molecular diagnosis requires different RealTime-PCR protocols, due to genomic characteristics and reagents used. We evaluated the use of FlexStar® (RT-)PCR Amplification Mix and FlexStar® LDT (RT-)PCR Detection Mix (altona Diagnostics GmbH, GER), using a unique protocol.

Materials and Methods: Positive samples were used to check amplification of: Orthoflavivirus (panflavivirus - PF), influenza B virus lineages (FluB), enterovirus (EV), EV-D68, rhinovirus (HRV), and monkeypox virus clade Ib (MPXV-Ib). Nucleic acids were extracted on AltoStar® Automation System M16 platform (altona Diagnostics GmbH, GER). The FlexStar® reagents, coupled targets-specific with custom primers and probes, were used to perform rRT-PCR, with the same amplification protocol.

Results: Among QCMD samples 37/37 was found positive for PF: three of them were negative for the specific target of the panel (i.e.: Chikungunya virus, West-Nile virus, Zika virus) but spiked with Japanese encephalitis virus. Further 172 flavivirus positive samples from diagnostic routine were positive with PF. The Victoria lineage was found in 52/53 FluB cases, while the last had both Victoria and Yamagata. EV and EV-D68 were tested in 133 positive specimens: all were confirmed as EV positive, while 49/133 were EV-D68 positive, in accordance with sequence analysis. A small proportion of HRV samples were tested, as duplex with EV: 5 were HRV positive, while in 7 cases the EV result by commercial assay was changed in HRV, as confirmed by sequence analysis. MPXV-Ib was assayed in 25 samples, with 22 positives, as per sequence classification, and 3 negatives (clade II); the reverse-transcription step did not influence results, even for a DNA target.

Conclusions: The reagents challenged in this study demonstrated high performance on the different DNA and RNA targets, with a unified rRT-PCR protocol, supporting the use of such approach for multitarget detection.

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Torque-teno virus viral load monitoring using metagenomics in liver transplant recipients.

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Background/Aims: In a study aimed to characterize the human blood virome by metagenomics (mNGS) in patients undergoing liver transplantation (OLT), we sought to evaluate the correlation between mNGS reads and torque-teno virus (TTV) load as determined by quantitative PCR (qPCR).

Methods: This prospective observational study included patients undergoing OLT and a control group who were not candidates for liver transplantation. Plasma samples were collected before transplantation and at 1, 3, 6, and 12 months thereafter for cases and at baseline and after 6 months for controls. DNA and RNA libraries were prepared separately from nucleic acids extracts and sequenced on the NovaSeq 6000 platform. Pathogen detection and classification were performed using Kraken2, with downstream virome analyses of vertebrate viruses. TTV viral load was determined using an TTV-specific quantitative PCR assay.

Results: A total of 70 liver transplant recipients and 8 non-transplanted controls were included. Indications for transplantation included hepatocellular carcinoma (MELD<15) and hepatic insufficiency (MELD≥15). Transplanted patients showed significantly higher abundance of TTV DNA reads at 6 months as compared to controls, likely reflecting a bloom due to immunosuppression. Considering all time points, and all samples with TTV reads detected by mNGS, a significant correlation was observed between normalized mNGS reads and TTV copies/mL in plasma measured by qPCR ($R=0.6$, $p<0.01$) whereas no significant correlation was observed for the RNA dataset ($R=0.021$, $p=0.73$). The same results were obtained when performing the correlation analysis for each time point separately.

Conclusions: The significant correlation of mNGS TTV normalised DNA reads with qPCR copies/mL supports the potential use of mNGS as a semi-quantitative approach for viral monitoring. Besides, our study confirms a blooming of TTV load in liver transplant recipients during the months following transplantation, likely as a consequence of immunosuppressive therapy.

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Laboratory-Driven Hierarchical BKPyV Testing After Kidney Transplantation: Urine Screening to Rationalize Reflex Plasma DNAemia Requests

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Introduction/Aim: Plasma BKPyV-DNAemia is the reference marker for risk stratification and clinical decision-making after kidney transplantation. Guidelines recommend monthly plasma screening until month 9 post-transplant. In routine practice, clinicians frequently co-order concurrent urine and plasma BKPyV testing. Whether urine results could inform conditional plasma processing, without modifying ordering behaviour, has not been evaluated as a laboratory stewardship strategy.

Methods: A single-centre retrospective analysis of 575 paired urine and plasma BKPyV real-time PCR episodes from 150 kidney transplant recipients (2025), reflecting routine co-ordering practice. Qualitative concordance and the predictive value of viruria for plasma DNAemia were assessed with stratified analyses by viral load level.

Results: Plasma DNAemia was detected in 54 episodes (9.4%; 29 patients) and viruria in 207 (36.0%; 106 patients), consistent with published prevalence ranges. Viruria demonstrated 98.1% sensitivity and 99.7% negative predictive value for plasma DNAemia. Only one episode showed detectable plasma BKPyV without viruria, a sub-quantification result; no quantifiable viraemia occurred in the absence of viruria. Higher plasma viral loads correlated with quantifiable viruria and higher urinary viral loads.

Conclusions: These findings support a laboratory-driven hierarchical algorithm in which plasma processing is conditionally deferred pending the urine result, with reflex plasma testing reserved for viruria-positive or clinically selected cases. Requiring no change to clinical ordering behaviour, this strategy may substantially reduce redundant plasma processing, rationalise laboratory resources, and align with guideline goals — without compromising clinical safety, given the near-complete absence of quantifiable viraemia in viruria-negative episodes.



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Ability of Influenza A virus non-structural 1 protein to inhibit the innate immune pathways

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Influenza A viruses (IAVs) are pathogens that cause mainly respiratory infections in humans, but they can also infect variety of animals including mammals and birds. IAVs are also responsible for seasonal epidemic outbreaks in humans, and the zoonotic strains pose a possible pandemic threat. In recent years, the H5N1 avian influenza virus, IAV subtype, has caused unprecedented bird mortality and infections in mammals around the world, raising concerns about adaptation to humans and highlighting the pandemic threat caused by IAVs. The objective of this research is to investigate the differences in the IAV non-structural protein 1 (NS1), a multi-functional virus protein primarily affecting the immune response of the host, between different human and avian IAV strains, with a focus on the strains from the H5N1 subtype. The inhibitory effect of thirteen NS1 proteins on the retinoic acid inducible gene 1 (RIG-I) pathway was determined with a luciferase assay, indicating that all NS1 proteins inhibit the pathway. However, some differences between NS1 proteins originating from different IAV types and variants were observed and the biological relevance of these differences is being further studied by revealing the interactions of NS1 proteins with host cell proteins (proximity-dependent labeling method and pull-down assay). This research offers valuable insights into the interactions between NS1 proteins from different IAV strains and the host cell, creating a basis for further research. Characterizing the function of the NS1 protein is important to better understand how IAV strains adapt to new host species.

Keywords: influenza A virus, interactome, NS1, RIG-I

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Influenza seroprotection among children in Portugal: a cross-sectional seroepidemiological study, August 2025

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Introduction: Children have the highest influenza infection rates and play a central role in community spread of influenza due to high contact rates, immature immunity and frequent exposure at school. Our aim was to estimate the prevalence of protective antibodies against influenza A subtypes and influenza B among children under 18 years, in Portugal, during the summer 2025.

Methods: We developed an annual cross-sectional seroepidemiological survey during August 2025. Thirty-three laboratories selected residual sera from children <18 years (y). Serological testing was done by haemagglutination-inhibition (HI) assay (HA1 titer $\geq$ 40 is protective). We estimated the prevalence of protective antibodies and geometric mean titer (GMT), with 95% confidence intervals (95% CI) against vaccine (A/Victoria/4897/2022, A/D.O.Columbia/27/2023, B/Austria/1359417/2021) and circulating (A/Missouri/11/2025, A/Norway/8765/2025 subclade K) strains by age groups: 0-2, 3-5, 6-9, 10-14 and 15-18 y.

Results: A total of 340 sera were selected from children younger than 18y. There were significant differences in prevalence of protective antibodies between age groups, with lower levels observed among children under 2y for all the tested viruses. The lowest prevalence was observed for AH3N2 subclade K (9.8%; 95%CI: 4.8-17.3). An increase in seroprevalence with age was observed from 3 to 10y, with seroprevalence frequently higher in children aged 6 to 10 years. In the 15-18y seroprevalence and GMT were frequently lower than in younger age groups, except for the AH3N2 that circulated in the previous season (A/D.O.Columbia/27/2023), for which seroprevalence (86.1%; 95%CI: 70.5-95.3) and GMT was higher (75.5; 95%CI: 51.1-114.5). Overall, children had low cross-protection against subclade K.

Conclusions: Age-related differences in seroprevalence were observed among children. Protective antibody prevalence was lower in the first two years of life, being higher in school aged children and lower in adolescents. Serological data are valuable for guiding vaccination strategies and disease prevention.

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Surveillance of the four seasonal human coronaviruses (HCoVs) in Portugal during the 2025/26 season

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Human coronaviruses (HCoVs) 229E, NL63, OC43 and HKU1 are respiratory viruses typically associated with mild respiratory illness, with potential for severe disease. Their impact is poorly understood due to limited global surveillance. We aimed to study the circulation and to genetically characterize HCoV in Portugal during the 2025/26 season.

We monitored HCoV circulation with data from the Portuguese Laboratory Network for the Diagnosis of Influenza and Other Respiratory Viruses (PT-LabNet) from 2025/Week40 to 2026/Week15. For the genetic characterization, we used HCoV-positive samples with Ct ≤30 from a monthly selection of 5 samples/hospital from the PT-LabNet and samples tested at the National Institute of Health Doutor Ricardo Jorge. Sequencing was performed using an amplicon-tiling methodology followed by Next-Generation Sequencing (Illumina). Bioinformatic processing was conducted in the INSaFLU-TELEVIR platform and phylogenetic analysis with Nextstrain. Genotyping was based on clustering with sequences with assigned genotype (Ye et al. 2023).

The four HCoVs co-circulated throughout the season, with a total of 653/10,111 (6.5%) detections. An increasing trend was observed from 2025/Week40 onwards, peaking in 2026/Week05 with 45 detections and 11.6% positivity. The most detected HCoV were OC43 with 164 (30.8%) and 229E with 141 (26.5%) detections. Regarding alphacoronaviruses, all 28 sequenced 229E belonged to genotype 7b, as in previous seasons, while NL63 showed genetic diversity (13 genotype C2 and 7 genotype B1). All detected genotypes from betacoronaviruses (OC43: 11 genotype K, 2 genotype J and HKU1: 5 genotype B, 1 genotype A) were previously in circulation.

In the 2025/26 season, there was co-circulation of the four HCoVs, with early predominance of alphacoronaviruses. A higher positivity during the winter months evidenced seasonality, a characteristic of HCoV. Their genetic diversity was similar to the latest seasons, except for NL63 with the reappearance of genotype B1 since the 2022/23 season, warranting future monitoring of emerging lineages.

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Urine CMV PCR in Neonatal Screening: Six-Year Institutional Diagnostic Yield, Incidence Trends and Clinical Referral Patterns

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Introduction/Aim: Congenital cytomegalovirus (cCMV) is the leading cause of non-hereditary sensorineural hearing loss and neurodevelopmental disability in children. Early diagnosis within the first three weeks of life is essential to distinguish congenital from postnatal acquisition and to guide antiviral therapy during the window of maximum neurological benefit. Despite its relevance, cCMV remains underdiagnosed and institutional yield data from suspicion-based screening are scarce.

Methods: Retrospective single-centre laboratory surveillance (January 2020–April 2026) of 1,055 infants under nine months evaluated for suspected congenital or perinatal CMV based on clinical and/or maternal risk factors. Diagnosis was established by urine CMV PCR and viral load quantification. Breast milk CMV PCR was performed selectively in four cases to investigate postnatal transmission or inform breastfeeding decisions.

Results: Among 1,055 infants, 55 confirmed infections were identified (5.2%). Annual incidence was stable 2020–2025 (mean 5.1%), with a higher partial-year rate in 2026 (9.0%; 6/67). The most frequent presentations were neonatal cholestasis and hepatobiliary dysfunction, followed by fetal growth restriction, maternal seroconversion, thrombocytopenia, and CNS abnormalities. All four breast milk samples were CMV-positive. In three urine-positive infants, breast milk positivity implicated breastfeeding as a probable transmission route. In one urine-negative neonate with acute myeloid leukaemia, breast milk CMV positivity supported suspension of breastfeeding as a precautionary measure.

Conclusions: Suspicion-based urine CMV screening yields a clinically meaningful diagnostic rate, with neonatal cholestasis and fetal growth restriction as the strongest referral indicators. Selective breast milk CMV PCR may clarify postnatal transmission routes and inform risk management in immunocompromised neonates, though its role remains exploratory pending guideline integration.

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Structure-guided in silico ORF2 mapping to prioritise candidate antigenic regions in Rocahepevirus rattii

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Aim: Rocahepevirus rattii (rat HEV) is an emerging zoonotic cause of hepatitis in immunocompromised patients. Current serology is optimised for Paslahepevirus balayani (HEV-A) and may inadequately discriminate rat HEV. Characterising these structural differences may inform hypotheses about antigenic cross-reactivity and diagnostic discrimination. This preliminary in silico study prioritised candidate ORF2 regions to support rational antigen design and experimental serology.

Methods: Aligned ORF2 sequences from twelve representative rat HEV strains and HEV-A comparators were curated to avoid single-reference bias and interpreted within a maximum-likelihood ORF2 phylogenetic framework. Four candidate windows from published E2s antibody-contact regions, 455 to 459, 468 to 472, 495 to 499, and 575 to 583, were evaluated for inter-genotypic divergence, intra-rHEV conservation, and host-derived signatures. Patches were mapped onto experimentally resolved rat HEV E2s dimers to qualitatively evaluate assembly-aware surface accessibility.

Results: Findings are consistent with a shared structural scaffold with local compositional divergence between rat HEV and HEV-A. Within the rat HEV panel, candidate regions were highly conserved, with no distinct human-derived versus rodent-derived signatures detected. Window 575 to 583 emerged as the lead Site 1 candidate, with eight of nine residues fully conserved and minor variation at 581. Window 468 to 472 provided a supportive Site 1 signal. Site 2 regions were either fully conserved, 455 to 459, or less stable, 495 to 499. Assembly-aware mapping showed that Site 1, notably the 575 to 583 apical spike patch, is more openly surface-displayed than the constrained, groove-associated Site 2.

Conclusions: This work represents a preliminary in silico prioritisation workflow. The highly conserved 575 to 583 apical patch provides a strong pre-experimental rationale for downstream prioritisation. Future work will test whether this prioritisation is retained in assembly-competent truncated ORF2 constructs and assess serological performance.

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Clinical Utility of Multiplex Respiratory PCR Screening in the Immunosuppressed Cohort

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Introduction/Aim: Asymptomatic respiratory multiplex PCR screening is widely used in cancer wards to prevent nosocomial transmission and as a part of a routine septic screen, but its clinical utility remains controversial compared to symptom-driven testing. This service evaluation examines whether delaying cancer therapy due to subclinical viral detection represents an overly cautious approach. Some clinical studies reveal that pre-transplant asymptomatic viral detection is not associated with increased mortality rates. Conversely, meta-analyses establish that every four-week delay in cancer treatment increases the risk of death by approximately 10%, with surgical delays of twelve weeks increasing breast cancer mortality by 26% and adjuvant systemic therapy delays of twelve weeks increasing bowel cancer mortality by 44%.

Material and Methods: We retrospectively reviewed 15,993 multiplex PCR assays (Roche Cobas 8800) from May 2025 to May 2026, comparing positivity and cycle threshold (Ct) values between a cancer centre screening cohort and a symptomatic ICU cohort. These findings were integrated with clinical details as well as reasons and impact of the test result.

Results: Cancer centre patients underwent testing significantly more frequently than ICU patients. While rhinovirus patterns were similar, influenza A and SARS-CoV-2 positivity rates were lower in the oncology cohort. Crucially, immunosuppressed patients exhibited significantly higher Ct values (Ct>30) for adenovirus, influenza A, and SARS-CoV-2, demonstrating potential false positives as well as low-level viral shedding or prolonged genomic clearance.

Conclusions: Qualitative screening of asymptomatic patients frequently detects clinically irrelevant, low-level viral shedding and potential false positives. Delaying life-saving chemotherapy or transplantation based on weak PCR positives, without clinical symptoms, may cause substantial harm. Guidelines must incorporate patient history, symptom correlation and specific Ct thresholds to avoid unnecessary, high-risk treatment delays.



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Genome resolution of measles cases with identical N450 genotype (D8) unveils hidden local transmission in Portugal, 2024

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Introduction: In 2024, 35 measles cases were confirmed in Portugal, including imported, sporadic, and outbreak-associated infections. This study applied whole-genome sequencing (WGS) to cases sharing the same sequence ID (DSId-8350) in WHO MeaNS database to identify hidden transmission links, unrecognised transmission chains, and possible multiple virus importations.

Material and Methods: Laboratory confirmation followed WHO guidelines, and genotyping was performed by Sanger sequencing of the 450-nucleotide region of the nucleoprotein gene (N-450). WGS was conducted using the Viral Surveillance Panel v2 (Illumina) according to the manufacturer's instructions, and bioinformatics analyses were performed using the INSAFLU-TELEVIR platform v2.2.0 (<https://insaflu.insa.pt/>).

Results: Between February and May 2024, 18 measles cases with identical DSId 8350 were identified across several Portuguese cities, with the first case being imported from Romania. While a few cases were epidemiologically linked, representing clear transmission chains, fourteen cases had no identified epidemiological link and appeared sporadic despite temporal and geographic proximity, suggesting unrecognised transmission and community spread. WGS-based phylogenetic analysis identified two distinct genomic clusters (A and B). Cluster A included three epidemiologically linked cases differing by up to one SNP, whereas Cluster B comprised 14 cases differing by no more than two SNPs, consistent with sustained community transmission.

Conclusions: These findings show that identical N450 sequences can mask distinct transmission dynamics and highlight the value of WGS in identifying multiple independent and hidden transmission clusters improving epidemiological surveillance.

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Improving Respiratory Viral Infection Diagnosis in elderly Patients Using the Type I Interferon Score

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Introduction/Aim: Respiratory viral infections (RVI) represent a significant burden in elderly patients, underscoring the need for rapid and reliable diagnostic strategies. In this study, we assess a novel host-response approach based on the type I interferon (IFN-I) signature in an elderly population. By leveraging a pathogen-agnostic antiviral response, this method enables broad viral detection, including emerging pathogens, and may improve both diagnostic performance and clinical decision-making.

Materials/Methods: Nasopharyngeal swabs (NPS) were collected from elderly patients admitted to Hospices Civils de Lyon (France) (n = 879) as part of routine care for suspected RVI and/or contact tracing during cluster investigations. NPS from healthy controls (HCs) were also included (n = 60). The IFN-I score was measured using the BioFire® IFN-I pouch prototype, detecting four interferon-stimulated genes. Diagnostic performance was evaluated against first-line RT-PCR targeting SARS-CoV-2, IAV, and RSV. The additional yield of the IFN-I score was specifically analysed in samples negative by first-line RT-PCR.

Results: Among the 659 NPS analysed, 99 (15%) were positive by first-line RT-PCR. The IFN-I score was significantly elevated in first-line positive samples (median [IQR]: 4.15 [2.48–30.33]) compared to healthy controls (1.44 [0.87–3.28]; p < 0.0001), with good diagnostic performance (AUC 0.80 [95% CI: 0.73–0.87]). Notably, preliminary analyses showed that 56% of the samples initially negative by first-line test but with a positive IFN-I score were subsequently confirmed to have viral detection using second-line testing (BioFire® RP2.1 plus panel) and viral metagenomics. These findings are currently under further investigation.

Conclusions: These results support the integration of the IFN-I score as a host response tool in routine diagnostics for elderly patients, with potential benefits for early viral detection and improved patient management, including infection control and isolation.

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West Nile virus detection in *Culex* mosquitos in Portugal, 2025

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Introduction/Aim: West Nile virus (WNV) is an emerging mosquito-borne pathogen, causing recurrent outbreaks in humans, horses and wild birds. Although WNV has been circulating in Portugal since the 1960s, most detections were only by serological methods, which limits the available genomic information concerning the circulating strains. The aim of this study was to investigate the presence and genetic diversity of WNV in mosquitoes collected from wetland ecosystems in Portugal, in 2025.

Material and Methods: Mosquitoes were collected from two wetlands areas in October 2025 with high concentration of wild birds. Mosquitoes were grouped into pools according to collection date, location, and species and screened for WNV RNA using RT-qPCR. Positive samples were further analysed by whole-genome sequencing using a hybrid-capture target enrichment approach. Bioinformatics analyses were performed using the INSAFLU-TELEVIR platform v.2.2.2 (<https://insaflu.insa.pt/>), including phylogenetic analyses using the embedded WNV Nextstrain workflow to assess the evolutionary relationships between the detected viruses and previously reported WNV strains.

Results: WNV RNA was detected in several mosquito pools, predominantly *Culex univittatus* group. Seven near-complete viral genomes were recovered and all belonged to lineage 1A. Phylogenetic analysis revealed two distinct genetic clusters: one grouped with strains previously described in the Iberian Peninsula, while the second represented a divergent variant not previously detected in the region. These findings demonstrate the co-circulation of genetically distinct WNV strains in Portuguese wetlands.

Conclusions: This study provides the phylogenetic characterization of WNV detected in mosquito populations in Portugal and reveals previously undocumented viral diversity in the western Iberian Peninsula. The results highlight the importance of integrating entomological surveillance with genomic approaches to better understand the introduction, maintenance, and spread of WNV in southern Europe.

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Diagnostic Conundrum in a Case of Subacute Chronic Encephalitis

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Background: Subacute and chronic encephalitis in immunocompromised patients presents a significant diagnostic challenge, particularly when standard microbiological investigations are repeatedly negative. Enteroviruses (EV) are an under-recognised cause of persistent neurological disease in patients with impaired humoral immunity.

Case presentation: A 25y old woman with mixed connective tissue interstitial lung disease, previously treated with cyclophosphamide, mycophenolate, and rituximab, developed secondary hypogammaglobulinemia. She presented with a 6m history of progressive encephalopathy characterised by behavioural change, cognitive decline and upper motor neurone signs. Serial MRI showed progressive cortical atrophy, while PET-CT excluded malignancy and vasculitis. Extensive investigations included repeated cerebrospinal fluid (CSF), blood, throat, and rectal swabs, all negative for infectious agents. CSF PCR was negative for a broad viral and bacterial panel including HSV-1/2, VZV, EV, PEV, JCV, HHV-6, CMV, EBV, astrovirus and bacterial 16S/18S targets. Serology for HIV, syphilis, toxoplasma, and TB were also negative. Brain biopsy was performed 10 months after symptom onset. Histology showed dense perivascular and parenchymal lymphocytic infiltrates, consistent with encephalitis. Metagenomic sequencing of brain tissue identified enterovirus, most closely related to Coxsackievirus B2. Outcome: The patient was treated with intravenous immunoglobulin and combination antiviral therapy (molnupiravir, favipiravir, remdesivir). Despite intervention, she clinically declined and died approximately 18m after first presentation.

Conclusion: This case highlights the diagnostic complexity of subacute chronic encephalitis in immunocompromised patients. EV infection should be considered even when CSF and ancillary PCR testing are repeatedly negative. Brain biopsy with metagenomic sequencing can improve diagnostic yield but is not widely available. Earlier targeted PCR testing of brain tissue may reduce diagnostic delay and enable more timely therapeutic intervention.

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Comparative Method Validation of Two Different Automated Immunoassay Systems for Hepatitis A, B, and C Serological Tests

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Introduction and aim: Test performance is critical for diagnosis and immune status determination in HAV, HBV, and HCV infections. Method validation is mandatory under CLSI EP12-A2 and ISO 15189. This study aimed to perform a comparative method validation of the Atellica IM (Siemens, Germany) against Alinity-i (Abbott, USA).

Materials and methods: Alinity-i, in routine use with completed verification studies and successful external quality assessment, was selected as the reference method. Nine assays were evaluated: HBsAg, HBeAg, Anti-HBc IgM, Anti-HBc IgG, Anti-HBe, Anti-HBs (quantitative), Anti-HCV, HAV IgM, and HAV IgG (quantitative). Weakly reactive samples within $\pm 20\%$ of the cutoff were included. Sensitivity, specificity, and Cohen's Kappa (κ) were calculated; CV% was reported for quantitative assays.

Results: Complete agreement was observed for HBsAg, HBeAg, Anti-HBc IgM, and Anti-HBs ($\kappa=1.000$, 100% sensitivity/specificity). Anti-HBc IgG ($\kappa=0.824$) and Anti-HBe ($\kappa=0.720$) showed good agreement. Anti-HCV showed good agreement ($\kappa=0.633$); two discordant samples tested HCV RNA-negative, suggesting false positivity. HAV IgM showed good agreement ($\kappa=0.615$); discordant samples were weakly reactive. For HAV IgG, differing units (Alinity-i: S/CO; Atellica IM: mIU/mL) precluded kappa calculation; however, samples below the Alinity-i cutoff were reported positive by Atellica IM with values exceeding the WHO protective threshold (≥ 10 mIU/mL). All assays met precision criteria ($CV\% \leq 5$).

Conclusion: Atellica IM demonstrated excellent agreement for the Hepatitis B panel. Discordances in Anti-HCV and HAV IgM were limited to weakly reactive levels. For HAV IgG, mIU/mL-based reporting appeared to offer more sensitive classification at low titres. Overall, Atellica IM is a reliable alternative for Hepatitis A, B, and C serology testing.

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Yellow fever virus vaccine-induced B cell activation

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Introduction: Mosquito-borne flaviviruses, including yellow fever virus (YFV), dengue virus, and Zika virus, are spreading into new regions due to climate change and globalization, increasing the risk of outbreaks in previously unaffected regions. Although the live-attenuated YFV vaccine (YF-17D) is one of the most effective viral vaccines, the molecular events that drive the initiation of an effective antibody response remain poorly characterized.

Materials and Methods: To characterize the key factors in YF-17D and wild type (wt) YFV-induced B cell activation, we are engineering a human B cell line displaying a YFV-specific B cell receptor (BCR) using CRISPR/Cas9 system. Engineered B cells are inoculated with YF-17D and wt YFV Asibi strain, and BCR-bound antigen internalization and processing is followed using super-resolution microscopy. In addition, we are developing immunopeptidomics assay to identify HLA class II-bound viral peptides using mass spectrometry.

Results: Our findings indicate that B cells are not directly infected by YFV, and the entry of YFV into B cells is restricted to BCR-mediated endocytosis. Ongoing analyses focus on comparing the differences in antigen processing pathways and in the repertoire of viral peptides presented on HLA class II molecules following exposure to YF-17D versus wt YFV.

Conclusions: Our approach enables detailed characterization of the mechanisms governing antigen uptake, processing, and presentation by B cells to T cells. These processes are critical for shaping lymphocyte fate and determining vaccine outcomes. A deeper understanding of how YF-17D elicits robust and durable antibody responses may inform the rational design of safer and more effective vaccines against other clinically important flaviviruses.

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Analytical Performance Evaluation of the Quantitative ELITE BeGenius CMV DNA Assay for Routine Diagnostic Use

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Introduction/Aim: Before routine laboratory implementation, a new assay's diagnostic reliability requires comprehensive analytical verification. This study evaluated the analytical performance of the ELITE BeGenius CMV DNA testing system (ELITechGroup, Italy) to determine its suitability for routine diagnostics.

Materials and Methods: Routine patient samples and external quality controls were evaluated for linearity, precision, and accuracy using the ELITE BeGenius platform and CMV ELITE MGB® kit. Accuracy was assessed with 12 CMV DNA-positive and 5 negative external quality controls. Categorical agreement was evaluated across 17 samples, while Bland-Altman analysis utilized eight sample pairs yielding quantifiable positive results in both methods. Intra-assay precision was determined by testing three low and four high viral load samples in triplicate. Inter-assay precision was assessed using one low, one medium, and one high positive sample across three days. Linearity was evaluated using serial dilutions of a high-positive reference sample.

Results: Verification yielded 100% sensitivity and specificity (Cohen's kappa = 1.0), indicating perfect agreement. Precision analysis demonstrated excellent reproducibility (log₁₀ SD: 0.01-0.13; CV: 0.42-3.11%). Bland-Altman analysis revealed a -0.176 log₁₀ IU/mL mean difference (95% limits of agreement: -0.609 to 0.256), showing that the reference method yielded approximately 1.5-fold higher results on average than the ELITE system. Linearity demonstrated a strong correlation (R² = 0.969). However, ELITE measurements were generally lower than expected (by ~0.33 log₁₀ IU/mL), which is consistent with the systematic negative bias observed in the accuracy analysis.

Conclusions: The ELITE BeGenius CMV DNA system demonstrated robust analytical performance regarding accuracy, precision, and linearity, supporting its reliability and feasibility for routine clinical use. These preliminary data are part of an ongoing verification study, and will be updated with additional sample data.

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Brain organoids as models for arboviral neuroinfection

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Introduction/Aim: West Nile virus (WNV), Oropouche virus and Zika virus are emerging, mosquito-borne, arboviruses that recently gained interest due to the growing incidence of neuroinvasive cases, and their spread across European countries. Conventional 2D approaches poorly reproduce the central nervous system complexity (CNS). Thus, this study aims at defining a 3D in vitro model to study their neuroinvasiveness, using human induced pluripotent stem cell-derived brain organoids (HBOs).

Materials and methods: HBOs were cultured and differentiated for 42 days prior to infection with the three arboviruses. Once mature, HBOs were exposed to viruses for 24 hours and monitored for up to 72 hours post-infection (hpi). Supernatants were collected at defined timepoints for downstream analysis. At 72 hpi, organoids were either fixed for cryosectioning or lysed for RNA extraction.

Viral replication was assessed by RT-qPCR in both culture supernatants and organoid lysates, while infectious particle production was evaluated by plaque assay.

Organoid sections were used for immunofluorescence coupled with high-resolution confocal imaging to identify and quantify infection of neurons and neural progenitors. RNA-seq was performed in infected and control organoids. WNV-induced neuroimmune responses were further examined by RT-qPCR.

Results: HBOs were permissive to arbovirus infection and supported viral replication, as demonstrated by RT-qPCR that showed a time increase in viral RNA levels. Plaque assay confirmed these results, although no structural abnormalities were observed in infected HBOs. Gene expression analysis revealed upregulation of inflammatory mediators, including IL-6, IL-10, CXCL5, TNF- γ and IFN- γ in WNV-infected organoids compared to non-infected controls, while IFN- γ expression was not detectable. RNAseq results showed the presence of numerous differentially expressed genes in infected organoids compared to controls.

Conclusions: these results demonstrate that HBOs represent a reliable in vitro model for the study of arboviruses and their neuropathogenesis, viral replication dynamics, and host neuroimmune response in the CNS.

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Genomic diversity of congenital cytomegalovirus: first findings from the cCMV Sequencing Consortium

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Introduction/Aim: Cytomegalovirus (CMV) is the most common congenital viral infection and a leading cause of sensorineural hearing loss and neurodevelopmental disability. CMV is the most genetically diverse herpesvirus, yet efforts to link viral genomics to disease severity have been hampered by small cohorts, sequencing limitations, and incomplete characterisation of CMV diversity. The Congenital CMV (cCMV) Sequencing Consortium is a multi-centre pilot study designed to close these gaps and identify viral determinants of cCMV outcome severity.

Material: Samples (urine, saliva, blood, and amniotic fluid) are collected from fetuses and congenitally infected newborns across UK and European sites, with standardised clinical data capture linked through cCMVnet. We present preliminary data from a pilot cohort of ~50–60 isolates from the UK and France.

Methods: The pilot tested a workflow built to scale up: each site extracts DNA locally and ships to UCL, where samples undergo CMV target-enrichment Illumina whole-genome sequencing (UCLG), processing and analysis. Sequence data are returned to contributing centres, and all sites share authorship on consortium publications.

Results: Babies with cCMV appear to carry a single dominant CMV strain, with broadly concordant sequences across body compartments (urine, oral swab, plasma). Phylogenetic analysis shows genomic diversity reflecting the cosmopolitan nature of our recruiting populations, with sequences tentatively clustering alongside European, African and Asian reference genomes.

Conclusions: These findings establish proof-of-concept for the Consortium's genomic framework. Continued recruitment across European and global sites will enable a genome-wide association study of viral determinants of cCMV disease severity.

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Metapneumovirus Myocarditis: An Unexpected Pathogen

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Introduction: Myocarditis is an inflammatory disease of the myocardium with a highly variable clinical presentation, ranging from mild chest pain to severe cardiac dysfunction. Although viral infections represent the most common aetiology, the spectrum of implicated pathogens continues to expand. Human metapneumovirus (hMPV), a common respiratory virus, is only rarely associated with cardiac involvement.

Materials and methods: We report the case of a 15-year-old male with cystic fibrosis (homozygous F508del) receiving triple CFTR modulator therapy, who presented to the Paediatric Emergency Department with chest pain and fever.

Results: The patient presented with retrosternal chest pain and fever (38.6°C) of 5 hours duration, in the context of rhinosinusitis lasting one week. Physical examination was unremarkable apart from fever. Laboratory evaluation revealed leukocytosis, C-reactive protein of 12.8 mg/L, and elevated high sensitivity I troponin (428.2 ng/L) in a decreasing trend (318.8 ng/L after 6 hours). The ECG demonstrated nonspecific repolarization abnormalities, while transthoracic echocardiography was normal. hMPV was detected by multiplex RT-PCR (Ct = 28.88) in a nasopharyngeal swab and a diagnosis of acute myocarditis associated with hMPV infection was considered. The patient remained hemodynamically stable during 24 hours of observation, with symptomatic improvement following treatment with ketorolac, and was discharged with scheduled follow-up.

Conclusions: We report a case of acute myocarditis in an adolescent with cystic fibrosis in whom hMPV was identified as the probable aetiological agent. Given the rarity of cardiac involvement associated with this virus, this case highlights the importance of considering less common viral pathogens when clinical, laboratory, and imaging findings are suggestive of myocarditis.

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Exploring the Testicular Tropism of Mpox Virus: Implications for Sexual Transmission

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The male genital tract can serve as a reservoir for certain viruses, facilitating their transmission to the population through sexual contact. In the summer of 2022, an outbreak of Mpox virus (MPXV) infections occurred outside of Africa, mostly affecting men who have sex with men. Skin lesions were mainly localised to the genital area. The virus has been detected in the semen of several patients. Here, we are investigating the pathophysiology of MPXV in the human testis.

Using an organotypic culture model of human testicular tissue obtained from post-mortem donations (n=10) and maintained for 9 days, we analyse viral replication and infectivity by qPCR and plaque-forming unit (PFU) measurement in culture supernatants after MPXV infection (clade IIb strain). At an MOI of 1, the MPXV begins to replicate from 6 days post-infection (dpi) and reaches a high viral load with a median of 1.97×10^{10} viral DNA copies/ml culture supernatant (10.3log) at 9 dpi (p<0.0001 between 3 and 9 dpi). At a lower MOI of 0.1, a comparable median of 4.28×10^9 viral DNA copies/ml (9.63log) is achieved at 9 dpi. Infectious virus detected in the supernatants follow the same kinetics (peak at 4 log PFU/ml at MOI 1 and 0.1). A release of lactate dehydrogenase at this late stage reflects a probable cytopathic effect of the virus. The viral tropism for the testis is investigated by in situ hybridisation and reveals also virus at 6 and 9 dpi. Infection spread from the interstitial tissue (peritubular cells, Sertoli cells) to the seminiferous tubules, where they infect Sertoli and germ cells. No significant transcriptional induction of key pro-inflammatory or antiviral cytokines is observed. Early treatment with the antiviral drug tecovirimat effectively inhibits infectious virus production.

MPXV robustly infects the testis ex vivo and produces infectious particles with relatively slow kinetics compared to other viruses (eg Mumps virus). The infection of seminiferous tubule cells could contribute to the release of MPXV into semen, promoting sexual transmission even in the absence of skin lesions.

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Assessing the prevalence and Genetic Diversity of HEV-3 in Pig Farm Wastewaters in Portugal, 2024–2025

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Introduction/Aim: Hepatitis E virus (HEV) is an emerging zoonotic pathogen of major public health relevance. In Europe, HEV genotype 3 (HEV-3) predominates and includes several sub-genotypes associated with pig production and trade patterns. Wastewater-based surveillance is increasingly recognized as a valuable One Health approach for monitoring HEV circulation and diversity. However, nationwide data from Portugal remain limited. This study aimed to assess the prevalence of HEV RNA in pig farm wastewater and characterize circulating HEV-3 sub-genotypes in Portugal.

Methods: Influent and effluent wastewater samples were collected from pig farms across Portugal during surveillance campaigns conducted between 2024 and 2025. Samples were filtered, concentrated, and viral RNA extracted using TRIzol reagent and the QIAamp Viral RNA Mini Kit. HEV RNA detection was performed by RT-qPCR using the ceeramTOOLS HEV detection kit. Positive samples were further characterized by RT-PCR and Sanger sequencing following the HEVnet protocol targeting a 493-bp ORF2 fragment. Genotypes and sub-genotypes were assigned using the HEVnet typing tool, and phylogenetic analyses were performed in MEGA software. Comparative analyses included Portuguese human, animal, and environmental HEV sequences, as well as sequences from neighbouring countries.

Results: A total of 197 wastewater samples were collected from pig farms across 14 districts in four Portuguese regions (Norte, Centro, LVT, and Algarve). HEV RNA was detected in 39% (n=45) of influent samples and 27% (n=22) of effluent samples, indicating reduced viral detection after wastewater treatment. Influent wastewater positivity declined from 52% in 2024 to 32% in 2025. Genotyping of twelve positive samples revealed co-circulation of HEV-3 sub-genotypes 3c, 3e, and 3f. Subtype 3f predominated in 2024, whereas subtype 3e became more frequent in 2025.

Conclusions: This study demonstrates widespread HEV circulation and considerable HEV-3 diversity in Portuguese pig farm wastewater, highlighting wastewater-based surveillance as a complementary One Health strategy for monitoring HEV epidemiology and strains of potential public health relevance.

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Epidemiology and molecular characterization of human parainfluenza viruses in Italy during 2022–2025: results from the GLIVIRE National Laboratory Network

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Introduction/Aim: Human parainfluenza viruses (HPIV) are common respiratory pathogens, but their epidemiological characteristics are not fully described. The aim of this study was to describe the epidemiology of HPIV in Italy from 2022 to 2025.

Material and Methods: We analyzed the results of molecular respiratory viral diagnostic tests performed in the laboratories of the Italian Working Group on Respiratory Viral Infections (GLIViRe) between January 2022 and December 2025. The requested data included: total tests performed, HPIV-positive results, HPIV type, and co-detection of other respiratory viru-

ses. Data were stratified by month and per age group, and positivity rates were calculated.

Results: Twenty-one laboratories contributed to the study, for a total of 264,230 tests and 9,860 HPIV-positive results, with a positivity rate of 3.7%. There was an increase in testing frequency across years: from approximately 37,000 tests in 2022 to approximately 92,000 tests in 2025, while the HPIV positivity rate remained consistent throughout the study period (range 3.4%-4.2%). HPIVs circulated with a seasonal pattern with positivity rates increasing in March/April, then peaking in May/June and slowly decreasing until year end. The highest positivity rate was observed in the 1-4 years age group (range 6.2%-8.4%), and more generally in the pediatric population (≤ 14 years). The positivity rate was consistently below 3.0% in individuals ≥ 15 years (range 1.4%-3.0%). Regarding viral types, HPIV-3 accounted for the largest proportion of HPIV detections (47.6% of total single-HPIV detections), followed by HPIV-4 (16.4%), HPIV-1 (12.0%), and HPIV-2 (8.8%). HPIV-2 and HPIV-4 showed a biennial circulation during summer and autumn, while HPIV-3 remained stable over the year with higher circulation from March to July. Interestingly, co-detection with at least one other respiratory virus was observed in 3.3% of samples.

Conclusions: HPIV is widespread in Italy, particularly in the pediatric population, with HPIV-3 being the most prevalent strain.

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Nanopore-based genomic surveillance of RSV in Tyrol, Austria, during the 2025–2026 season

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Introduction/Aim: Respiratory syncytial virus (RSV) remains a major cause of hospitalisation in infants and severe illness in older adults. The introduction of RSVpreF vaccines and the monoclonal antibody nirsevimab has altered the landscape of circulating RSV variants. Genomic surveillance is essential for detecting resistance-associated mutations and monitoring intervention effectiveness.

Material: A total of 287 clinical respiratory samples and wastewater collected in Tyrol, Austria, during the 2025–2026 respiratory season were included in this study.

Methods: RNA was extracted, reverse-transcribed, amplified, and sequenced using Oxford Nanopore PromethION. Data were analysed using a bioinformatic pipeline, and an automated RSV mutation analysis tool was developed. Mutations were classified as nirsevimab resistance-associated or as binding-site polymorphisms without confirmed resistance.

Results: RSV-A and RSV-B genomes were characterised with sufficient coverage for lineage and clade assignment. Seasonal patterns showed RSV-A predominance early in the season, followed by a shift to RSV-B. Two clinical samples breakthrough harboured the N201S substitution in the RSV-B F protein, associated with reduced nirsevimab binding, representing the first detection of resistance-associated mutations in this cohort. Other mutations at nirsevimab binding sites, including S211N and R209Q, were not associated with reduced efficacy, and no additional resistance mutations were found in breakthrough infections. Mutations at the clesrovimab binding site were also analysed, with no resistance-associated substitutions detected. Mutations at the clesrovimab binding site — a monoclonal antibody recently approved in the United States targeting a distinct F protein epitope — were also analysed, with no resistance-associated substitutions detected.

Conclusions: Nanopore-based RSV surveillance provides a scalable approach for real-time monitoring of circulating variants and the emergence of resistance.

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Ribavirin and its use in different respiratory viral infections in specific instances

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The use of ribavirin as antiviral for respiratory viral infections is not well standardised within practice guidelines, including in immunocompromised patients, where they cast a significant burden for hospitalisation and poor outcomes.

Ribavirin is a synthetic guanosine analogue that effects antiviral actions mostly against RNA viruses. Its metabolised form ribavirin tri-phosphate (RTP) effects a range of antiviral actions through 1) depleting intranuclear guanosine triphosphate pool by competitive inhibition of cellular enzyme inosine monophosphate dehydrogenase, 2) inducing lethal mutagenesis when RTP pairs either with cytidine (found in RNA, DNA) or uridine (found in RNA), 3) inhibition of viral RNA polymerase, 4) interference with viral mRNA capping affecting viral stability and protein translation, 5) immunomodulation by stimulating host immunity Th1 response, upregulating interferon-related genes.

Here we describe our centre's experience in using ribavirin as antiviral therapy for respiratory viral infections over the past 2 years in 10 instances (respiratory syncytial virus = 2; human para-influenza virus type 3 = 2, human metapneumovirus = 3, adenovirus = 3). The locally determined criteria for ribavirin in symptomatic patients was based on clinical/radiological features, significant viral load [crossing threshold (Ct) <25] and high risk for clinical deterioration [immunocompromised, significant respiratory deterioration]. Clinical outcome was successful in all 10 instances, with significant reduction in viral load [Ct value], relief from supplemental oxygen and successful discharge from care.

We will share detailed data from our experience, describing our ribavirin initiation criteria, ribavirin safety and virological monitoring and therapy endpoints. In the absence of consensus guidelines in ribavirin use for different respiratory viruses, our proposed criteria and thresholds could potentially be studied in larger cohorts of risk group/immunocompromised patients, towards building consensus clinical guidelines in the use of ribavirin for specific respiratory viral infections in those patients where there are no other alternative antiviral options.

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Shift from A/H1N1 to A/H3N2 driven by expansion of a dominant “K” sublineage during the 2025–2026 influenza season in Spain.

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Introduction/Aim: Influenza circulation in the Valencia region has fluctuated since the SARS-CoV-2 pandemic, shifting from A/H3N2 to A/H1N1 predominance in recent seasons. We aimed to characterise influenza subtype dynamics and genetic diversity during the 2025–2026 season, assessing changes relative to previous trends.

Material and Methods: A hospital-based prospective active surveillance study conducted within the Valencia Hospital Network for the Study of Infectious Diseases (VAHNSI). Respiratory samples from ILI and SARI cases who required hospital admission were collected (total of 3,642 respiratory samples between November 2025 and May 2026) and screened for respiratory viruses by multiplex rRT-PCR. Influenza virus subtyping was performed by specific rRT-PCR, and a subset of samples (up to 21 December 2025) underwent whole genome sequencing for clade assignment and temporal analysis (further sequencing ongoing).

Results: Influenza A was detected in 601 samples, with one influenza B case and one A/B co-infection (positivity rate: 16.56%), representing the highest influenza activity since the SARS-CoV-2 pandemic onset. The epidemic showed a biphasic pattern. Early circulation (November) was dominated by A/H1N1, consistent with previous seasons. From mid-November, A/H3N2 increased rapidly and became dominant at the epidemic peak (weeks 52/2025–1/2026), reversing prior trends. Available sequencing (n=157) revealed limited diversity: A/H1N1 isolates belonged to clade 6B.1A.5a.2a.1, while A/H3N2 isolates clustered in 3C.2a.1b.2a.2a.3a.1. Notably, ~70–75% of H3N2 sequences corresponded to the sublineage (“K”), present in the early epidemic and dominant at the peak, indicating rapid expansion.

Conclusions: The 2025–2026 season shows a shift back to A/H3N2 dominance after two A/H1N1-predominant seasons, with a clear biphasic pattern. The expansion of a dominant A/H3N2 sublineage suggests a potential epidemiological advantage. These findings highlight the importance of ongoing molecular surveillance to detect subtype shifts and emerging variants.

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Hepatitis A outbreak linked to severe social deprivation in western France in 2025

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Introduction: Hepatitis A (HAV) incidence has declined worldwide thanks to improvements in sanitation and hygiene. However, the risk of a resurgence of this faecal-oral infection remains among populations living in poor sanitary conditions. On 19 June 2025, the Regional Health Agency for the Pays de la Loire (PDL) was alerted to an unusual number of cases.

Material and Methods: The National Reference Centre received serum from patients who tested positive for IgM, who lived in or had links to the PDL region, and wastewater from treatment plants serving the town most affected. Sanger VP1/2A sequences, and whole genome (WG) sequences from a subset of samples were submitted to phylogenetic analysis.

Results: Between 1 December 2024 and 31 December 2025, 186 sequences were obtained from patients residing in or with links to the PDL region; 22 were excluded because they had been exposed whilst travelling, and 143/164 cases could be investigated thanks to the mandatory reporting system. Of the cases investigated, 70% (100) were infected by a subgenotype IA strain (FR09-HA44.2) or closely related variants. This strain was initially detected within the Roma population (most of whom lived in slums); 38% (38/100) of the cases infected with this strain were Roma and 2% (2) were Travellers. A IB strain (FR25-HA24.9) was found in 29% of cases (42), which concerned only 2% (1) of Roma but 19% (8) of homeless people. Patients in these vulnerable situations accounted for the majority of cases between April and July, with the outbreak then shifting to the general population from August onwards. Wastewater analysis revealed the presence of both strains as early as late March and early April.

Conclusion: This outbreak highlights the role of social inequalities in the spread of infectious diseases and underscores the urgent need for targeted prevention and vaccination among vulnerable groups.

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Post-pandemic circulation and phylogenetic characterization of Enterovirus D68 in ILI and SARI admissions, Valencia, Spain (2020–2024)

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Introduction/Aim: Enteroviruses (EVs) are relevant pathogens due to their wide tissue tropism and association with neurological complications. Enterovirus D68 (EV-D68), initially described as a respiratory virus in 1962, has re-emerged since 2014 with links to acute flaccid paralysis and shows a biennial circulation pattern worldwide. We aimed to study enterovirus circulation in influenza-like illness (ILI) and severe acute respiratory infection (SARI) emergency admissions in Valencia, Spain.

Material and Methods: A hospital-based prospective active surveillance study conducted within the Valencia Hospital Network for the Study of Infectious Diseases (VAHNSI). Respiratory samples from ILI and SARI cases who required hospital admission were collected over four post-SARS-CoV-2 influenza seasons (2020/2021–2023/2024) and screened for respiratory viruses by multiplex rRT-PCR. EV-positive and undistinguishable RV/EV samples (n=85) were analysed by RT-PCR and partial Sanger sequencing of the VP4–VP2 region was performed, complemented with phylogenetic analysis.

Results: VP4–VP2 sequencing was successful in 67 samples, identifying EV-D (n=30), RV-A (n=20), RV-C (n=13), EV-B (n=3), and EV-C (n=1). All EV-D isolates corresponded to EV-D68. Most EV-D68 cases occurred in children (28/35), with a slight predominance in females (19 vs 16). Phylogenetic analysis of 27 sequences revealed co-circulation of subclades A2 (n=3) and B3 (n=24), mainly associated with adults and children, respectively. EV-D68 circulation followed a biennial pattern, with peaks observed in 2022 and 2024.

Conclusions: Our findings highlight the sustained circulation of EV-D68 in the Valencia region after the COVID-19 pandemic, with clear temporal patterns and age-related distribution of subclades. Continuous molecular surveillance is essential to monitor EV epidemiology and support early detection of strains potentially associated with severe clinical outcomes.

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Uridine-depleted codon optimization enables a potent unmodified mRNA vaccine against canine influenza virus in beagles

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Introduction/Aim: N1-methylpseudouridine (m1Ψ) is widely used in mRNA vaccines to limit innate immune activation, but it raises production costs, complicates intellectual property, and may promote ribosomal frameshifting. We aimed to enhance the in vivo potency of unmodified mRNA vaccines using a rational, uridine-depleted codon optimization strategy that remodels mRNA secondary structure, avoiding complex computational design.

Materials and Methods: Uridine-depleted, codon-optimized mRNAs encoding model antigens (Human papillomavirus 16 E7 or ovalbumin) and the hemagglutinin of canine H3N2 influenza virus were generated. In vitro protein expression and antigen-specific T cell and antibody responses were assessed in mice. Protection and viral clearance were evaluated after challenge, and durability was compared with a conventional inactivated vaccine in beagles.

Results: The optimized variants markedly increased in vitro protein expression and elicited robust antigen-specific CD8+ T cell responses in mice. The unmodified hemagglutinin mRNA vaccine induced potent humoral and cellular immunity comparable to m1Ψ-modified mRNA and superior to the inactivated vaccine, conferring complete protection and faster viral clearance. In beagles, it sustained a neutralizing antibody titer 24-fold higher than the inactivated vaccine at 15 weeks post-boost.

Conclusions: Rational uridine-depleted codon optimization alone can establish unmodified mRNA as a potent, cost-effective vaccine platform, offering an accessible alternative to chemically modified mRNA for veterinary and human use.



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Development of a system of primers for determining the causative agent of Crimean–Congo hemorrhagic fever using a real-time polymerase chain reaction method based on reverse transcription

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Introduction/Aim: Crimean-Congo hemorrhagic fever (CCHF) is a highly fatal tick-borne zoonosis with expanding endemicity in Kazakhstan due to climate-driven changes in Hyalomma tick distribution and livestock movement. Rapid laboratory confirmation is essential. This study aimed to develop, optimize, and evaluate a novel real-time reverse transcription polymerase chain reaction (RT-PCR) primer-probe system for sensitive and specific detection of CCHF virus (CCHFV) RNA in ticks and clinical samples.

Material: The study included 118 adult ticks collected in May–June 2024 from camels (*Camelus dromedarius*) and cattle (*Bos taurus*) in CCHF-endemic areas of the Kyzylorda region, Kazakhstan. Dominant species were *Hyalomma asiaticum* (n=53), *H. scupense* (n=31), and *H. anatolicum* (n=34), confirmed morphologically and by COI DNA barcoding. Acute-phase serum samples from two confirmed CCHF patients (Zhambyl region outbreak, 2024) were also tested. Synthetic RNA standards and nucleic acids from heterologous pathogens served as controls.

Methods: Ticks were pooled (up to 5 per pool) by species and site, then homogenized for RNA extraction using the QIAamp Viral RNA Mini Kit. A TaqMan real-time RT-PCR assay targeting the conserved 5'UTR of the CCHFV S segment was developed with degenerate primers and probe for broad clade coverage (I–IX). The assay was run on a QuantStudio 5 platform in triplicate. Analytical sensitivity (LOD₉₅ via Probit analysis), efficiency, specificity, and concordance with the WHO 2017 reference assay were evaluated under BSL-3 conditions.

Results: The assay demonstrated high sensitivity (LOD₉₅ corresponding to 10²–10³ copies/reaction) and no cross-reactivity with other pathogens. CCHFV RNA was detected in two *H. scupense* tick pools from Kyzylorda and in both patient serum samples.

Conclusion: The developed primer-probe system shows excellent sensitivity, specificity, and reproducibility, supporting its application in domestic diagnostic kits and surveillance of CCHF virus in natural foci in Kazakhstan.

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Broad-spectrum antiviral activity by targeting host cellular functions

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Introduction/Aim: Broad-spectrum antivirals that target conserved host-cell pathways offer a promising alternative to conventional virus-directed drugs. Because RNA viruses mutate rapidly, drugs aimed at viral proteins can quickly lose effectiveness. In contrast, host-cell processes used by different viruses for entry, replication, or spread are more stable, making them attractive targets for durable antiviral therapy.

Materials and Methods: A library of 880 FDA-approved compounds was screened at 10 μM using two pseudoviral systems: VSV-G pseudoparticles and SARS-CoV-2 S-spike (D614G Δ18) pseudoparticles. Viral inhibition was measured by luciferase activity in HEK-293T and HEK-293T/hACE2 cells. Compounds showing at least 50% inhibition of both systems with no more than 40% cytotoxicity were selected for further testing. Hits were then validated against whole REO and HSV-1 in Vero cells, a mini-HIV dual-reporter system, and NDV in embryonated eggs.

Results: The primary screen identified 59 non-toxic compounds that inhibited both pseudoviral systems. Among them, 31 protected Vero cells from REO-induced cytotoxicity, 7 protected against HSV-1, and 5 showed activity against both viruses. Two lead compounds, 1G10 and 2A11, selectively reduced HIV LTR-driven GFP expression without affecting host EF1Y-driven mKO2 expression, suggesting specific inhibition of viral transcription rather than general toxicity. In embryonated eggs infected with NDV, 1G10 increased embryo survival to 60% at 33 μM, compared with 0% survival in untreated infected controls. Under the same conditions, 2A11 achieved 40% survival. Natural extracts VC10 and VC7 also showed dual antiviral activity against REO and mini-HIV without detectable cytotoxicity.

Conclusions: These findings support the feasibility of repurposing FDA-approved compounds as host-directed broad-spectrum antivirals. The lead candidates showed activity across several viral models and provided meaningful protection in an in vivo embryo system. Ongoing work will focus on dose optimization, combination treatments, and evaluation in live poultry infection models.



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Evaluating the role of Geenius™ in discordant HIV serology: a retrospective quality improvement project

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Introduction: The local HIV serology pathway at UKHSA, Cambridge University Hospitals included reflex Geenius™, a third-generation immuno-chromatographic antibody assay, after a reactive first-line screen. This quality improvement project evaluated whether Geenius adds diagnostic value when the initial Centaur® HIV Ag/Ab assay was reactive, but the second-line VIDAS® HIV Duo Ultra assay was negative, aiming to reduce unnecessary testing safely.

Methods: A retrospective review of reactive HIV serology specimens tested between 1 Sep 2022-7 Oct 2024. Of 739 reactive specimens, 11 without a hospital number and 454 without confirmatory samples within the time-period were excluded. Final dataset comprised 287 specimens with serial serology from 105 patients. Concordance between Centaur, VIDAS, and Geenius repeated serology results was assessed alongside viral load and reference laboratory investigations to determine true HIV status. The subgroup of interest was Centaur-reactive, VIDAS-negative cases.

Results: Among 287 analysed specimens, 141 specimens from 57 patients were concordantly positive and 4 specimens from 2 patients were concordantly negative, across all three assays. Within the discordant subgroup, 117 specimens from 41 patients were Centaur reactive, VIDAS negative; only one patient was confirmed HIV positive. This case represented seroconversion and was resolved through repeat serology and viral load testing rather than Geenius alone. The true-positive proportion in Centaur-reactive, VIDAS-negative cases was 2.44% at patient level (1/41; 95% Wilson CI: 0.43-12.6). Geenius frequently produced indeterminate results in patients who were later confirmed HIV negative by follow-up testing.

Conclusions: The Geenius added minimal diagnostic value when VIDAS was negative and did not reliably resolve discordant serology. Omitting automatic Geenius testing was deemed appropriate, repeat serology and HIV RNA testing where indicated, was recommended, aligning local practice with national guidance, including the Infectious Diseases in Pregnancy Screening programme and the UK Standards for Microbiology Investigations.

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IgG3-skewed Response Underestimates Pediatric Varicella Vaccine Immunity Measured by Commercial gpELISA Without Compromising Fc-Mediated Antibody Functions

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Introduction: Accurate assessment of varicella vaccine-induced immunity in early childhood is critical. However, commercially available Varicella-Zoster Virus (VZV) glycoprotein ELISA (gpELISA) kits often classify pediatric vaccine recipients as seronegative or borderline, despite protective responses by fluorescent antibody to membrane antigen (FAMA), suggesting suboptimal gpELISA cut-off values.

Materials & Methods: We re-calculated gpELISA cut-off values using receiver operating characteristic (ROC) curve analysis with FAMA as the reference standard, utilizing 304 pediatric sera samples obtained from a Phase III primary live-attenuated varicella vaccine (LAV) clinical trial, including a subset of paired sera. To investigate age-dependent differences in the performance of gpELISA cut-offs, we assessed VZV-specific IgG subclass responses across cohorts representing children and adults. In parallel, antibody-dependent cellular phagocytosis (ADCP) and cytotoxicity (ADCC) were evaluated to assess Fc-mediated antibody functions.

Results: Re-calculated gpELISA cut-offs derived from ROC curve analysis lowered the seropositivity threshold and significantly improved the agreement with FAMA. While adults exhibited markedly higher gpELISA titers than children despite comparable FAMA titers, this discrepancy aligned with distinct IgG subclass responses; children predominantly generated VZV-specific IgG3, whereas adults elicited a dominant IgG1 response. Crucially, despite having lower gpELISA titers, children demonstrated significantly higher ADCP activity compared to adults, confirming that vaccine-induced viral clearance capacity is fully preserved in pediatric vaccinees.

Conclusions: Commercial VZV gpELISA kits substantially underestimate protective immunity in young children due to an age-dependent bias toward IgG1 detection, which overlooks the highly functional IgG3 response prevalent in pediatric population. Re-calculation of kit-specific cut-off values and the incorporation of Fc-mediated functional assays are required to ensure accurate evaluation of primary LAV efficacy in children.

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Enhanced Fc-Mediated Antibody Effector Functions and Minimal T-Cell Responses Following Live Attenuated Varicella Vaccination in Infants

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Introduction/Aim: Live attenuated varicella (LAV) vaccination induces protective immunity through multiple immune mechanisms. Although conventional serological assays, including glycoprotein ELISA (gpELISA) and fluorescent antibody to membrane antigen (FAMA), primarily evaluate antibody quantity, assessment of functional antibody quality and antigen-specific T-cell responses provides deeper insight into vaccine-mediated protection. This study evaluated humoral, Fc-mediated antibody, and cellular immune responses following LAV vaccination in infants.

Materials and Methods: Paired pre- and post-vaccination sera and peripheral blood mononuclear cells (PBMCs) were collected from 53 healthy infants (12–16 months) who received LAV vaccination. VZV-specific antibody responses were evaluated using FAMA and gpELISA. Fc-mediated antibody functions were assessed by antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC) assays against VZV-infected MRC-5 cells. VZV-specific T-cell responses were measured using IFN- γ ELISpot and an activation-induced marker (AIM) assay following PBMC stimulation with a VZV glycoprotein E (gE) peptide pool.

Results: Following vaccination, FAMA titers and gpELISA antibody levels increased significantly compared with pre-vaccination levels (both $p < 0.0001$). This increase was accompanied by robust Fc-mediated functional antibody responses, with both ADCP and ADCC showing significant post-vaccination enhancement (both $p < 0.0001$). In contrast, VZV-specific T-cell responses remained largely quiescent, demonstrating minimal activation in the AIM assay and no detectable responses in the IFN- γ ELISpot assay.

Conclusions: LAV vaccination in infants elicited robust humoral immunity characterized by increased antibody quantity and enhanced Fc-mediated effector functions, whereas vaccine-induced T-cell responses remained minimal. These findings suggest that Fc-mediated functional antibody responses contribute to vaccine-induced protection in this age group and support the integration of functional antibody assays with conventional serology for evaluating varicella vaccine immunogenicity.

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Genomic characterization of the first complete Italian Molluscum contagiosum virus genomes

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Molluscum contagiosum is a common cutaneous infection caused by Molluscum contagiosum virus (MOCV), a human-specific poxvirus responsible for benign but highly transmissible skin lesions. Despite its global prevalence, the genomic diversity and evolutionary dynamics of MOCV remain poorly characterized, particularly in Italy, where complete genome data are lacking. In this study, we generated and analyzed the first complete Italian MOCV genomes to expand current knowledge of viral variability and adaptation.

Ten complete MOCV genomes were sequenced from clinical samples using a hybrid strategy combining short and long read sequencing, enabling high quality assembly and an in deep characterization of genome architecture. To assess phylogenetic placement and genotype distribution, Italian genomes were analyzed together with all publicly available complete MOCV genomes using comparative genomic and phylogenetic approaches. Our results confirmed the previously described global distribution of MOCV genotypes, with Italian strains clustering within known major genotypes and showing no evidence of unique lineage diversification.

Evolutionary analyses identified a subset of genes under positive selection. These genes were enriched for functions linked to immune evasion and host interaction, including inhibition of NF- κ B activation, interference with apoptosis, and proteins with immunoglobulin-like domains or similarity to host immune receptors. Additional selected genes included factors involved in viral entry/fusion, morphogenesis, and virion structure. Overall, this study provides the first genomic insight into Italian MOCV diversity and highlights evolutionary mechanisms potentially contributing to adaptation in this human-restricted pathogen.

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Blood-donor surveillance reveals heterogeneous population immunity against JN.1 descendants and BA.3.2-lineage SARS-CoV-2 variants

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Introduction/Aim: Newly emerging SARS-CoV-2 variants may necessitate vaccine strain updates when they escape pre-existing immunity. Population-level immunity remains a key parameter for vaccine strain decisions. Yet, most neutralization studies rely on selected cohorts rather than scalable population-based surveillance. To overcome this limitation, we used routine blood donor samples as a pragmatic proxy for SARS-CoV-2 immunity in the healthy adult population.

Materials and Methods: Plasma samples were collected from whole-blood donors in North Tyrol, Austria, in October 2025 and March 2026 (n=78 per time point). SARS-CoV-2 neutralizing antibodies were analysed using a vesicular stomatitis virus-based pseudovirus assay. We selected seven SARS-CoV-2 spike variants, covering previous vaccine antigens (BA.5, XBB.1.5, and JN.1), current 2025/2026 season vaccine antigen formulation (LP.8.1), and variants currently circulating globally (NB.1.8.1, XFG, and BA.3.2.2), to assess population immunity.

Results: We validated the pseudovirus neutralisation assay against an authentic virus focus-forming assay using WHO reference standards and samples obtained from individuals after an XBB.1.5 booster immunization. We found a good correlation (Spearman) of both assays using the XBB.1.5 booster sera (BA.5 $r=0.8014$, XBB.1.5 $r=0.7912$, and JN.1 $r=0.7618$). At both time points, neutralisation antibody titres in the blood donor were highest against earlier Omicron variants but declined against more recent JN.1 descendants and BA.3.2.2, with many titers against recent variants falling below the limit of detection. The within-variant spread of titers was rather high and titers were generally lower compared to those reported earlier in selected cohort studies. Between the two time points, neutralizing antibody titers increased significantly only for BA.3.2.2 and XFG, consistent with the detection of these variants in public wastewater monitoring.

Conclusions: Our study highlights the value of blood donor populations as a scalable approach for monitoring variant-resolved population immunity and informing SARS-CoV-2 vaccine-strain update decisions.

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Evaluation of Copan FecalSwab™ performance for molecular diagnostic applications

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Background: Rapid and accurate identification of gastrointestinal pathogens is essential for appropriate patient management. While culture-based methods have traditionally been used, molecular multiplex assays have improved diagnostic sensitivity and turnaround time. This study evaluated the suitability of Copan FecalSwab™ for the molecular detection of gastrointestinal viruses.

Methods: FecalSwab™ devices were inoculated with Human Adenovirus 1 (ATCC VR-1), Human Astrovirus (ATCC VR-1936), and Rotavirus A (ATCC VR-2018). Viral suspensions were diluted to achieve Ct values between 25 and 35 after spiking into the device. Samples were processed immediately (T0) or stored at 5 °C ± 3 °C and 22.5 °C ± 2 °C for up to 15 days to simulate transport conditions, followed by nucleic acid extraction. Extracted nucleic acids were stored at -80 °C and subsequently analyzed by RT-PCR in single, target-specific runs to minimize inter-run variability. Additional tubes were subjected to storage at -20 °C to assess the impact of up to three freeze-thaw cycles. Potential interference with molecular assays was evaluated using non-inoculated FecalSwab™ controls.

Results: Performance was considered acceptable when a maximum increase of 3.5 Cts was detected between T0 and the stability time under evaluation.

Across all tested viruses, FecalSwab™ maintained stable Ct values within the acceptance threshold after storage at both refrigerated and room temperatures for up to 15 days, including after three freeze-thaw cycles. No assay interference or invalid results were observed.

Conclusions: Copan FecalSwab™ showed reliable molecular performance for the detection of gastrointestinal viruses, ensuring nucleic acid stability under simulated transport and storage conditions, without evidence of interference across the evaluated molecular assays

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Performance evaluation of a novel guanidine-free medium for viral inactivation and nucleic acid preservation

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Introduction: Reliable molecular diagnostics require the preservation of nucleic acid integrity throughout the preanalytical phase. In addition, the COVID-19 pandemic highlighted the need for transport media capable of inactivating pathogens while maintaining compatibility with molecular assays. This study aimed to evaluate Copan UBM™, a guanidine-free medium developed for viral inactivation and nucleic acid preservation.

Methods: The inactivation efficacy of UBM™ was assessed using Influenza A virus (ATCC VR-1679) after a 10-minute incubation at room temperature. Residual infectivity was evaluated by monitoring cytopathic effect in MDCK cells, and viral titers were quantified using the TCID₅₀ assay. Molecular compatibility was investigated in the presence and absence of viral targets (Influenza A and Adenovirus ATCC VR-1) to detect potential assay interference. Nucleic acid extraction was performed with the AutoMate Express™ system using the PrepSEQ™ Express Kit (Life Technologies). Detection was performed with RIDAGENE Flu & RSV (R-Biopharm) and QIAGEN Adenovirus qPCR assays on the Applied Biosystems 7500 platform. Compatibility with closed systems was further assessed using the Xpert Xpress Flu/RSV assay (Cepheid).

Stability of viral nucleic acids was examined at baseline and after four weeks of storage at 5 °C ± 3 °C, 18 °C ± 3 °C, and 35 °C ± 2 °C.

Results: A >3 log₁₀ TCID₅₀ was observed after 10 minutes of incubation in UBM™, indicating effective inactivation of Influenza A virus. Ct variations remained within ±3 cycles compared to baseline under all storage conditions, demonstrating preservation of nucleic acids over time. No assay interference or invalid results were detected across all tested platforms.

Conclusions: Copan UBM™ effectively inactivates viral samples while ensuring user safety through its guanidine-free formulation. It showed full compatibility with molecular assays, even after prolonged storage at different temperatures, making it a reliable solution for safe handling and testing of infectious samples.

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Comparative analysis of a novel, guanidine-free inactivation medium and conventional viral transport media for FluA and RSV molecular detection

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Introduction: In the preanalytical phase, transport media play a critical role in preserving nucleic acids integrity while samples are transported to the laboratory for molecular diagnostics.

This study aims to compare Copan UBMTM, a novel, guanidine-free inactivating medium, with standard viral transport media for influenza A virus (FluA) and respiratory syncytial virus (RSV) detection using molecular techniques.

Materials and Methods: UBMTM and conventional, non-inactivating viral transport media from 3 different providers were inoculated with identical viral suspensions, using 100µL in 3mL for RSV and FluA. For each device and microorganism, one sample tested in duplicate at baseline (T0) and two samples tested in duplicate after 48 hours of storage (T48) at room temperature were prepared. A volume of 300µL from each sample was tested using the Xpert Xpress CoV-2/Flu/RSV plus assay (Cepheid).

Results: Mean Ct values for UBMTM remained stable between T0 and T48, with minimal variations observed:

- FluA (T0: 22.25 γT48: 22.15, ΔCt: -0.10)
- RSV (17.70 γ17.58, ΔCt: -0.13)

while the other devices generally showed a higher difference in ΔCt:

- Device 1 à FluA (T0: 23.25 γT48: 24.95, ΔCt: 1.70)
- Device 1 à RSV (T0: 19.15 γT48: 19.73, ΔCt: 0.58)
- Device 2 à FluA (T0: 24.50 γT48: 25.08, ΔCt: 0.57)
- Device 2 à RSV (T0: 19.40 γT48: 19.40, ΔCt: 0.00)
- Device 3 à FluA (T0: 23.15 γT48: 24.65, ΔCt: 1.50)
- Device 3 à RSV (T0: 18.85 γT48: 19.20, ΔCt: 0.35)

In addition, UBMTM consistently generated lower Ct values at both time points compared to the other transport media for both FluA and RSV.

Conclusions: UBMTM provided enhanced performance for the viral target tested for both T0 and T48 storage at room temperature. These preliminary data on stability of nucleic acids supports its applicability as a medium for sample transport for molecular diagnostics.



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A case of infection with a putative new subtype M of human immunodeficiency virus type 1 of group M

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Introduction: HIV-1 emergence is estimated to have occurred c.a. 1920s in Democratic Republic of the Congo (DRC). Only its group M is pandemic, which currently comprises 10 established subtypes (A-D, F-H, J-L). Here, we describe a near-complete HIV-1 genome that meets the definition of a putative new HIV-1 group M subtype.

Materials and methods: In-house-designed PCR primers, the DeepChek Assay Whole Genome HIV-1 Genotyping kit (ABL), or the Comprehensive viral panel (Twist) were used for PCR amplification or enrichment of HIV-1 RNA/DNA from blood. Sequencing used Sanger and next-generation (Oxford Nanopore, Illumina) technologies. Bioinformatic analyses used Samtools, Minimap2, ViroScore, Bootscan, IQ-TREE, and MEGA12. Structural analyses used Mutate, HyperChem, and Molegro Molecular Viewer.

Results: The patient, HIV-1-diagnosed in 1989, originates from DRC. The HIV-1 western blot shows atypical reactivities. CD4+ T cell counts fluctuated between 351 (at antiretroviral therapy (cART) initiation) and 2394/mm³, and plasma viral load, initially 5.0 log₁₀copies/mL, reached undetectability on cART (tenofovir AF, emtricitabine, and bictegravir since 2019 in our center). The obtained near-full length genome (GenBank no. PZ307881) showed a mean nucleotide divergence of 12.7% (range, 11.9-13.7%) with reference genomes of the 10 established subtypes, while mean divergence is 12.6% (10.0-13.7%) for these 10 subtypes between each other. Similar results were obtained when cutting the genomes in 8 fragments. Phylogenies showed that sequences of this putative new subtype comprised a distinct branch. No recombination, or natural antiretroviral drug resistance, were detected. Reverse transcriptase (RT) structure exhibited slight changes compared to subtype B RT that could alter processivity.

Discussion: These data support that the obtained genome is of a new HIV-1/M subtype. They indicate that HIV-1 diversity is still not comprehensively deciphered, and confirms DRC as an epicenter of the HIV-1/M pandemic. This original HIV-1 genome might allow detecting new HIV-1 recombinants. Genotypic drug resistance testing did not identify particularities.

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Non-RBD Spike-reactive human monoclonal antibodies from a SARS-CoV-2 convalescent donor show binding without detectable pseudovirus neutralization

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Introduction/Aim: Human monoclonal antibodies recovered after SARS-CoV-2 infection provide insight into humoral specificity and function. This study characterized Spike-reactive monoclonal antibodies isolated from an unvaccinated convalescent donor sampled during South Africa's Beta-dominant epidemic wave.

Material and Methods: Donor serum reactivity to Spike and receptor-binding domain (RBD) was assessed by ELISA, and neutralization by pseudovirus assay. Antigen-reactive B cells were single-cell sorted using biotinylated Beta and D614G Spike antigens. Paired heavy- and kappa light-chain variable regions were recovered, cloned and expressed as recombinant human IgG1 antibodies. Antibodies were characterized by ELISA, surface plasmon resonance, pseudovirus neutralization, antibody-dependent cellular phagocytosis (ADCP), and molecular docking.

Results: From 161 sorted B cells, eight recombinant human IgG1 monoclonal antibodies were generated. Three antibodies, NM296, NM295 and NM215, bound wild-type Spike by ELISA but did not bind recombinant RBD. Surface plasmon resonance confirmed binding to immobilized wild-type Spike, with apparent submicromolar affinities and distinct kinetic profiles. None neutralized D614G, Beta, BA.1, BA.4, BA.2.87, or XBB.1.16 pseudoviruses, and no detectable ADCP activity against Beta Spike was observed. Docking predicted non-RBD Spike-binding interfaces, with NM296 and NM295 showing interactions involving the S2 subunit.

Conclusions: These data identify donor-derived non-RBD Spike-reactive antibodies that bind SARS-CoV-2 Spike but lack detectable pseudovirus neutralization or ADCP activity. The findings contribute to the characterization of non-RBD antibody responses and support further evaluation of variant reactivity, epitope specificity, and Fc-mediated functions.

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Analytical and Clinical Validation of the cobas® UCT-TIB-FluA-GT Assay for Influenza A Detection and Subtyping

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The recent emergence of human infections caused by highly pathogenic avian influenza A(H5N1), including cases presenting with conjunctivitis, highlights the need for validated molecular assays capable of rapidly detecting and accurately subtyping influenza A viruses from multiple clinically relevant specimen types. We performed an analytical and clinical validation of the cobas® UCT-Tib-FluA-GT assay (Roche Diagnostics) for influenza A detection and subtyping using nasopharyngeal swabs, bronchoalveolar lavage fluids and conjunctival fluids. The validation included assessments of reproducibility, specificity, robustness and analytical sensitivity, with the limit of detection determined using titrated virus stocks and expressed as PFU/mL. Reproducibility was evaluated using 20 clinical H1N1 and H3N2 specimens of each subtype and 20 matrices spiked with H5N1 viruses representing the two predominant circulating genotypes, B3.13 and D1.1. Analytical specificity was demonstrated using a panel of influenza A viruses including H1N1, H2N2, H3N2, H5N1, H6N2, H7N9, H9N2 and H11N9. Clinical performance was evaluated by comparison with the laboratory-developed RT-qPCR assay routinely used. The cobas® UCT-Tib-FluA-GT assay demonstrated excellent reproducibility (overall %CV <2.5%) across all specimen types and influenza A subtypes tested. No cross-reactivity or incorrect subtype assignment was observed during specificity testing, and robustness experiments confirmed consistent assay performance. Complete qualitative agreement was observed between the cobas® assay and the reference RT-PCR for influenza A detection, although fluorescence values differed between platforms as expected due to differences in instrument optics. The assay correctly differentiated seasonal influenza A viruses from H5N1 viruses and accurately identified both circulating H5N1 genotypes. These findings demonstrate that the cobas® UCT-Tib-FluA-GT assay is a robust and reliable molecular diagnostic tool for influenza A detection and subtyping across multiple clinically relevant specimen types and supports enhanced diagnostic capacity, surveillance and preparedness for emerging avian influenza viruses.

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Detection and genetic analysis of Jingmen tick virus in Greece

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Introduction/Aim: Jingmen tick virus (JMTV) is an emerging, segmented RNA virus in the Flaviviridae family. Since its first identification in 2010 in China, JMTV has been detected in several parts of the world, primarily in ticks, but also in other arthropods, mammals, and even humans. The aim of this study was to screen for JMTV ticks collected in Greece, obtain whole genome sequences of the viral strains, and identify the genetic relationships with strains detected in other parts of the world.

Material and Methods: The study included 400 adult ticks (333 Rhipicephalus bursa, 40 Ixodes ricinus and 27 Ixodes gibbosus) previously collected from livestock and the environment in various administrative regions of Greece. The ticks were grouped into 107 pools and screened for JMTV using an in-house real-time RT-PCR protocol.

Results: JMTV was detected in 41 (38.3%) tick pools in five of the seven regions tested. The virus positivity differed among tick species, hosts and regions. A single JMTV positive R. bursa tick was coinfecting with Aigai virus. Five positive pools with the highest viral load (Ct range 14.61-20.36) were subjected to metagenomic next-generation sequencing. Phylogenetic analysis based on the complete coding sequences of all four JMTV RNA segments revealed that the sequences of the Greek strains share 95.41% to 97.16% nucleotide identity with their closest relatives from Kosovo, Romania, and Türkiye.

Conclusions: The results of the study confirm the wide distribution of JMTV in ticks in Greece, providing the first comprehensive genomic data for the virus in the country and highlighting the need for surveillance studies to assess any public health risk.

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Evaluating the use and impact of multiplex lateral flow (M-LFD) testing for influenza A, B and SARS-CoV-2 viral infections in a South London hospital

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Introduction: SureScreen M-LFD testing was introduced in paediatric/adult emergency and ward settings for influenza A/B and SARS-CoV-2 to inform antiviral and infection prevention and control (IPC) decisions. Following discordant results, including influenza A with low Ct values, we audited concordance with respiratory PCR in symptomatic patients.

Method: All PCR results positive for influenza A, influenza B or SARS-CoV-2 from 1 November to 16 December 2025 were reviewed. Patients were included if M-LFD and PCR were performed within 24 hours of each other. Data included timing, semi-quantitative Ct values, clinical presentation, location, IPC actions and antiviral treatment.

Results: Forty-three patients met inclusion criteria. Median sampling interval was 5.5 hrs (IQR 0.02–18.1) with PCR was usually after M-LFD. Cases were influenza A(35/43, 81.4%), mainly H3(31/35, 88.6%); 7/43(16.3%) were SARS-CoV-2 and 1/43(2.3%) influenza B. Only 9/43(20.9%) had concordant positive results, 34/43(79.1%) were M-LFD negative/PCR positive. M-LFD detected 6/35(17.1%) influenza A and 3/7(42.9%)SARS-CoV-2 cases. Median AusDiagnostics Ct for was 19.2 (IQR 14.5–23.9), indicating high viral burden in many discordant cases.

Median PCR turnaround time was 25.1 hrs (IQR 20.4–32.2) and increased during the audit (p=0.031). All six concordant influenza A cases received prompt oseltamivir. Of 29 discordant influenza A cases, 18(62.1%) received oseltamivir after a median 2.5-day delay; 11 received none. Many M-LFD-negative/PCR-positive patients remained in bays or overflow areas and moved wards before PCR confirmation, increasing nosocomial transmission risk.

Conclusion: Negative M-LFD results were insufficiently reliable to guide treatment or IPC decisions during peak respiratory virus activity, especially for influenza A/H3. Delayed PCR compounded treatment delays and transmission risk.

Point-of-care PCR should be considered for admitted or high-risk symptomatic patients before ward transfer. Where unavailable, PCR should be taken alongside M-LFD. Structured M-LFD recording, clearer dual-testing rationale, and EPR based contact tracing and prophylaxis should be introduced. Re-audit is recommended.

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Measles-associated immune amnesia, secondary infections and long-term health consequences

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Introduction: Bangladesh is experiencing a major measles outbreak following a decline in vaccination coverage, with a marked increase in paediatric hospitalizations. Infection with measles virus has been associated with 'immune amnesia', resulting in increased susceptibility to secondary infections. However, its impact in endemic settings remains poorly understood. Here, we describe a prospective cohort study that aims to study the relationship between early-life measles and viral load, viral dissemination, disease severity, immune dysregulation, secondary infections, and neurological complications in Bangladesh.

Materials and Methods: Children (<11 years, n=100) presenting with suspected measles (within 48 hour of onset of rash) are being enrolled at the Infectious Diseases Hospital, Dhaka, Bangladesh. Clinical data and blood, respiratory, urine and stool samples will be collected during acute measles virus infection. A subset of cases (n=50) will be followed for three years, together with age- and sex-matched uninfected controls.

Laboratory analyses include the detection of measles virus genome by RT-qPCR followed by whole genome sequencing, and measles virus-specific antibodies. To study measles-associated immune amnesia, lymphocyte counts will be determined, combined with spectral flow cytometry for immune phenotyping of infected cells, and a longitudinal assessment of measles virus-specific and heterologous immune responses. Associations between virological, immunological and clinical parameters will be analysed using multivariable statistical models.

Results: We will include 100 pediatric cases, with an additional 50 cases and 50 matched controls enrolled in longitudinal follow-up. Preliminary analyses will be presented, focusing on epidemiological data, viral diagnostics and viral dynamics.

Conclusions: This study will provide insight into measles pathogenesis and immune amnesia in a high-transmission setting. The findings will inform vaccination strategies, patient management, and preparedness for future measles outbreaks which are likely to occur in areas with declining vaccination rates.



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Peptide-based ELISA for the Detection of Antibodies Against Enteroviruses Associated with human hand-foot-and-mouth disease

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Hand-foot-and-mouth disease (HFMD) is a highly contagious infectious disease caused by Enterovirus A predominantly affecting children. Among the serotypes responsible, enterovirus A71 (EV71), coxsackievirus A6 (CVA6), CVA10, and CVA16 are the most frequently detected and show an increasing prevalence globally. Effective serological tools capable of detecting antibodies against these serotypes are crucial for both clinical diagnosis and epidemiological surveillance. In our previous work, we successfully identified B-cell epitopes on the proteins of EV71, CVA6, CVA10, and CVA16 using sera from HFMD patients possessing elevated neutralizing titers (NT) against EV71, CVA10, and CVA16. Building on these findings, the present study aimed to develop peptide-based ELISA tests capable of detecting antibodies against the three major serotypes. We tested HFMD patient sera with mixtures of peptides representing immunodominant epitopes from (i) one serotype to define serotype-specific antibodies and (ii) mix of all four serotypes as HFMD universal ELISA tests. Our peptide-based ELISAs showed positive reactivity with sera having neutralizing titer (NT) > 4 and NT < 4; however, the low reactivity (OD₄₅₀) was also observed in some samples. Correlation analyses between OD₄₅₀ and NT revealed R² scores ranging from < 0.1 to 0.45. Importantly, our universal ELISAs were able to detect antibodies elicited by all three serotypes. Nevertheless, the serotype-specific ELISA tests not only reacted with antibodies elicited by their target serotypes but also cross-reacted with antibodies elicited by other serotypes. Altogether, in this work, we successfully developed universal peptide-based ELISA tests for detecting antibodies against multiple Enterovirus A serotypes, which could help inform antibody status in HFMD patients.

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Genomic Characterization of Mpox Virus Clade Ib in Spain: First Imported and Autochthonous Cases

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Introduction/aim: Mpox virus (MPXV) clade Ib, declared a Public Health Emergency of International Concern by WHO in 2024, has spread beyond Africa, with the first autochthonous cases in Spain reported since October 2025. This clade shows enhanced person-to-person transmission and a high number of APOBEC3-related mutations. To explore the introduction and community transmission of MPXV clade Ib, we analyzed whole genomes from imported and locally acquired cases from different Spanish regions.

Material and Methods: A total of 49 cases from 13 hospitals/health centres located in southern (Andalusia), central (Community of Madrid), and north-eastern (Catalonia) Spain were sequenced using Illumina platforms between September 2025 and March 2026. Phylogenetic inference was performed with IQ-TREE3. Genomic analyses were conducted using a Squirrel-based pipeline, and mutational profiles were evaluated for APOBEC3 signatures.

Results: A total of 44 complete MPXV clade Ib genomes were obtained. The phylogenetic tree showed short internal branches and limited resolution, consistent with the narrow sampling window and the low genetic diversity of orthopoxviruses. The sequences from the north-eastern and southern regions grouped in the same monophyletic branch, suggesting a single introduction into both regions. In contrast, in the central region, three distinct introductions may be inferred: one imported case without secondary transmission, and two autochthonous transmission chains. The most recent introduction clustered with the monophyletic north-east-south branch. APOBEC3 mutations were predominant in all samples, consistent with their role in the recent evolution of MPXV. No inter-clade recombination

events were observed.

Conclusions: The molecular epidemiology of the first MPXV clade Ib cases in Spain reveals at least three independent introductions, though only two of them led to sustained local transmission. The limited phylogenetic resolution elsewhere suggests the need for increased genomic surveillance and indicates that further introduction events could be identified if the study is extended to other Spanish regions.

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Emergence of a Resistance-Associated Mutation During Prolonged RSV Infection in an Immuno-compromised Patient

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Introduction/Aim: Persistent RSV infection in immunocompromised hosts provides an opportunity for viral evolution, but the emergence of monoclonal antibody (mAb) resistance-associated mutations in the absence of mAb treatment, has rarely been described. We report the emergence of an RSV fusion gene mutation, previously associated with nirsevimab resistance, in a ribavirin-treated immunocompromised patient with prolonged RSV infection.

Methods: From February to April 2025, six respiratory swabs were collected from a hospitalized immunocompromised individual, all testing positive for RSV. Samples were sent to the Wadsworth Center Virology Laboratory for further genetic analysis including RSV subtyping with real-time RT-PCR and whole genome sequencing (WGS). All six samples were additionally cultured in Hep2 cells. Consensus sequences were assembled and percentage single nucleotide polymorphisms (SNP's) determined with Geneious Prime. Lineage was determined using Nextclade.

Results: All samples typed as RSV A, with Ct values indicating an increase in viral titer over the two-month period. Virus was successfully cultured from five of the six samples. WGS analysis indicated the presence of lineage A.D.3 in all samples. Analysis showed the emergence of a nirsevimab resistance-associated substitution (RAS) at position 209 of the fusion gene (K209E) within a month of collection of the initial RSV positive sample, followed by its proportional increase in the virus population to >99% in the final specimen collected two months later. Similar proportional increases of the mutant virus were seen in the cultured isolates, suggesting a fitness advantage over the parent strain.

Conclusions: The emergence and eventual predominance of K209E, a nirsevimab RAS, during prolonged RSV infection, but without RSV-specific mAb treatment, is notable. While ribavirin treatment did little to reduce viral titer, its mutagenic mechanism during persistent viral replication may have potentiated the emergence of mutations that reduced the efficacy of RSV therapeutics.



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Impact of an inactivating, non-toxic transport medium on molecular assay performance: a paired multi-platform comparison with conventional media

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Introduction: The choice of viral transport media may influence nucleic acid stability, extraction efficiency, and the analytical performance of molecular assays. Copan UBMTM was developed as a non-guanidine-based inactivating medium, overcoming limitations of guanidine salts, which are incompatible with some extraction chemistries and may inhibit downstream assays. This study assessed the performance of UBMTM across multiple commercial molecular platforms for the diagnosis of respiratory viruses, compared with routinely used transport media.

Methods: For each viral target (SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus), 15 positive and 15 negative samples were included. Paired aliquots from the same specimen were tested using Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay (Seegene), Xpert® Xpress CoV-2/Flu/RSV plus (Cepheid), and Panther Fusion® SARS-CoV-2/Flu A/B/RSV Assay (Hologic). When multiple genetic targets were detected, Ct values were analyzed separately. Comparator media were Copan UTM® for Xpert® Xpress and Allplex™, and the manufacturer-provided Hologic lysis buffer for Panther Fusion®. Paired comparisons were performed using paired t-tests or Wilcoxon signed-rank tests.

Results: Xpert® Xpress showed high concordance between UBMTM and UTM®, with no significant differences for SARS-CoV-2 ($p=0.626$), Influenza A ($p=0.607$ and $p=0.532$), or RSV ($p=0.492$), and a significant difference only for Influenza B ($p=0.012$). Allplex™ showed significant differences for SARS-CoV-2 N and RdRp genes and for Influenza A, while Influenza B and RSV were comparable ($p=0.906$ and $p=0.164$). Panther Fusion® showed significant differences for SARS-CoV-2 ($p=0.040$) and RSV ($p=0.011$), whereas Influenza A and B showed no significant differences ($p=0.589$ and $p=0.900$).

Conclusion: All assays demonstrated good comparability between UBMTM and standard diagnostic media. Observed differences were largely non-significant and, when statistically significant, did not affect result interpretation. These findings support the use of UBMTM as a suitable alternative transport media for molecular diagnosis of respiratory viral infections.

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Evaluation of a novel inactivating transport medium for HPV molecular detection across multiple molecular platforms

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Background: Human papillomavirus (HPV) testing is central to cervical cancer screening programs and requires reliable sample preservation. Following the COVID-19 pandemic, there has been increased interest in platform redundancy and interoperable sample media to ensure continuity of diagnostic workflows. However, many transport media are limited by chemical incompatibility or lack of pathogen inactivation. Copan Universal Biomolecular Medium (UBMTM), a novel inactivating liquid medium, has been developed to support interoperability across diagnostic systems. This study aimed to evaluate the analytical accuracy and stability of UBMTM for HPV detection across multiple molecular assays.

Methods: Common molecular assay/platform for HPV detection from Abbott, Hologic, Roche (cobas 5800/6800) and Seegene were included in this study.

Contrived clinical specimens were created using vaginal matrix and HeLa cells. Copan FLOQSwabs® were loaded with the contrived clinical material. The devices were then resuspended in either Copan UBMTM or in manufacturer-validated assay buffer. Five replicates were tested immediately, while five were stored at room temperature for 28 days before testing. Comparative analysis was performed between UBMTM and the validated standard media on each platform.

Results: UBMTM demonstrated comparable clinical accuracy to manufacturer-validated collection systems at both baseline and after 28 days of storage. Performance was consistent, with stable results across workflows. A single discrepancy was observed for one HPV-positive sample in the Seegene assay.

Conclusions: Overall, UBMTM maintained stability and compatibility across diverse molecular platforms for HPV detection. These findings support its potential use as a versatile, inactivating transport medium for HPV testing, enabling greater flexibility in molecular diagnostic workflows. Further studies including additional analytes, specimen types, and molecular platforms will help to confirm whether UBMTM can function as a truly universal molecular medium beyond HPV detection in vaginal samples.



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