Validation of a High-Throughput Laboratory Developed Test for the Detection and Quantitation of HHV-6A and HHV-6B Nucleic Acid in Plasma

Presenting Author:
Sean Thomas, PhD, University of Rochester Medical Center, Rochester, NY

Objective: Human Herpesvirus 6 (HHV-6) is a double-stranded DNA virus that can cause primary infection, become latent, and reactivate upon immunosuppression, causing potentially severe disease. HHV-6 refers to two closely related viruses, HHV-6A and HHV-6B. There is no FDA-approved test for HHV-6 in plasma; quantitative testing is available at select reference laboratories, but may result in increased test cost and turn-around-time. Such tests may also not distinguish between HHV-6A and HHV-6B. Here, a quantitative HHV-6 test for plasma using the high-throughput DSQ Alert HT HHV-6 A&B Research Use Only Detection Reagent (ELITechGroup MDx) and the cobas omni Utility Channel reagent kit was evaluated for use on the cobas 8800 System (Roche Molecular Systems).

Methods: The primers/probes used in DSQ Alert HT HHV-6 A&B RUO Detection Reagent target the HHV-6A envelope glycoprotein H and HHV-6B hypothetical protein genes. Authentic clinical and HHV-6-spiked plasma specimens were tested using a specimen volume of 200 ul to determine the limit of detection, the limit of quantitation, specificity, reproducibility, and for accuracy verification. Results from this test were compared to those from the quantitative HHV-6 assay performed at a reference laboratory.

Results: The limit of detection for both HHV-6A and HHV-6B was established as 100 copies/ml with 95% confidence (detected in 19/20 specimens for HHV-6A and 20/20 for HHV-6B). Quantitation was linear from 100 copies/ml to the highest concentration of standard tested (5,000,000 copies/ml) for both HHV-6A (R²=0.99) and HHV-6B (R²=0.99). For all specimens tested for both HHV-6A and HHV-6B, inter- and intra-assay reproducibility was established with a SD <0.13 log copies/ml and <0.08 log copies/ml, respectively. HHV-6 was detected in 36/36 known positive and spiked specimens tested and not detected in 10/10 HHV-6 negative specimens using this assay compared to an approved test performed at a reference laboratory. The mean difference in log copies/ml for result produced by this test versus the reference assay was 0.17 log copies/ml. Due to a lower limit of detection/quantitation, this test was capable of quantifying 8/36 positive specimens that were detectable but not quantified by the reference assay.

Conclusions: This test was reproducible, sensitive, and specific for the quantitation of HHV-6A and HHV-6B in plasma. Test results were concordant with those from a reference laboratory assay and demonstrate acceptable performance of a high-throughput LDT to aid in the diagnosis of HHV-6 infection.
**Poster Board # 002**

Adaptation of an Adenovirus Real-Time PCR Assay for the Detection and Quantitation of the Virus in the Plasma from Immunocompromised Individuals

**Presenting Author:**
Christine Stellrecht, PhD, Albany Medical Center Hospital, Albany, NY

**Objective:** To develop a real-time PCR assay for the detection and quantitation of adenovirus (ADV) in plasma samples, primarily for transplant recipients

**Methods:** A real time PCR Assay for the detection of ADV was developed using primers and probe targeting a 131 bp region of the Adenovirus hex gene1, described by Heim et al. The assay was optimized for amplification on the Q-cyclers, using PerfeCTa® SuperMix and an internal control targeting Φ X174 DNA. AdV-1 infected A549 cells, quantified with genomic ADV DNA, both from ATCC, were used for limit of detection (LOD), linearity and precision analyses, spiking clinical specimens and assay controls. ADV strains 2, 3, 5, 7, 8, 11 and 21 were obtained from the NYS DOH Proficiency Testing Program. Analytical specificity was assessed by testing 28 types of viruses, bacteria, and parasites. Clinical plasma specimens were obtained from specimens sent to our laboratory for other testing.

**Results:** Plasma spiked with serial 10-fold dilutions of quantified ADV infected cells, extracted on the eMAG, were used for LOD analysis. These studies demonstrated the assay had an LOD of 11.67 copies/reaction and was highly linear (R2=0.962). The precision ranged from 0.60 to 2.5% for the Ct values of these dilution. The assay demonstrated good linearity and amplification efficiency with all serotypes tested, except ADV-5. The ADV-5 dilutions were linear but lost efficiency. The assay did not cross react with the various other organisms tested. The ADV Real-Time PCR assay verification studies were also performed by analyzing 40 ADV negative plasmas that were either un-spiked or spiked at various concentrations of ADV-1 and tested in a randomized fashion. All samples tested as expected.

**Conclusions:** An optimal ADV detection and quantitative real-time PCR assay was developed which will greatly aid clinicians in the monitoring and treatment of immunocompromised patients.
Objective: Sexually-transmitted infection (STI) diagnostic testing remains a critical aspect of clinical microbiology laboratories, with a need to distinguish viral STI pathogens such as herpes simplex viruses 1 and 2 (HSV-1, HSV-2) and monkeypox virus (MPXV). While these infections can vary in terms of clinical presentation, both cause the formation of vesicles on the skin and mucous membranes, requiring laboratory testing to confirm a definitive diagnosis. Considering their overlapping presentations, a diagnostic test to discriminate HSV-1, HSV-2, and MPXV in a point-of-care setting offers a convenient and flexible option for laboratory STI diagnosis.

Methods: We developed a novel real-time PCR assay for the detection of HSV-1, HSV-2, and MPXV using the Coyote Biosciences Mini8, a portable small-footprint real-time PCR platform, using a lyophilized shelf-stable PCR mix (NEB). Viral stock of HSV-1, HSV-2, and MPXV were propagated and titered by TCID50 or plaque assay. Analytical sensitivity (limit-of-detection [LOD]) and precision studies were assessed; further assessment of assay specificity, clinical correlation, and other performance parameters are ongoing.

Results: MPXV LOD was determined at 6.7 copies/mL while LODs for HSV-1 and HSV-2 were below 10 TCID50/mL. MPXV inter- and intra-assay variation, assessed by coefficient of variation (%CV), ranged from 0.09-3.26% and 0.47-0.77%, respectively. Evaluation of specificity is ongoing and will include viral and bacterial pathogens and skin flora. Assay tolerance to potentially contaminating interfering substances are also being assessed. Finally, clinical correlation is being performed using patient specimens characterized by an alternative PCR-based gold-standard method. Full validation data will be fully available at the PASCV 2024 Annual Meeting.

Conclusions: We developed a novel assay for the detection and distinction of HSV-1, HSV-2, and MPXV on a portable real-time PCR platform fitted for rapid standup testing using a shelf-stable reagent mix. Our preliminary results demonstrate a robust sensitivity for all three viruses with a high rate of reproducibility.
Poster Board # 004
A High-Throughput Laboratory-Developed Test for Quantitative Detection of HSV-1/2 in Plasma on the Cobas 8800 System

Presenting Author:
Bailey Mosher, PhD, University of Rochester Medical Center, Rochester, NY

Objective: Herpes simplex virus 1 and herpes simplex virus 2 (HSV-1 and HSV-2) cause asymptomatic, mild, and severe disease in all age groups. Severe infection can lead to multi-organ viral dissemination and central nervous system (CNS) involvement. Here, we evaluated a new high-throughput laboratory-developed test (LDT) for PCR-based quantitative detection of HSV-1 and HSV-2 nucleic acid in plasma. This test is intended for our patient population, which includes both immunocompromised and immunocompetent adults and children.

Methods: This test uses the DSQ Alert HT HSV-1&2 Research Use Only Detection (RUO) Reagent (ELITEchGroup MDx) and the cobas omni Utility Channel reagent kit with the cobas 8800 System (Roche Molecular Systems). The assay targets the glycoprotein D and glycoprotein G genes in HSV-1 and HSV-2, respectively. The limit of detection (LOD) was determined at 95% confidence using HSV-1/2-spiked normal human plasma (NHP). Linearity of the analytical sensitivity data was also assessed. Authentic clinical and spiked specimens were used to assess specificity, inter-assay reproducibility, intra-assay reproducibility, and for accuracy verification. For accuracy, specimens were tested in parallel using an approved assay available at a commercial reference laboratory.

Results: Using a minimal specimen volume of 200 μl, the assay was capable of detecting HSV-1/2 nucleic acid as low as 250 copies/ml with 95% confidence. The assay exhibited linearity between 250 - 2,000,000 copies/ml for both HSV-1 and HSV-2 (R2 = >0.99). No PCR inhibition was detected in 264 plasma specimens tested. The assay detected 18/18 known positive HSV-1 specimens, 16/16 known positive HSV-2 specimens, and 0/20 known negative specimens. The mean difference in log copies/ml between quantitative results produced using the assay and compared to an approved reference laboratory test method was 0.08 log copies/ml and -0.13 log copies/ml for HSV-1 and HSV-2, respectively.

Conclusions: With the DSQ Alert HT HSV-1&2 RUO Detection Reagent on the cobas 8800 system, a sensitive, specific, and reproducible quantitative nucleic acid amplification test was developed for the detection of HSV-1/2 in plasma. This assay uses a relatively small specimen volume which better serves our pediatric population. This assay also allows HSV-1/2 quantitative testing to be moved to an automated high-throughput system that expands our test capacity with little additional labor costs and improves turnaround time.
Poster Board # 005
Molecular Characterization of a Reptarenavirus Detected in a Colombian Red-Tailed Boa (Boa Constrictor Imperator)

Presenting Author:
Mohamed Abouelkhair, DVM, MS, PhD, The University of Tennessee Comparative and Experimental Medicine, Knoxville, TN

Objective: The objective of this study was to contribute to the understanding of reptarenavirus diversity by molecularly characterizing a reptarenavirus detected in a Colombian Red-Tailed Boa (Boa constrictor imperator), with a specific focus on its potential zoontic implications.

Methods: A metagenomics approach was utilized to identify and de novo assemble the complete genomic sequences of the reptarenavirus found in the symptomatic Colombian Red-Tailed Boa. The sample was collected from a boa displaying clinical symptoms consistent with Boid Inclusion Body Disease (BIBD). Molecular analyses were conducted to determine the genotype and characteristics of the identified reptarenavirus.

Results: The analysis revealed the presence of University of Giessen virus (UGV-1) S or S6 (UGV/S6) segment and L genotype 7 in the Colombian Red-Tailed Boa under scrutiny. The prevalence of the UGV/S6 genotype suggests potential advantageous traits or adaptations, possibly conferring competitive advantages within the host population.

Conclusions: This research underscores the importance of monitoring and characterizing viral pathogens within captive and wild snake populations, especially considering potential zoontic implications. Such knowledge is essential for the development of effective diagnostic methods, potential intervention strategies, and the conservation of vulnerable reptilian species. Furthermore, our study provides valuable insights for future research focusing on the evolutionary history, molecular epidemiology, and biological properties of reptarenaviruses in boas and other snake species.
Analytical Performance of the Co-Dx™ PCR COVID-19 Test

Presenting Author: Daniel Garey, Co-Diagnostics, Salt Lake City, UT

Objective: The Co-Dx PCR Pro™ is an easy-to-use instrument designed to provide fast and affordable at-home and Point-of-Care testing using gold standard RT-PCR technology. The instrument and the corresponding COVID-19 test are designed to perform comparably to lab grade testing. Here we evaluate the analytical performance of the Co-Dx PCR COVID-19 Test on the Co-Dx PCR Pro.

Methods: Testing was conducted using contrived anterior nasal swabs (ANS). ANS from donors were confirmed negative then spiked with intact chemically inactivated SARS-CoV-2 virus. The limit of detection was initially established in copies per swab. Inclusivity testing used seven prominent SARS-CoV-2 variants. 46 organisms were tested for cross-reactivity and microbial interference. 19 substances, potentially present in ANS samples, were tested for interference. Cross-contamination risk was assessed by alternating 20x the limit of detection positive samples with negative samples eight times each on five instruments. Nine additional studies were conducted to evaluate potential operator errors and the effects of environmental factors.

Results: The limit of detection was determined to be 2100 copies/swab. All seven SARS-CoV-2 variants were identified at 3x the limit of detection. No cross-reactivity was identified in negative samples. No microbial interference with the SARS-CoV-2 analyte was identified from the 46 organisms tested. In silico analysis corroborated these findings. The system showed no susceptibility to carry-over contamination. In several instances the system was forced to failure by exaggerated user error, but these errors were mitigatable through proper use of the system. All tests had a near 30-minute run time.

Conclusions: The Co-Dx PCR COVID-19 Test on the Co-Dx PCR Pro system demonstrated analytical performance comparable to gold-standard central laboratory RT-PCR testing. The system was demonstrated to be robust, fast, and easy to use while maintaining high analytical sensitivity and specificity.
Enhanced Enterovirus Surveillance in New York State Following a Confirmed Poliomyelitis Case in July 2022

Presenting Author:
Simon Ogbamikael, MS, Wadsworth Center, New York State Department of Health, Albany, NY

Objective: In July 2022, the Wadsworth Center detected Sabin type 2 poliovirus, with reversion to virulence, in a hospitalized patient with paralysis and no relevant travel history. Subsequent wastewater surveillance demonstrated community poliovirus transmission, with positive samples from treatment plants in neighboring counties and the county of residence of the index case. To help investigate the extent of virus circulation, the New York State Department of Health initiated enhanced enterovirus surveillance.

Methods: Patients under investigation (PUI) for poliovirus infection included those with 1) mild prodromal poliovirus symptoms, unimmunized, incompletely immunized or unknown polio immunization status, who lived or worked in areas of wastewater positivity, or low vaccination rates and tested positive for enterovirus; 2) enterovirus positive meningitis and lived or worked in wastewater positive areas; and 3) suspected acute flaccid myelitis (AFM). Preferred sample types included stool, CSF, and nasopharyngeal swabs. Asymptomatic surveillance was accomplished by collecting stool samples from healthy baby diapers during pediatrician well visits. Samples were tested by enterovirus-specific two-step real-time RT-PCR. Positive samples were genotyped by PCR amplification of the VP1 region, followed by Sanger sequencing, and NCBI BLAST analysis.

Results: From July 2022 to December 2023, 445 samples from 368 patients were tested. A total of 91 PUIs were tested, of which 60 were enterovirus positive. Additionally, 36 stool samples were collected from asymptomatic children, of which 8 were enterovirus positive. Among the enterovirus-positive samples, subtyping identified 76 Coxsackieviruses, 59 echoviruses, and 37 cases of enterovirus D68, but 0 polioviruses.

Conclusions: Despite evidence from wastewater testing of ongoing community poliovirus transmission, no additional cases of polio were identified, although surveillance continues. However, testing did result in the identification of diverse enteroviruses in both symptomatic and asymptomatic patients, including a statewide enterovirus D68 outbreak in 2022 and a Coxsackievirus B4 outbreak in 2023.
Analytical and Clinical Evaluation of the Cobas Epstein-Barr Virus Test at a Tertiary Care Cancer Hospital

Presenting Author: Cindy Lee, Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Epstein-Barr Virus (EBV) viral load monitoring in hematopoietic stem cell transplant (HSCT) recipients are critical within the first-year post-transplant as the risk of developing post-transplant lymphoproliferative disorder (PTLDs) is highest. The Cobas EBV test (Roche Molecular, Pleasanton, CA) is FDA-cleared for the monitoring of EBV viral loads in plasma of transplant patients. This study compares viral loads obtained by a laboratory-developed test (EBV LDT) using Altona analytic specific reagents (ASR) to those obtained on the Cobas EBV test.

Methods: Verification experiments including lower limit of detection (LLOD), reproducibility, accuracy, and clinical sensitivity were performed on previously tested EBV patient plasma from allogeneic SCT recipients. Samples were stored at -70°C until testing. Cobas 6800 testing was conducted per manufacturer’s instructions with 350 µL of plasma.

Results: LLOD with 95% confidence was calculated to be 5 IU/mL. Reproducibility was 100% with inter and intra-assay coefficient of variation of 1.40-3.25% and 0.84-4.64% respectively. The assay accurately measured concentrations of each member of the EDX EBV LOD Verification Panel with an r² value of 0.99. Accuracy testing was performed on 344 samples (235 positive, 109 negative). 187 positives and 108 negatives resulted in 85.6% qualitative agreement (Kappa score=0.71; Substantial agreement). 49 samples were discordant; repeat testing resolved discrepancies for 33 samples, mostly due to previous viral loads at or below the LLOQ for the 6800 and LDT. Viral loads from the Cobas were generally lower than with the Altona EBV PCR (mean bias of -1.39). Viral load trending on 6 patients, confirmed that while the 6800 EBV viral loads were lower, trends were similar.

Conclusions: The Cobas EBV test provides an accurate and valid, IVD option for monitoring EBV viral loads in transplant patients. As an IVD option, the EBV Cobas test should provide an opportunity for increased standardization and commutability of tests results across laboratories.
**Poster Board # 009**  
**PlexPlus® Mass Multiplex Assay Detects 14 Viral Respiratory Targets in a Single Well**

**Presenting Author:**  
Alison Todd, PhD, SpeeDx Pty Ltd, Eveleigh, New South Wales, Australia

**Objective:** PlexPlus® technology allows detection of multiple targets within each channel during qPCR. The method uses specialised probes which are both temperature and target dependant with fluorescence acquired at two temperatures across multiple wavelengths in real time. Data obtained within each channel at each temperature is discrete, with no cross talk between temperatures, meaning analysis does not require complex algorithms to ascertain contributions from different targets measured at the same wavelength.

**Methods:** A RespiV PlexPlus® prototype assay was assessed for its capacity to simultaneously detect 14 respiratory viral targets, namely FluA, FluB, SARS-CoV-2, RSV A and B, AdV B and C, HPIV 1-4, hMPV, Rhinoviruses (RhV) and Human enterovirus (hEV). Fluorescence was acquired at 52°C and 76°C in six channels during PCR cycling on the QuantStudio™ Real-Time PCR system. The performance of the assay was characterised by retrospective analysis of 679 nasopharyngeal swab samples using PlexPCR® RespiVirus and PlexPCR® Flu/RSV/SARS-CoV-2 tests (SpeeDx) as comparators. A subset of non-concordant results were resolved by sequencing.

**Results:** Preliminary analytical studies indicate the assay has the capacity to detect as little as 5-20 copies of target across the entire panel. Analysis of the nasopharyngeal swabs showed overall concordance of 96% or greater for all 14 targets. Further, the assay successfully detected and discriminated RhV and hEV samples in the clinical specimens and in a QCMD 2023 Rhinovirus RNA EQA Programme (RVRNA23S) panel.

**Conclusions:** The single-well, highly multiplexed assay delivered comparable results on 679 clinical samples compared to two current IVD cleared assays. The assay expedites high throughput analysis, allowing determination of up to 1,302 individual results relating to the presence or absence of 14 respiratory viral targets in 93 patient samples, analysed simultaneously in one 96 well plate on a standard qPCR instrument.
The Stability of Virus Detection in Raw Saliva Supports the Sustainable Clinical Diagnostic and Surveillance of Respiratory Infection

Presenting Author:
Yasmine Ali, MPA, MBA, SalivaDirect Inc., New Haven, CT

Objective: Respiratory viruses pose a significant threat to global public health. Traditional diagnostic approaches often rely on complex sample collection and transportation requirements. We previously demonstrated with SARS-CoV-2, that sampling saliva can eliminate the dependence on specialized collection devices and cold-chain transportation, simplifying logistics and significantly reducing costs. Looking to further enhance clinical diagnostics and surveillance, we evaluated the stability of detection of other respiratory viruses in raw, unsupplemented saliva.

Methods: Saliva collected from individuals positive for SARS-CoV-2, influenza A (IAV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and mpox virus was incubated at room temperature (RT, -19°C), 30°C, and 40°C for 72H (hMPV, mpox) or 168H (7 days, SARS-CoV-2, IAV, RSV), at 4°C and -20°C for ≤6 weeks, and cycled through temperatures representing seasonal shipping conditions. Virus detection was tested using qPCR prior to and after incubation.

Results: The detection of SARS-CoV-2, IAV, RSV, and hMPV remained stable throughout their incubation at -19°C and 30°C. Detection following storage at -20°C and 4°C remained stable for >4 weeks. For mpox, detection remained relatively stable for 24-48H at 4°C, RT, and 30°C, and was stable for 24H at 40°C. Detection of all viruses remained stable following simulated shipping conditions.

Conclusions: Validating the use of saliva for respiratory virus detection holds immense promise for clinical diagnostics, surveillance and outbreak response efforts. Building upon the practical advantages related to its ease of collection, our findings provide supporting evidence for discrete, at-home, and/or remote sampling options; enabling individuals to self-collect in the comfort of their homes, can facilitate early detection, minimizing transmission risks, while opening avenues for longitudinal surveillance. As such, saliva offers an ideal suitable sample type for widespread adoption in decentralized testing scenarios, reducing logistical challenges, and enhancing the accessibility and scalability of diagnostic and surveillance programs, particularly in remote and resource-limited settings.
Objective: Norovirus is a highly contagious virus belonging to the Caliciviridae family, and the number one viral cause of food related illnesses in the United States. In this retrospective review, we examine the frequency of Norovirus at an oncology center from January 2018 to December 2023.

Methods: This is a retrospective review of all BioFire® FilmArray GI Panel testing performed from January 2018 to December 2023 at Memorial Sloan Kettering Cancer Center in New York City. The GI Panel detects Campylobacter, Salmonella, Y. enterocolitica, Vibrio, P. shigelloides, C. difficile, Cryptosporidium, C. cayetanensis, E. histolytica, G. lamblia, Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus, Enteroaggregative E. coli, Enteropathogenic E. coli, Enterotoxigenic E. coli, Shiga-like-toxin-producing E.coli, and Shigella/Enteroinvasive E. coli.

Results: 33,203 tests were performed during the study period. 4,541/33,203 (13.7%) positive for at least one target on 3,424 unique patients. Of the 4,541 positive results 1,483 (32.7%) were positive for Norovirus GI/GII on 1,132 unique patients. The number of GI PCR tests performed yearly was 4,544 in 2018, 5,103 in 2019, 5,084 in 2020, 5,501 in 2021, 6,101 in 2022 and 6,870 in 2023. During these years, 88/226 (38.9%), 161/357 (45.1%), 75/424 (17.7%), 92/593 (15.5%), 295/903 (32.7%), and lastly, 491/1,218 (40.3%) unique patient samples were positive for Norovirus. The average distribution of samples from outpatients accounted for more than 70% of the total. The distribution of patient service for inpatients tested was very similar between both solid and liquid services.

Conclusions: A significant reduction in Norovirus cases was observed in 2020 and 2021 while testing remained stable. This reduction is likely linked to public health measures implemented during the SARS-CoV-2 pandemic. A rebound in the number of cases in 2022 and 2023 following the end of the pandemic highlight the importance of public health measures to prevent the spread of Noroviruses.
**Poster Board # 012**

Analytical Performance of the Alinity m HR HPV Assay in SurePath Preservative Fluid

**Presenting Author:**
Richard Cullum, PhD, Abbott, Des Plaines, IL

**Objective:** Fourteen high-risk (HR) HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) account for almost all cervical cancers. Molecular HPV tests capable of detecting HR HPV genotypes are utilized in routine cervical cancer screening. Here, we evaluated the analytical performance of the Alinity m HR HPV assay, which reports HPV 16, 18, and 45 individually and aggregates other genotypes as Other A (HPV 31/33/52/58) and Other B (HPV 35/39/51/56/59/66).

**Methods:** All analytical studies were performed using the SurePath Preservative Fluid. Precision and reproducibility were evaluated using HPV panels VP-62-M1 (16/18/45), VP-62-M2 (31/33/66), and VP-62-M3 (39/51/52) that were obtained from Microbix (Mississauga, Ontario, Canada). These panels were tested in triplicate over three consecutive days. Limit of detection was evaluated for HPV genotypes 16 and 18 using AcroMetrix HPV Genotype Controls obtained from Thermo Fisher Scientific (Waltham, MA, USA). Potential interfering substances that may be present in cervical specimens were assessed in HR HPV negative samples and HR HPV positive samples.

**Results:** The Alinity m HR HPV assay demonstrated 100% reproducibility across the tested panels. The assay exhibited intra-run precision of <4.0% CV and inter-run precision of <3.0% CV across all HPV genotypes tested. The limit of detection was 150 cells/mL and 500 cells/mL for HPV genotypes 16 and 18, respectively. No interference was observed for any of the substances tested.

**Conclusions:** The Alinity m HR HPV assay demonstrated acceptable analytical performance for reproducibility, precision, limit of detection, and interference for each HPV genotype tested. These data support the utility of the Alinity m HR HPV assay for the detection of HR HPV genotypes in SurePath Preservative Fluid.
Poster Board # 013
Respiratory Viral Trends Over Three Seasons at a Pediatric Healthcare System in Colorado, USA

Presenting Author:
Sarah Jung, MS, PhD, D(ABMM), Children’s Hospital Colorado, Aurora, CO

Objective: The COVID-19 pandemic (2020-2023) and related non-pharmaceutical interventions (e.g., mask use and increased hand hygiene) altered respiratory virus circulation. The goal of this study was to examine positivity rate patterns of four respiratory viruses-influenza A (flu A), influenza B (flu B), respiratory syncytial virus (RSV), and SARS-CoV-2 (CoV-2) – over three 7-month seasons (October through April), two of which were during the pandemic: 2021-2022, 2022-2023, and 2023-2024. These trends were investigated at Children’s Hospital Colorado (CHCO, Aurora, CO).

Methods: Molecular detection of flu A, flu B, RSV, and CoV-2 was performed using the following platforms across three CHCO sites: Abbott m2000 RealTime, Applied Biosystems 7500 (CDC 2019-nCoV RT-PCR assay), BIOFIRE® FILMARRAY® Torch System, Cepheid GeneXpert® IV, Cepheid GeneXpert® Infinity-48s, and Diasorin LIAISON® MDX. Sample types included nasopharyngeal swab, mid-turbinate swab, nasal wash, tracheal aspirate, and bronchoalveolar lavage. Test volumes and positivity rates were collected over the 7-month respiratory season (October 1 through April 30) for three seasons.

Results: Respiratory test volume for each season was the following: 76,232 tests in the ‘21-’22 season, 38,984 tests in the ‘22-’23 season, and 26,000 tests [predicted at time of abstract submission] in the ‘23-’24 season. Each virus displayed different seasonal dynamics. Flu A peaked at the end of April during the ‘21-’22 season, shifting to mid-December during the ‘22-’23 season, and then maintaining the December peak in the ‘23-’24 season. Flu B displayed the most dramatic shift. The positivity rate was <1% across the entire ‘21-’22 season, followed by a resurgence in the ‘22-’23 season with a peak in early April, and then another peak at the end of December in the ‘23-’24 season. RSV peaked in November, a typical emergence, during pandemic respiratory seasons but peaked later during the ‘23-’24 season (early January), which aligns with other RSV resurgence studies after the mask regulation lifting in CO Summer 2021. CoV-2 surged in January of the ‘21-’22 season, peaked in February during the ‘22-’23 season, and then steadily declined over the ‘23-’24 season [at time of abstract submission]. Positivity rates did not vary by test platform or sample type.

Conclusions: The COVID-19 pandemic led to societal and human behavior shifts that altered respiratory viral circulation patterns. In fact, the ‘21-’22 respiratory season observed a global decline in respiratory pathogens other than CoV-2, displaying the effectiveness of non-pharmaceutical interventions. Our and others’ data show that respiratory virus circulation could be resetting to pre-pandemic seasonal peaks. Continued shared and collaborative surveillance will shed further insight on whether these predictions hold true in future respiratory seasons.
Poster Board # 014
The Significance of Third-Party External Quality Controls in In Vitro Diagnostic (IVD) Assays Utilizing Assay Manufacturer Quality Controls

Presenting Author:
Mikayla Quinton, BS, MLS, MSDS, Johns Hopkins Hospital, Baltimore, MD

Objective: Utilizing In Vitro Diagnostic (IVD) assay manufacturer quality control (QC) materials is a common practice in molecular virology. However, there is a concern that assay manufacturer QC might be suboptimal for adequately detecting the assay’s analytical performance. In this study, we assessed the potential significance of incorporating a third-party QC and compared its performance to that of an IVD assay manufacturer QC.

Methods: The performance of the Bio-Rad ExactDx HCV Low Run Control (HCV LRC) was compared to the Roche cobas 6800-manufactured HCV/HIV/HBV Positive Control kit (HCV PCK). Over a 6-month period, 90 samples of HCV LRC were concurrently tested with HCV PCK. Three different lots of HCV PCK (lot A, lot B, lot C) and HCV Quantitative reagent kits (RA, RB, RC) were used in this analysis, while only one lot of HCV LRC was utilized. The average control value, standard deviation (SD), and the number of points (N) were compared between the two instruments (I1, I2). Additionally, the performance of the control material during changes in reagent lots was assessed in comparison to the fixed mean set at the beginning of the control lot.

Results: The average control value for the HCV LRC was 2.93 (I1) and 2.86 (I2), representing an average 0.80 higher than the HCV PCK. A noticeable shift in QC results occurred in the HCV LRC during the change of reagent lots: 0.10 from reagent lot A to B and 0.05 from lot B to C (I1), 0.02 from lot A to B and 0.18 from lot B to C (I2).

Conclusions: Both the HCV LRC and HCV PCK demonstrated consistent performance, with no major differences between the control materials. However, a minor shift in QC during the switch in reagent lots was observed only with the HCV LRC, attributed to the consistent use of a single control lot. In the HCV PCK data, frequent changes in control lots around the same time as reagent lot switches led to mean adjustments for the new control, potentially contributing to missing the slight change in the assay’s performance. The consistent use of the control lot of a third-party QC could allow for the identification of changes that could be missed when multiple lots of assay manufactured QC are used.
Epidemiology of High-Risk HPV Genotypes in the Post-Vaccine Era at a Tertiary Cancer Center

Presenting Author:
Alexis Jaramillo Cartagena, PhD, Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Human Papillomavirus (HPV) is a widespread sexually transmitted infection with oncogenic potential, comprising over 200 genotypes, 40 targeting mucosal epithelium. These genotypes are high-risk or low-risk, based on their association with cervical, vaginal, anal, and vulvar cancers. The CDC reports significant reductions in HPV infection rates and related outcomes since HPV vaccination introduction in 2006, primarily targeting HPV 16 and 18 initially, followed by subsequent vaccines targeting other high-risk genotypes. This study aims to investigate year-over-year changes in high-risk HPV genotypes in a post-vaccine era at a tertiary care cancer center and trends in HPV genotype detection by age group.

Methods: Nucleic acid-based molecular testing used the FDA-approved cobas® HPV tests on cobas® 4800 and 6800 platforms. This HPV test detects 14 high-risk genotypes, with results: not detected, HPV 16, HPV 18, or other high-risk (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). Testing targeted patients suspected of HPV-related cancers and patients with other malignancies or immunodeficiencies receiving gynecological care at our cancer center. Results from January 2018 to October 2023 were retrieved from our laboratory and clinical information systems. Linear regressions of year-over-year trends and statistical comparisons among age groups used GraphPad Prism version 10.0.3.

Results: 8410 tests during this period revealed high-risk HPV in 17.86% of samples. No significant annual changes in genotype distribution were observed. HPV 16 detection remained consistently high at 19.8%, HPV 18 at 4.6%, and other high-risk genotypes at 67.7%. Co-infections, notably HPV 16 with HR-other, occurred in 6.1%, with 0.2% detecting both HPV 16 and 18, and 1.4% showing HPV 16 with HR-other. Stratifying results by age groups revealed varying high-risk HPV detection percentages: 20-29 years (27.7%), 30-39 (20.7%), 40-49 (14%), 50-59 (15.3%), 60-69 (18.3%), 70-79 (22%), 80-89 (21.4%). Significant statistical differences were observed comparing genotypes in women aged 20-29 to the aggregate 30-89 group: HPV 16 (4.3% vs. 19.78%), HPV 18 (0% vs. 4.5%), HR-other (91.4% vs. 67.8%).

Conclusions: Despite two decades of HPV vaccination, HPV 16 remains highly prevalent, exceeding HPV 18. The majority of high-risk HPV in our cohort belonged to the HR-other group, potentially emerging as more common. Lower prevalence of HPV 16 and 18 is observed in younger patients. Further genotyping could provide insight into specific genotypic trends in our patient population, laying the groundwork for future research.
Poster Board # 016
Prevalence of High-Risk HPV Genotypes in Anal Pap Specimens Collected in a High-Risk Population

Presenting Author:
Richard Cullum, PhD, Abbott, Des Plaines, IL

Objective: Like cervical cancer, most anal cancers are associated with human papillomavirus (HPV) infection. Also like in cervical cancer, HPV genotype 16 is the most carcinogenic HPV type in anal cancer. However, due to low anal cancer screening rates, less is known about the prevalence of the 14 high-risk (HR) HPV genotypes in anal pap samples. Here, we observed the prevalence of HR HPV genotypes in anal pap samples at Montefiore Medical Center, a large academic medical center in the United States.

Methods: The study included 58 HR HPV positive, de-identified residual anal pap specimens collected by clinicians as part of anal cancer screening. All specimens were collected in SurePath and processed using the Alinity m HR HPV assay. The Alinity m HR HPV assay was designed to stratify HPV 16, 18, and 45 individually, and aggregate HPV 31, 33, 52, and 58 into Other HR HPV A and HPV 35, 39, 51, 56, 59, 66, and 68 into Other HR HPV B groups. All specimens were processed for cytology using the BD Totalys SlidePrep system.

Results: Of the 58 HR HPV positive specimens, 43.1% (25/58) tested positive for HPV 16, 13.8% (8/58) tested positive for HPV 18, 20.7% (12/58) tested positive for HPV 45, 37.9% (22/58) tested positive for Other HR HPV A, and 63.8% (37/58) tested positive for Other HR HPV B. 41.4% (24/58) of the specimens tested positive for a single HR HPV genotype. 58.6% (34/58) of the specimens tested positive for multiple HR HPV genotypes. 79.3% (46/58) of the specimens were graded with atypical cytology.

Conclusions: In this study, Other HR HPV B had the highest prevalence in anal pap specimens followed by HPV 16, HR HPV Other A, HPV 45, and HPV 18. Over 50% of the specimens tested positive for multiple HR HPV genotypes.
Poster Board # 017  
Genomic Analysis and Outcomes of the 2023-2024 Influenza Season in the Johns Hopkins Health System

Presenting Author:  
David Villafuerte, BS, Johns Hopkins, Baltimore, MD

Objective: The influenza season in the northern hemisphere is variable, but usually spans from October through May with peak activity between December and January. The genomes of circulating influenza A and B viruses were analyzed and associated with clinical outcomes.

Methods: Left-over influenza positive samples after the standard of care testing at the Johns Hopkins Health system (JHHS) from October of 2023 to January of 2024 were used for the study. Samples were randomly selected for whole genome sequencing using the Oxford-Nanopore GridION. Phylogenetic analysis was performed for all genome segments. Clinical data was collected from the JHHS electronic medical database and associated with genomic data.

Results: A total of 2507 influenza-positive samples were diagnosed at JHHS in the time frame October 2023 to January 2024. Of the total, 84.2% (n=2111) were influenza A, and 15.8% (n=396) were influenza B. Of the total influenza positive samples, 526 were randomly selected for whole genome sequencing, and 335 were deemed high-quality. The dominant influenza A subtype of the 2023-2024 season was H1N1 followed by H3N2, making up 86.1% and 13.9% of the total influenza A cases, respectively. The most encountered H1N1 clade was 6B.1A.5a.2a.1, and the most frequent clade for H3N2 was 3C.2a1b.2a.2a.3a.1. This contrasts with the homogeneity of influenza B, with V1A.3a.2 existing as the only sequenced clade. Influenza infected patients were primarily between the ages of 5-17 (n=93), and most intensive care admissions were attributed to influenza A.

Conclusions: H1N1 was the most frequently detected subtype influenza A virus in samples collected from JHHS during the 2023-2024 season. Sequenced influenza B was composed completely of the V1A.3a.2 clade.
Limitation of A Commercial Analyte Specific Reagents for Detection of Adenovirus Subtype

Presenting Author:
Tracy McMillen, MS, Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Human adenoviruses (HAdV) are double-stranded DNA viruses with more than 70 different subtypes. Common conditions caused by adenoviruses can include pneumonia, colitis, nephritis, and hepatitis. These infections in immunocompromised patients can become acute resulting in a significantly higher morbidity and mortality. Several analyte specific reagents (ASR) are available for use in laboratory-developed tests (LDT). In this study, we report an investigation of a CAP proficiency failure associated with the limitation of ASR primers used in an Adenovirus LDT PCR.

Methods: The Adenovirus LDT PCR was developed using ASR primers based on MultiCode technology (Luminex Corporation, Austin, TX). After a failed CAP proficiency sample a root cause investigation was performed. Repeat testing of remnant CAP samples was performed using both Adenovirus LDT PCR and a secondary PCR (BioFire RVP). Genotyping was performed using dual primers, 10µM each, 3µL of PerfCTa 5X SuperMix, PCR grade water and 5µL of DNA. Post-PCR products were purified with ExoSAP-IT. Sanger sequencing was performed on an ABI 3500 with BigDye reagents according to manufacturer’s directions. Sequencing results were analyzed with NCBI Basic Local Alignment Search Tool (BLAST).

Results: 3 CAP survey samples had adequate remnant sample for further testing. Two samples were concordant with reported CAP results, negative and positive respectively. One sample had a discordant result with expected survey results, this sample was reported as Not Detected by routine Adenovirus LDT PCR testing. Repeat LDT PCR was negative but testing by alternative method and genotyping resulted in the identification of subtype HAdV Type 1. The concordant positive sample also underwent genotyping and was identified as HAdV subtype 66.

Conclusions: Analyte specific reagents are a valuable resource when developing laboratory-developed tests. Caution should be exercised especially when used for targets that have multiple genotypes, as is the case for Adenovirus, and a disclaimer added to results indicating possible limitations.
**Poster Board # 019**  
**Evaluation of QIAGEN’s PyroMark Q48 Pyrosequencer**

**Presenting Author:**  
G. Stephanie Feumba, Wadsworth Center, New York State Department of Health, Albany, NY

**Objective:** The Laboratory of Viral Diseases, Wadsworth Center, New York State Department of Health, conducts influenza antiviral susceptibility surveillance with several methods including pyrosequencing. QIAGEN is the sole supplier of PyroMark instruments for pyrosequencing and has recently discontinued the PyroMark Q96ID system, used for this work since 2005. We conducted a validation on the only available alternative, the PyroMark Q48.

**Methods:** Nucleic acid was extracted from residual clinical specimens and cultured isolates, that had tested positive for A/H1pdm09 and A/H3N2 viruses by real-time RT-PCR assays. Virus RNA was amplified by conventional RT-PCR, amplicons confirmed by gel electrophoresis, and pyrosequencing performed on both the Q96ID and Q48 sequencers. Limits of detection (LOD) were determined on serial dilutions of cultured isolates, after determining relative viral load by Ct value from real-time RT-PCR. Additionally, assay and sequencing modifications, including the addition of Q solution, varying annealing temperatures, and concentrating amplicons prior to sequencing, were investigated to optimize the pyrosequencing assays.

**Results:** The LOD for A/H1pdm09 and A/H3N2 were tested across Ct ranges of 13-37 and 16-37 respectively. The Q96ID produced sequence on samples up to Ct 33 and 29 respectively, while the Q48 produced no sequence on any samples. None of the optimization experiments improved the performance of the Q48 and numerous performance issues were encountered with the instrument. For example, the injector repeatedly failed to deliver enzyme to the reaction well, and the reaction wells were too small for the reaction volume. Additionally, total assay time on the Q96ID was 70 minutes, compared to 116 minutes on the Q48.

**Conclusions:** The PyroMark Q48 did not perform as expected and is not an acceptable replacement to the PyroMark Q96ID. Qiagen is working with their engineering team and Field Application Specialist to improve the performance of the Q48 on these assays.
**Poster Board # 020**

**Genetic Diversity and Molecular Epidemiology of Human Rhinoviruses in Maryland from 2018 to 2023**

**Presenting Author:**
Amary Fall, John Hopkins School of Medicine, Baltimore, MD

**Objective:** Human Rhinoviruses (HRVs) are leading causes of upper respiratory tract infections. In this study, we describe the genetic diversity of HRV and explore the association between HRV types, illness severity, and clinical outcomes in a cohort diagnosed at the Johns Hopkins Hospital System (JHHS).

**Methods:** From May 2018 to February 2023, Rhinovirus/Enterovirus-positive samples were randomly selected and amplified using RT-PCR targeting the VP4-VP2 region of both viruses to determine their genotypes.

**Results:** Out of the 1207 sequencing attempts, 87.4% were successfully sequenced of those, 96.8% were rhinoviruses and 3.2% enteroviruses. HRV circulated throughout the year, with higher frequencies observed between August and October and specifically in September. HRV-A (50.9%) and HRV-C (38.2%) were detected in the majority of cases, while HRV-B accounted for less than 11% of cases. HRV-C was significantly prevalent among the under five years old (44.6%), while HRV-A exhibited a higher prevalence in patients aged 65 and above (18.7%). In terms of clinical data, HRV-B exhibited higher odds for symptoms such as chest pain (10.7%; OR = 3.18; 95%CI 1.53-6.21), while HRV-C showed elevated odds of bronchiolitis (5.4%; OR = 3.53; 95%CI 1.68-7.92), breathing problems (5.4%; OR = 2.05; 95%CI 1.07-4.00), and wheezing (6.9%; OR = 2.53; 95%CI 1.38-4.74). Additionally, HRV-C exhibited a higher prevalence in asthmatic patients (10.3%; OR = 2.01; 95%CI 1.25-3.24). HRV-A demonstrated a higher likelihood of hospitalization (63.7%; 1.16; 95% CI: 0.90-1.50), while HRV-C (25.3%; OR = 1.35, 95% CI: 1.01-1.80) appeared to have a higher likelihood for ICU care. A total of 139 HRV genotypes were identified, comprising 72 from HRV-A, 49 from HRV-C, and 18 from HRV-B. The most commonly detected genotypes were HRV-C11 (28), HRV-A21 (24) and HRV-C15 (24).

Interestingly, seven of our strains consisting of 4 HRV-As and 3 HRV-Cs could not be definitively categorized within known HRV genotypes.

**Conclusions:** The present study offers a recent description of the high genetic diversity of HRV detected in respiratory specimens from patients diagnosed at JHHS. Our findings further support the hypothesis that HRV-C is associated with more severe disease compared to other types.
Poster Board # 021

Optimizing Syndromic Panel Pathogen Detection Protocols: When to Repeat Testing?

Presenting Author:
Filipe Cerqueira, PhD, The University of Texas Medical Branch, Galveston, TX

Objective: The BioFire FilmArray® gastrointestinal (GI) and respiratory (R) panel assays play a crucial role in identifying infectious pathogens within clinical microbiology laboratories. While the manufacturer’s protocol recommends a retest of the patient specimen for which 4 or more pathogens are detected, our laboratory adopted a more stringent approach, retesting panels that detected 3 or more pathogens upon launching these syndromic panel assays. This study aimed to assess the benefits of utilizing our 3-pathogen protocol compared to the manufacturer’s 4-pathogen BioFire® protocol in terms of cost, clinical impact and decision-making at our institute.

Methods: A retrospective analysis of 12,027 GI and R panels conducted at our laboratory was performed to evaluate differences in the detection of the pathogens, the cost, and clinical decisions made based on results from each of these protocols.

Results: 39 out of 48 (81%) GI panels and 26 out of 34 (76%) R panels had identical results upon retesting in accordance with our laboratory policy. 17 (21%) panels (9 GI & 8 R) initially detected 3 organisms that yielded discrepant results upon retesting prompted further analysis. Accounting for the cost of materials and labor, the difference in total hospital expense between our protocol versus BioFire® protocol was $9,820.32. There was no evidence suggesting that repeating panels had any change in the decision for antibiotic prescription or clinical outcomes.

Conclusions: Implementing a more stringent threshold for repeating panels positive for 3 targets rather than 4 did not significantly impact clinical decision-making or outcome. However, it incurred unnecessary costs for the clinical laboratory, and during periods of supply shortages, it heightened the risk of depleting testing kits critical for severely ill patients.
Objective: The SARS-CoV-2 JN.1 lineage, descendant of the BA.2.86, emerged in late 2023 and displaced the prior Omicron lineage HV.1. The predominance of JN.1 was associated with an increased number of COVID-19 cases in the respiratory viral season of 2023/2024. We analyzed SARS-CoV-2 whole genome sequencing data collected between June 2023 and December 2023 to study the association between the evolution of JN.1, the changes in SARS-CoV-2 positivity rate, and the clinical outcomes. We also evaluated the recovery of JN.1 in cell culture when compared to the previously circulating lineages.

Methods: Samples positive for SARS-CoV-2 between June and December 2023 were randomly collected after testing at the Johns Hopkins Hospital System (JHHS) for whole genome sequencing. The clinical outcome of JN.1 infections was compared to infections with HV.1. A subset of clinical samples was collected for viral recovery in VeroE6-ACE2-TMPRSS2 after controlling for genome coverage and depth.

Results: SARS-CoV-2 positivity rate increased in August and September to 7% and 7.5% followed by a slight reduction to 5.7% in October, then an increase in November and December to 6.1% and 8.6%. The JN.1 variant was first detected in our system in October 2023, then increased quickly to become the most predominant variant in December 2023. A notable corresponding increase in COVID-19 influenza like illness and admissions for ages 18 years and older were associated with the increase in JN.1. Isolation of infectious virus in cell culture was comparable between JN.1 and HV.1 positive samples.

Conclusions: Our data suggest that the JN.1 has a fitness or transmissibility advantage compared to the prior Omicron variants, contributing to its quick predominance in December 2023. Our data shows that the increased COVID-19 cases and admissions in the respiratory season of 2023/2024 were correlated with the predominance of JN.1.
**Poster Board # 025**

**False Positive Influenza B Virus Detection with FDA-Cleared Multiplex Reverse Transcription Real-Time PCR Assay**

**Presenting Author:**
Saress Hale, BS, Cleveland Clinic Department of Laboratory Medicine, Cleveland, OH

**Objective:** Several rRT-PCR assays have been FDA cleared/authorized for simultaneous detection of SARS-CoV-2, influenza A/B, and respiratory syncytial virus (RSV). Clinical labs must verify assay performance characteristics before performing patient testing. The Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (Hologic) was approved by the FDA in May 2023, and underwent verification in our laboratory in October 2023. This abstract describes unexpected findings during verification and subsequent clinical testing, related to failure of thermocycler/detectors to account for spectral overlap.

**Methods:** Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay (Hologic) accuracy was assessed on 35-38 remnant clinical respiratory specimens tested using comparator assays previously-validated for clinical testing. Four Panther Fusion instruments (Instruments A-D) were assessed.

**Results:** Instruments A, B, and D each returned expected results. On Instrument C, 16/23 (70%) of specimens expected to be SARS-CoV-2 positive-only resulted erroneously positive for SARS-CoV-2 and influenza B (false-positive cycle threshold [Ct] range 14.2-27.8). The low peak fluorescence of influenza B (RED647 channel) suggested the signal was due to spectral overlap from SARS-CoV-2 (ROX channel). After additional verification studies, patient testing was started on instruments A, B, and D. On the first clinical run for Instrument D, 20/110 (18.2%) specimens initially resulted as dual positive for SARS-CoV-2 and influenza B. Of these, only 1 repeated as SARS-CoV-2 and influenza B dual positive on a different instrument. The other 19 results had similar low-fluorescence influenza B signal indicative of spectral overlap artifact. However, failure on Instrument D was sporadic, and not limited to strong positives (false-positive Ct range 17.2-37.1). Replacement of defective thermocycler/detector units in Instruments C/D resolved the issue.

**Conclusions:** Uncorrected spectral overlap can cause false positive results in multiplex rRT-PCR. A report was made to FDA MedWatch on 12/4/2023, and Hologic issued a Medical Device Recall on 12/14/2023 noting similar findings discovered at 7 other testing sites. These findings reinforce the value of lab-based verification of FDA-cleared tests. They also suggest the following best practices during multiplex PCR verification: 1) include strong-positives to assess for spectral overlap artifact, and 2) evaluate all positions of a thermocycler unit.
Detection of Enteroviruses in a Pediatric Oncology Patient Population

Objective: Enteroviruses are responsible for around 10 to 15 million infections and tens of thousands of hospitalizations annually. Infants, children, and people with weakened immune systems are more likely to get hospitalized and can have serious complications from enterovirus infection. Non-polio enteroviruses like EV-D68 can cause mild to severe symptoms like wheezing, difficulty breathing and acute flaccid myelitis (AFM). Therefore, it is crucial to continue to monitor the prevalence of enteroviruses, especially those that can potentially cause severe symptoms like EV-D68. This study focused on determining the prevalence of enteroviruses in a pediatric oncology patient population.

Methods: Residual Nasopharyngeal swabs (NPS) specimens, collected from pediatric patients (<18 years old) presenting to Memorial Sloan Kettering Cancer Center (MSKCC) between September 2022 and December 2022, and September 2023 and December 2023 for cancer care were included in the study. Rhinovirus/Enterovirus (RV/EV) was detected using the BioFire FilmArray Respiratory Panel (bioMerieux, Inc.) or the ePlex Respiratory Pathogen Panel (GenMark/Roche Molecular). For Enterovirus identification, RNA was extracted from the RV/EV positive NPS using the KingFisher™ Flex Magnetic Particle Processors (Thermo Scientific, USA), followed by RT-PCR in the ABI 7500 Fast real-time PCR instrument (ThermoFisher, USA) for enteroviruses and EV-D68 using pan-Enterovirus and EV-D68 specific primers, respectively.

Results: A total of 880 and 694 NPS samples from patients <18 years old were tested during the study period in 2022 and 2023, respectively. Of these, 238 (238/880; 27%) and 225 (225/694; 32%) RV/EV positives NPS were detected in 2022 and 2023 respectively. A total of 370 (370/463; 80%) RV/EV positive samples (182 samples from 2022 and 188 samples from 2023) were selected for additional testing and 14 samples (14/370; 4%) were positive for Enteroviruses. Of note, EV-D68 was not detected.

Conclusions: In this study, we report a high prevalence of RV/EV which is consistent with the higher detection rate in the fall seasons, when our samples were collected. The low prevalence of EV suggests a high prevalence of RV, although this was not tested. Despite a nationwide surge in the USA in 2022, there was no EV-D68 circulating in these patients. Further analysis, using Whole genome sequencing of the enteroviruses detected in this study will provide additional information on specific circulating Enterovirus genotypes.
Unmasking the Viral Culprit: Insights into the Prevalence and Histopathological Patterns of Gastroenteritis

Presenting Author:
Poonam Sharma, MD, PhD, University of Mississippi Medical Center, Jackson, MS

Objective: Gastroenteritis is a common cause of morbidity and mortality globally. Its cause encompasses a spectrum of agents, including viruses, bacteria, parasites, toxins, and drugs. Viruses account for a considerable portion of gastroenteritis cases across all age groups. While sporadic cases occur, viral gastroenteritis is more frequently observed in outbreaks within closely-knit communities such as daycare facilities, nursing homes, and cruise ships. Therefore, it becomes necessary to determine when healthcare providers should consider this condition in their differential diagnosis and to develop the most effective strategy to confirm the diagnosis.

Methods: De-identified data of patients with gastroenteritis were collected over a five-year period utilizing the patient cohort explorer, an electronic health record at the University of Mississippi Medical Center. Confirmatory laboratory tests employed the BioFire® FilmArray® multiplex polymerase chain reaction for gastrointestinal pathogens. Out of the 22 most common agents associated with gastroenteritis, only viral pathogens, specifically adenovirus, astrovirus, norovirus, rotavirus, and sapovirus, were included in the analysis. These were correlated with histopathology.

Results: Among the various causes of gastroenteritis, both infectious and non-infectious, our findings revealed that 25.46% of the cases were linked to viral pathogens. This included a significantly higher percentage of pediatric patients (72.73%) when compared to adults (27.07%), with a p-value of 0.015. Norovirus genogroup I and II emerged as the most frequently detected viruses across all age groups, with a significant prevalence among adults. No discernible gender-based differences were observed. The histopathological findings included acute inflammation, ulceration, erosion, architectural distortion, and pathognomonic viral inclusion bodies specially in adenovirus.

Conclusions: Our comprehensive analysis of viral gastroenteritis cases highlights the substantial burden of this condition, particularly among pediatric patients. Norovirus emerges as a prevalent culprit which emphasizes the importance of vigilant surveillance and timely diagnosis, especially in settings where outbreaks are common.
Poster Board # 028  
Comparison of Multiple Methods for the Whole Genome Sequencing of Respiratory Syncytial Virus  

Presenting Author:  
Victoria Schnurr, Wadsworth Center, New York State Department of Health, Albany, NY 

Objective: To evaluate and compare the performance of four whole genome sequencing (WGS) methods for respiratory syncytial virus (RSV).

Methods: A total of 32 RSV-positive pediatric respiratory swabs (18 RSV-A and 14 RSV-B, with real-time RT-PCR Ct values ≤24) collected during the 2022-2023 respiratory season, were selected for analysis. Three methods utilized large amplicon sequencing with Illumina instruments: Dong et al. (2023), Wang et al. (2022), and an IDT xGen custom assay. The fourth method consisted of a custom small amplicon sequencing panel (AmpliSeq) on the fully automated Ion Torrent Genexus sequencer. The Wang assay had separate primer sets for RSV-A and RSV-B while the other assays used conserved primers designed to cover both subtypes. Genome coverage, read depth, ease of use, and cost were compared between the four methods. All sequences were analyzed using identical bioinformatic methods.

Results: Genome coverage was highest with the Dong assay, yielding genomes with >90% coverage at ≥10X depth for 30/32 samples. The highest rate of ambiguous bases was found in xGen and AmpliSeq assays. The four assays exhibited reduced coverage in different genomic regions, with xGen and Wang assays displaying the most substantial gaps. Primer mismatches were identified in the Dong and Wang assays; however, the xGen and AmpliSeq primer designs were proprietary and unable to be assessed.

Conclusions: With the approval and use of RSV vaccines, tracking its genomic evolution is of increased importance. In this evaluation we found that the Dong assay provided the highest genome coverage with lowest cost, and Dong and xGen the highest throughput. AmpliSeq had the best turnaround time and ease of use, but the highest cost and lowest throughput. Mutational changes in circulating lineages caused reduced primer binding and resultant reduced genome coverage. Therefore, with evolution, repeated optimization of amplicon based WGS methods may be required.
Poster Board # 029

Evaluation of Contamination in Hepatitis C Virus (HCV) Antibody Positive Specimens Reflexed for Hepatitis C Virus (HCV) RNA Testing

Presenting Author:
Emily Hallock, BS, Cleveland Clinic Foundation, Cleveland, OH

Objective: Hepatitis C virus (HCV) antibody screening and reflex HCV RNA testing on the same specimen is an operational strategy that allows for single-visit sample collection, thus increasing likelihood of timely diagnosis and treatment for patients with active HCV infection (Cartwright EJ et al, 2023, PMID: 37440452). However, this strategy can introduce risk of contamination, reported in up 4% of specimens tested after HCV-antibody positive specimens on chemistry instruments (Rondahl E et al, 2014, PMID: 24735614). A carryover evaluation was conducted in a Molecular Microbiology laboratory receiving residual specimens from 5 Regional Chemistry laboratories, with subsequent implementation of a mitigation strategy to reduce risk of false positives.

Methods: Contrived HCV RNA-positive (>6 log IU/mL) and negative samples were created by pooling residual serum specimens. Ten pools (5 positive, 5 negative) were distributed to each Regional Chemistry lab (Labs A–E), with instructions to test for HCV antibody in alternating sequence. These samples were tested on analyzers such as the Cobas e 801 (Roche), Atellica IM (Siemens), and Alinity M (Abbott) analyzers. The Cobas e 801 and Atellica IM use disposable tips, while the Alinity M uses a fixed needle for specimen transfer. Afterwards, specimens were returned to the central Molecular Microbiology laboratory and tested for HCV RNA using the Cobas HCV assay on the Cobas 8800 (Roche).

Results: All 5 samples from Labs B (Cobas e 801), Lab C (Atellica IM), and Lab E (Cobas e 801) respectively were negative for HCV RNA as expected. Lab A (Alinity M) and Lab D (Cobas e 801) each returned 1/5 (20%) samples that tested falsely-positive for HCV RNA. These 2/25 (8%) samples falsely positive for HCV RNA had viral loads of 43.7 and 59.9 IU/mL respectively. Low quantities of HCV RNA may contaminate residual samples initially tested on automated chemistry instruments both with and without disposable tips. To mitigate the risk of releasing a false positive HCV RNA result, the Molecular Microbiology lab stopped auto-verification of reflexed HCV RNA tests with detectable RNA levels <1000 IU/mL. In such cases, the medical director reviews the patient history. These tests are canceled and redraws are requested, unless the patient has a recent diagnosis of active HCV made on another specimen. From June–October 2023, a total of 1119 HCV RNA tests were run on residual specimens previously positive for HCV antibody. Of these, 9 (0.8%) tested positive for HCV RNA with a viral load <1000 IU/mL. In these individuals, false positivity rate was 3/9 (33%), and failure to present for redraw rate was 3/9 (33%).

Conclusions: Our findings that HCV RNA contamination can affect specimens processed on automated chemistry instruments using both fixed needles and disposable pipettes, and that false-positive results are limited to <1000 IU/mL, are corroborated by prior studies (Rondahl E et al, 2014, PMID: 24735614; Albert C, 2023, CAP Today June 2023). A retrospective review revealed that contamination is an infrequent (<0.8%) risk of reflex testing for HCV RNA on residual serum. The mitigation strategy described here can be used to further reduce risk of contamination while allowing single-visit sample collection, as recommended by the CDC. Public health authorities should consider contamination risk and possible mitigation strategies when recommending reflex testing strategies on a single specimen.

Poster Board # 030

Validation of a Qualitative Real-Time PCR for Toxoplasma gondii from Amniotic Fluid, CSF and Vitreous Fluid
Presenting Author: Katharine Uhteg, Johns Hopkins Hospital, Baltimore, MD

Objective: Toxoplasma gondii is a serious risk for immunocompromised individuals and a cause of congenital toxoplasmosis. While the gold standard for detection is serology, direct testing of the organism can be achieved through molecular methods. We validated a qualitative real-time PCR on the Liaison MDX (DiaSorin) for detecting Toxoplasma gondii in amniotic fluid, CSF and vitreous fluid.

Methods: 50 µL of sample and 50 µL of reagents were pipetted in an 8-well Direct Disk and run with the Toxoplasma gondii protocol. Reagent mix (target primers, IC and IC primers, master mix) were from DiaSorin. We assessed performance using different target concentrations, including the limit of detection (LOD), specificity, agreement and reproducibility. Contrived samples were made by spiking in various concentrations of quantified Toxoplasma gondii (ME49 strain) into each sample matrix.

Results: The LOD of the assay was found to be 0.25 parasites/µL (amniotic fluid, vitreous) or 0.5 parasites/µL (CSF). Six other organisms were tested, none of which showed cross reactivity. Two other strains of Toxoplasma gondii were also run. A panel of 10 contrived positive samples per source showed 100% agreement, along with 100% negative agreement of unspiked samples (n=10 per source). The assay showed 100% reproducibility in both inter and intra assay studies.

Conclusions: The qualitative real-time PCR for Toxoplasma gondii from amniotic fluid, CSF and vitreous fluid is sensitive and specific. Because there was no in house molecular assay available previously, implementation of this test in the clinical laboratory should improve turn around times.
Diagnostic Testing Preferences Can Help Inform Future Public Health Response Efforts: Global Insights from an International Survey

Presenting Author:
Laura Burke, SalivaDirect Inc., New Haven, CT

Objective: Public perception regarding clinical diagnostic sample types as well as personal experiences can influence willingness to test. As such, public preferences for specific sample type(s) should be used to inform diagnostic and surveillance testing programs to improve public health response efforts. To understand where preferences lie, we conducted an international survey regarding sample types used for SARS-CoV-2 tests.

Methods: A Qualtrics survey regarding SARS-CoV-2 testing preferences was distributed via social media and email. The survey collected preferences regarding test sample types and key demographic data. Python was used to analyze survey responses.

Results: From March 30th to June 15th, 2022, 2,094 responses were collected from 125 countries. Participants were 55% female and predominantly aged 25-34 years (27%). Education and employment were skewed: 51% had graduate degrees, 26% had bachelor’s degrees, 27% were scientists/researchers, and 29% were healthcare workers. By rank sum analysis, the most preferred sample type globally was the oral swab, followed by saliva, with parents/guardians preferring saliva-based testing for children. Respondents indicated a higher degree of trust in PCR testing (84%) vs. rapid antigen testing (36%). Preferences for self- or healthcare worker-collected sampling varied across regions.

Conclusions: This international survey identified a preference for oral sample types when testing for SARS-CoV-2. Notably, respondents indicated that if they could be assured that all sample types performed equally, then saliva was preferred. Overall, survey responses reflected the region-specific testing experiences during the COVID-19. Public preferences should be considered when designing future response efforts to increase utilization, with oral sample types providing a practical option for large-scale, accessible diagnostic testing.
**Poster Board # 032**  
**An Increased Circulation of Human Adenovirus in 2023 is Associated with a Predominance of B3 Type**

**Presenting Author:**  
Omar Abdullah, BSc, Johns Hopkins University, Baltimore, MD

**Objective:** An increase in the circulation of human adenoviruses (HAdV) in 2023 was notable. HAdV genotypes circulating were characterized. Viral loads, clinical presentations, and outcomes were associated with the genotypes.

**Methods:** Remnant respiratory samples positive for HAdV after the standard of care testing were collected for genotyping by next generation sequencing of the hexon gene. Viral loads were assessed using droplet digital PCR. The association between predominant types, outcomes, and viral loads were evaluated.

**Results:** Of a total of 249 samples with characterized HAdV genotypes, 179 (71.9%) were genotype B3. HAdV-B3 was associated with a statistically significant increase in viral loads in respiratory samples, specifically in patients 5 years and younger. Patients infected with HAdV-B3 were primarily in the age group 3-5 years in contrast to patients infected with non-B3 genotypes who were younger than 3 years. Strict criteria for defining HAdV-related admission identified a hospitalization rate of 14.8%. Infections with HAdV-B3 were not associated with an increased likelihood of HAdV-related admissions.

**Conclusions:** The circulation of HAdV-B3 in 2023 after at least 2 years of reduced detection likely contributed to the increased number of cases. We show that HAdV-B3 was predominant in 2023 in a cohort characterized by the Johns Hopkins Hospital System. We also show that HAdV-B3 was associated with an increase in viral loads in respiratory samples and provide a correlation with the clinical presentations and outcomes.
**Poster Board # 033**  
**Decreased Analytical Performance of a Commercial PCR Assay to Detect Influenza A During the 2022-23 Winter**

**Presenting Author:**  
**Chris Attaway, MD, MSc**, Cleveland Clinic, Cleveland, OH

**Objective:** Influenza A is a significant respiratory pathogen of great public health concern, especially due to its capacity for genetic shift and drift. Influenza A infections are detected in laboratories primarily by nucleic acid amplification tests (NAAT). Current circulating influenza A virus subtypes are H3N2 and H1N1pdm09. The diagnostic performance of NAATs for influenza A can be impacted by genetic mutations occurring as part of genetic drift. The target of most commercial assays is the matrix (M) gene due to its conservation across influenza A variants. In March of 2022, it was reported that false negative test results for influenza A subtype H3N2 are possible due to two single base changes in the center of the matrix gene.1 In December of 2022, another laboratory reported decreased sensitivity of influenza A H1N1 subtype.2 Assays that rely on a single matrix gene target are vulnerable to false negatives due to genetic drift. One platform that uses a single matrix gene target is the cobas SARS-CoV-2 Influenza A/B Assay (Roche Diagnostics; Indianapolis, Indiana). We investigated the relative sensitivity and performance of this cobas reverse transcription real-time PCR (rRT-PCR) assay for detection of influenza A subtypes H1N1pdm09 and H3N2 circulating during the winter of 2022-2023. We compared the cobas SARS-CoV-2 influenza A/B Assay to two other validated molecular diagnostic influenza assays: GeneXpert Infinity Cov/Flu/RSV assay (Cepheid; Sunnyvale, CA, USA) which utilizes multiple gene targets, and Panther Fusion Flu A/B/RSV RT-PCR assay (Hologic; Marlborough, MA, USA) which also uses a single gene target.

**Methods:** In December 2022, inter-instrument comparison for detection of influenza A was performed as part of routine quality monitoring in Cleveland Clinic Main Campus laboratory. Ninety-three (93) remnant samples in which influenza A was detected by standard-of-care using the Panther Fusion Flu A/B/RSV RT-PCR assay with cycle threshold (CT) < 30 were tested using cobas. A subset (n=21) of specimens were subtyped using Luminex NxTAG Respiratory Pathogen Panel (Diasorin; Saluggia, Italy). Additionally, a separate set of 20 consecutive specimens that were positive for influenza A and subtyped on the Luminex NxTAG Respiratory Pathogen Panel were tested on all three assays: cobas, Panther, and GeneXpert. In all testing methodologies, the CT values were recorded as well as the final diagnostic result. Exact Clopper-Pearson binomial confidence intervals were calculated for sensitivity and specificity.

**Results:** Of the remnant specimens that were identified as influenza A by Panther, ninety-eight percent (98%; 91/93) were also detected by cobas. Two specimens testing negative by cobas were subtyped as H1N1pdm09. The specimens that were subtyped as H1N1pdm09 (n = 11) showed a median CT value on the Panther and cobas of 20.4 and 31.8, respectively. Separately, the additional 20 consecutive previously identified subtyped influenza A positive specimens were analyzed using all three platforms (Panther, cobas, and GeneXpert). Two samples that were subtyped as H1N1pdm09 were not detected by cobas but were detected by the other two platforms, yielding 90% instrument sensitivity in detecting influenza A.

**Conclusions:** Our quality investigation identified that the cobas assay had decreased sensitivity for detection of influenza A virus, specifically the H1N1pdm09 subtype. The United States Centers for Disease Control and Prevention’s (CDC) most recent data from the 2022-2023 influenza season shows that, of specimens that were subtyped, influenza A H3N2 was the most prevalent circulating subtype (70.8%) in all age groups; about a third of cases were subtyped as H1N1pdm09.3 Despite our limited sample size, the subtyped specimens from our laboratory overall approximate the data collected nationwide. We estimate that the cobas assay did not detect approximately 10% of influenza A
infections in Northeast Ohio during the winter of 2022-2023, which would have been detectable using similar nucleic acid diagnostic testing. While this study is limited by small sample size, our findings are corroborated by reports from another laboratory, and an FDA recall of the cobas assay. A statement from Roche attributes the loss of sensitivity in their assay for H1N1pdm09 subtype viruses to two mutations (C124A and C124A + G141A) in the M gene. This H1N1 variant began circulating at low frequency in 2021 but has since become the dominant H1N1 variant in Europe but with reported cases in the Americas. Subsequently, Cleveland Clinic diverted influenza testing from cobas to the Panther assay in January 2023. NAATs used for viral detection are at risk of target failure; using more than a single gene target could help mitigate the risk. For example, the GeneXpert assay evaluated here contains targets for not only matrix gene, but also PB2 and PA genes, which were added by Cepheid in response to genetic drift in the H3N2 subtype which resulted in decreased sensitivity of molecular assays during the 2015 influenza season. Continuous in-silico and in-vitro monitoring of diagnostic assays targeting viruses known to mutate frequently should be performed by manufacturers, laboratories, and regulatory agencies.
**Poster Board # 034**

**A Generic, Saliva-Based PCR Test for the Detection of RSV Offers a Low-Burden Approach for Sustainable Surveillance of RSV**

**Presenting Author:**

Anne Wyllie, PhD, Yale School of Public Health, New Haven, CT

**Objective:** With the introduction of RSV vaccines, large-scale surveillance is crucial for monitoring their safety, performance, and impact on reducing the global burden of RSV infection. Saliva, being non-invasive, offers a low burden approach to support surveillance efforts.

**Methods:** We modified our saliva-based, RNA-extraction-free test (originally developed for SARS-CoV-2) for the detection of RSV. To facilitate broad implementation, we validated the assay using numerous reagents and PCR instruments from different suppliers. To determine the feasibility of using simple, laboratory plastic tubes for sample collection, we assessed the stability of RSV detection in raw saliva. Additionally, we developed a full genome sequencing protocol for RSV, based on the Oxford Nanopore SARS-CoV-2 sequencing workflow.

**Results:** We achieved an assay limit of detection of 4 copies/µl. RSV remained detectable for 24 hours at 40°C; ≥7 days at +4°C, 19°C and 30°C; ≥8 weeks in a ‘frost-free’ freezer (-20°C); and through cold-chain-free postal conditions typically encountered in summer and winter. Sequencing RSV from saliva yielded 80-95% genome coverage with minimal adjustments to the SARS-CoV-2 protocol.

**Conclusions:** Saliva, due to its non-invasive nature and low resource requirements, is ideal for large-scale surveillance. Combined with an open-source, extraction-free PCR test, this significantly reduces costs, ensuring ongoing assessment of vaccination programs and incidence of RSV. The ability to sequence RSV from saliva further enhances surveillance capacity and monitoring of seasonal epidemics. Importantly, we have demonstrated that this RSV test can be expanded to simultaneously screen for SARS-CoV-2 and influenza A/B. This multiplexing capability offers the potential for a cost-effective, all-in-one diagnostic solution.
Validation of an Epstein-Barr Virus BamHI-W Real-Time PCR for the Monitoring of EBV in Patients with Nasopharyngeal Carcinoma

Presenting Author: 
Krupa Jani, MS, Memorial Sloan Kettering Cancer Center, New York, NY

Objective: Epstein-Barr Virus (EBV) is a transforming herpesvirus capable of inducing the uncontrolled proliferation of infected cells. Because of this property, EBV is associated with a variety of malignancies including nasopharyngeal carcinoma (NPC). EBV genome contains BamHI-W region (8-11 repeats), which can lead to increased sensitivity as a target for PCR than assays targeting a single gene copy. EBV-BamHI-W levels can be used to track the effectiveness of chemoradiation therapy in patients with advanced stage NPC. The goal of this study was to validate a real-time PCR for detection of EBV-BamHI-W DNA levels in plasma as aid in the management of patients with NPC.

Methods: Primers and probes were obtained from IDT (Coralville, IA). DNA extraction was performed using EMAG (Biomerieux) followed by amplification on an ABI 7500 (Applied Biosystems, Foster City, CA). Lower limit of detection (LOD) was determined by testing a dilution series of EBV genomic DNA spiked in negative plasma, with additional twenty replicates tested at the estimated LOD. Specificity was determined by testing a panel of potentially cross-reacting organisms (n=33). Accuracy was established by testing 40 plasma samples including 30 spiked positive samples and 10 negative samples. Analysis of all validation experiments was performed using Microsoft Office Excel.

Results: The LOD of the EBV BamHI-W PCR was 10 IU/mL. There was no cross-reaction with other pathogens tested. Agreement between EBV BamHI-W PCR and expected results was 100% with cut-off Ct value of <39 for positives. Additionally, 30 spiked positive samples were detected by EBV BamHI-W PCR at Stanford Clinical Virology laboratories. Reproducibility was determined to be 100% with coefficient of variation of inter-assay and intra-assay being 0.62%-4.53% and 1.18%-2.86% respectively.

Conclusions: Our validation testing indicates, this assay is both highly specific and sensitive for monitoring of EBV infections. EBV BamHI-W PCR will allow measurement of low levels of EBV DNA in patients with advance (stage II to IVb) NPC.
Poster Board # 037
MUC5AC Levels as Potential Biomarker for Influenza Disease Severity in Children

Presenting Author:
Patricio Acosta, MD, Hospital de Niños Ricardo Gutierrez, Ciudad de Buenos Aires, Argentina

Objective: Numerous factors can increase the risk of severe Influenza, however, the majority of severe cases occur in previously healthy children. Mucin MUC5AC is a significant component of airway mucus. We aimed to analyze MUC5AC levels in nasal aspirates from Influenza-infected children and explore its potential use as biomarker.

Methods: Prospective cohort study conducted during 2019. Children (<16yo) with acute respiratory infection (ARI) and confirmed Influenza infection were enrolled. Patients were defined to have mild disease when hospitalized but not requiring supplemental oxygen; moderate disease when hospitalized, requiring supplemental oxygen but not admitted to the intensive care unit (ICU); and life-threatening disease (LTD) when admitted to the ICU. Nasal aspirates were tested for MUC5AC levels using an ELISA kit. MUC5AC gene expression was analyzed by RT-qPCR. Epidemiologic and clinical risk factors were assessed.

Results: 342 patients were hospitalized with ARI, 49 (14%) had confirmed Influenza infection. The mean age was 19.1 (IQR 7-24) months. 11 (22%) had mild disease, 32 (65%) had moderate disease, and 6 (12%) developed LTD. We did not find significant differences in age, sex or vaccination status between groups. MUC5AC levels in nasal aspirates were higher in those patients with mild disease, when compared to moderate and LTD group (P<0.001). We further investigated if MUC5AC mRNA expression levels correlated with disease severity. MUC5AC mRNA expression was decreased in those children with moderate or LTD (P<0.001). We did not find significant differences in viral load between groups. No statistically significant associations were found between MUC5AC levels and age, gender, vaccination status or time after the onset of illness. (P=ns). A shorter hospital length of stay (P<0.001) and fewer days of supplemental oxygen (P<0.0001) were associated with higher MUC5AC levels.

Conclusions: Our results show that MUC5AC levels correlate with Influenza disease severity. This molecule could be a potential biomarker for disease severity.