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Session: 233. Diagnostics: Virology
Saturday, October 6, 2018: 12:30 PM

Background. Early determination of viral etiology among febrile children with suspicion of systemic infection by use of rapid molecular assay could impact patient care. BioFire® FilmArray® Childhood Systemic Infection (CSI) Panel is a non-FDA cleared research use only sample-to-answer PCR-based assay that includes identification of seven viruses from 200 µl of whole blood collected from children suspected of systemic infection. The aim of this prospective multicenter study was to determine the viral diagnostic yield and potential impact of CSI panel on management of pediatric sepsis.

Methods. Children <18 years with suspected systemic infections were prospectively enrolled in emergency rooms at seven healthcare facilities. Febrile children with a clinician order of blood culture for sepsis evaluation were enrolled and additional blood was collected with the standard of care (SOC) blood culture. Blood samples were tested by the CSI Panel on the FilmArray platform. Demographic and laboratory test results from SOC blood, urine and cerebrospinal fluid (CSF) cultures were recorded.

Results. Among 1,022 children enrolled, data for 1,002 was complete. The CSI Panel testing of whole blood detected 203 (20%) viral infections including 14 (7%) with dual/multiple viruses. The median age of children with viral detections (20 months) was significantly lower than children without viral detections (54 months) ($P < 0.01$).The viruses detected were enterovirus (54%), adenovirus (22%), cytomegalovirus (15%), parvovirus B19 (15%) and parechovirus (3%). Herpes simplex virus 1 and 2 were not detected. Among 203 positive and 799 negative viral detections with the CSI Panel, blood culture was positive in 2 (1%) and 24 (3%) children respectively ($P = 0.14$). All CSF bacterial cultures ordered were negative. Urine culture was positive in 7/83 (8.4%) and 31/266 (11.7%) viral positive and negative children respectively ($P = 0.55$).

Conclusion. The CSI Panel detected virus in blood from 20% of febrile children suspected of systemic infection. Concurrent bacterial infection of blood and urine was lower in children that were viral positive vs. negative. Prompt determination of viral etiology using the CSI Panel has the potential to optimize care of children with suspected systemic infection.

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2085. Evaluation of Panther Fusion Flu A/B/RSV, AdV/hMPV/RV and Paraflu Assays for the Detection of Respiratory Viruses in Children

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Session: 233. Diagnostics: Virology
Saturday, October 6, 2018: 12:30 PM

Background. There are many FDA cleared multiplexed respiratory assays available in the United States, ranging from 3 to 20 targets per assay. FilmArray Respiratory Panel (RP) is one of the mega-multiplexed assay, includes 20 targets and results are available in 1 hour. Recently Hologic has received FDA-clearance for several smaller respiratory "Panther Fusion assays" (3–4 targets/assay) and results are available in <3 hours. The aim of this study was to evaluate the performance of three panther fusion multiplexed assays: (i) Flu A/B/RSV assay, (ii) adenovirus/human metapneumovirus/rhinovirus assay, and (iii) paraflu (parainfluenza virus 1–4) assay in comparison to RP assay.

Methods. A total of 194 frozen nasopharyngeal swab samples (from 2016 to 2018) obtained from children aged ≤18 years and previously tested by RP as a routine clinical testing were included in this study. These samples were tested by all three fusion assays. Positive percent agreement (PPA) and negative percent agreement (NPA) of Fusion assays were calculated against RP assay.

Results. Among 194 samples, 58.0% were from male. Median age was 36 months (IQR 13–72 months). Overall agreement between two assays was 82.5% (95% CI 77.6–88.1). PPA and NPA of Fusion assays for each target was; Flu A-100.0% and 100.0%, Flu B-95.0% and 100.0%, RSV-90.1% and 94.0%, adenovirus-80.0% and 98.2%, hMPV-95.2% and 99.4%, Rhino-79.1% and 95.8%, Parainfluenza virus-100.0 and 100.0%, respectively. There were total 34 discrepant samples. Among these, majority were rhino ($n = 12$), RSV ($n = 12$) and adenovirus ($n = 7$). Of 12 rhinovirus discrepant samples, Fusion assay detected additional seven but missed five samples. For 12 RSV discrepant samples, fusion assay detected 10 more RSV but missed two. Fusion assay missed four adenoviruses and detected additional three samples. All discrepant samples, especially rhinovirus positive samples need further investigation since RP detects both rhinovirus and enterovirus, whereas the Fusion assay detects rhinovirus only.

Conclusion. Performance of fusion Flu A/B/RSV and Paraflu assays were comparable with RP assays. Fusion AdV/hMPV/RV assay had the highest discrepancy with RP assay. Overall, Panther fusion respiratory assays provide the opportunity to customize testing with smaller respiratory panels at a reduced cost.

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2086. Perils of CMV PCR Primer/Probe Design: Emergence of Mutations in Clinical Samples from Two Pediatric Patients

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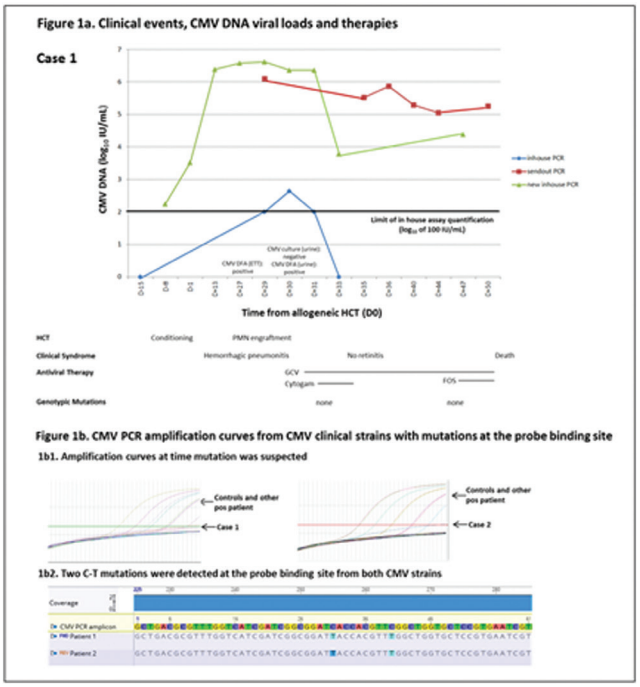
Session: 233. Diagnostics: Virology
Saturday, October 6, 2018: 12:30 PM

Background. Detection of CMV by PCR is the preferred method for both diagnosing infection and monitoring therapy. The design of CMV PCR depends on analysis of all available nucleic acid sequences to maximize performance. We describe two patients in whom our in-house CMV PCR was falsely negative (FN) due to two recently emerged mutations in the DNA polymerase gene.

Methods. In-house CMV PCR targeting a specific 61 bp fragment of the polymerase gene (*UL54*) has been in use in our lab since 2003. Confirmatory CMV PCR was sent to a reference lab which uses PCR targeting *US9* gene.

Results. *Case 1:* 4 months F with familial hemophagocytic lymphohistiocytosis (homozygous *PRF1*) underwent 10/10 MUD BMT (CMVD+/R−). Plasma CMV was not detected on admission and monitoring was performed weekly. She developed respiratory failure, intubated on D+13 with hemorrhagic respiratory secretions. Repeat PCR of tracheal secretions and plasma detected CMV on D+33, prompting ganciclovir and cytogam. She developed refractory hypoxemia and asystolic cardiac arrest on D+51 (Figure 1a). *Case 2*: Thirty-two-week F born via C-section for fetal distress noted to have SGA, microcephaly, thrombocytopenia and hyperbilirubinemia at birth, concerning for congenital CMV; urine CMV + (Ct 43.18). Repeat urine and blood PCRs on Day 5 of life were indeterminate. Given initial CMV detection and clinical stigmata, ganciclovir was started. Close analysis in Case 1 of the amplification curve (Figure 1b1) on the 21st sample submitted lead us to sequence the amplicon region and to discover two mutations (C-T) in the probe binding site affecting the sensitivity of UL54 PCR(Figure 1b2). These previous FNs delayed CMV diagnosis and the start of antivirals. For Case 2, the distinct curve was noted on the first sample and was sent for confirmation, resulting in no adverse clinical implications. We subsequently developed a CMV PCR targeting US9 that can detect these mutations.

Conclusion. Periodic assessment of all available CMV sequences and close review of amplification curves are essential to prevent FN PCR. With conflicting laboratory and clinical data, clinicians with a high suspicion for CMV should question negatives and if appropriate, ask for PCR using an alternate target.



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