Clinical Performance of the GenMark Dx ePlex Respiratory Pathogen Panels for Upper and Lower Respiratory Tract Infections

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Abstract
The GenMark Dx ePlex Respiratory Pathogen (RP) panel is a multiplexed nucleic acid test for the qualitative detection of common viral and a few bacterial causes of respiratory tract infections. We evaluated the performance of the ePlex RP, compared to the Luminex NxTAG Respiratory Pathogen Panel (NxTAG-RPP) for nasopharyngeal swab (NPS) specimens and bronchoalveolar lavage (BAL) specimens. We also evaluated the ePlex RP2 panel, which added the SARS-CoV-2 target to the RP for NPS and the test turn-around time (TAT) of the ePlex RP. Verification of the performance of the ePlex RP for both NPS and BAL showed 93.3% and 84.9% total agreement with the NxTAG RPP, respectively. An overall comparison of the TAT of the ePlex RP to the NxTAG-RPP assay showed an average decrease of almost sevenfold.

Introduction
Molecular respiratory syndromic panels represent a great advancement in the diagnosis of a broad range of respiratory viruses in clinical microbiology laboratories. Extended multiplex respiratory panels allow the simultaneous detection of multiple common viral causes of respiratory tract infections, in addition to a few bacterial targets. In this study, we compared the performance of the ePlex RP panel to the NxTAG-RPP. Our evaluation consisted of a verification of the performance of the ePlex RP for NPS and an analytical and clinical validation of the ePlex RP for BAL. The evaluation of the ePlex RP was performed prior to its clinical implementation in our laboratory to replace the NxTAG-RPP. The ePlex RP2 was also evaluated to replace the ePlex RP to consolidate SARS-CoV-2 testing with the extended panel during the current SARS-CoV-2 pandemic.

Materials and Methods
Study site and ethics: This study was performed at the Molecular Microbiology Laboratory, Johns Hopkins Hospital. Clinical specimens were analyzed after the study approved by the Institutional Research Board committee.

Clinical samples: Residual clinical specimens archived after standard of care testing on NxTAG-RPP assay were used for the study. 242 NPS (175 positives [115 tested with RP and 58 tested with RP2] and 69 negatives [64 tested with RP and 5 tested with RP2]) and 77 BAL (52 positives and 25 negatives [tested with RP only] were either used in the same day or frozen at -80°C for evaluating the performance of the ePlex RP and RP2 panels.

Contrived samples: To determine the analytical sensitivity (LOD) of the ePlex RP for BAL, tenfold dilutions were prepared using the quantified stocks of each of the analytes spiked into pooled negative BAL patient specimens and tested in at least 9 replicates for each pathogen on the panel.

Luminex NxTAG-RPP: 200 μL of respiratory specimen samples were aliquoted for nucleic acid extraction and purified on EasyMag (BioMerieux, Inc.) using the generic protocol to yield 110 μL of extracted material of which 35 μL was used for the PCR reaction. PCR was performed on an Eppendorf thermocycler with the following cycling parameters: 1 cycle at 42°C for 20 min, 1 cycle at 95°C for 2 min and 30 s, 15 cycles (95°C for 20 s, 60°C for 60 s, 72°C for 10 s), 26 cycles (95°C for 20 s, 56°C for 60 s, 72°C for 10 s) and 1 cycle at 37°C for 45 min. Analyte detection was performed on the Luminex MAGPIX.

ePlex RP: NPS received in Copan Universal Transport Medium tubes and BAL samples were briefly vortexed prior to pipetting 200 μL of the sample to the sample delivery device, then vortexed again for 10 s. The sample/lysis mixture was then transferred into the ePlex RP cartridge and loaded onto the ePlex instrument.

ePlex RP2: NPS were vortexed and 200 μL of the sample was pipetted straight into the RP2 cartridge using a calibrated pipet.

TAT: We compared TAT soon after the ePlex RP was implemented (January 2020) to the same month from the previous year when the NxTAG-RPP test was the clinical assay in use (January 2019). TAT was measured from the time the samples were received in the laboratory to the time the results were released to the hospital’s electronic health record system.

Results

Accuracy of ePlex RP in NPS: The total agreement between the two assays was 93.3%, with 93.9% agreement of total positives (108/115) and 92.2% agreement of total negatives (59/64). Discrepant analysis confirmed the ePlex RP results in 8 of the 13 discrepancies with a notable prominence of human parainfluenza virus 4 as a recurrent potential false positive by the NxTAG-RPP.

Accuracy of ePlex RP2 in NPS: All positive and negative NPS tested on the ePlex RP2 were detected as expected except for four SARS-CoV-2 positive samples that were not detected by the ePlex RP2. Total agreement between the ePlex RP2 and the standard of care diagnostic assay was 73.79% (93.91% positive agreement and 100% negative agreement).

Accuracy of ePlex RP in BAL: The total agreement between the two assays was 84.9% (62/73 after excluding 4 invalid results), with 79.6% agreement of total positives (39/49) and 95.8% agreement of total negatives (23/24). Discrepant analysis confirmed the ePlex RP results in all 9 tested samples.

Analytical sensitivity of ePlex RP in BAL: The LOD (copies/mL) was defined as the minimum concentration at which ≥95% of the replicates were positive (Table 1).

TAT comparison: The TAT during January 2019 averaged to 1,373.3 min (approx. 23 h). The TAT during January 2020 had an average of 208.3 min (approx. 3.5 h).

Table 1 – LOD of the ePlex RP in BAL matrix.

Discussion
The diagnosis of viral pathogens responsible for symptoms of upper respiratory tract infection is essential for triaging patients to appropriate therapy. Specific antivirals are available for influenza [1,2] and early diagnosis can be associated with a marked reduction in the unnecessary use of antibiotics [3]. Also, the use of the extended panels for diagnosis of viruses revealed a large role for viruses in causing hospital and ventilator associated pneumonia [4,5]. Our contrived studies and LOD data show that the panel is capable of reliably detecting all the targets in the BAL matrix with good reproducibility in target concentrations above the LOD. This approach is useful for detecting viral causes of pneumonia, as well as difficult to culture bacterial targets included in the panels as M. pneumoniae and C. pneumoniae. Limitations of implementing extended respiratory panels clinically include the cost, defining proper test utilization, and challenges with reimbursement [6,7].

With the recent pandemic caused by SARS-CoV-2 and its widespread prevalence, SARS-CoV-2 made it to the top of the causative pathogens of influenza-like disease. Adding this target to the respiratory syndromic panels will assist with rapid diagnosis and infection control. This will be especially valuable in immunocompromised patient populations.

Our data show that implementing the ePlex RP in our laboratory was associated with a great reduction in TAT that was largely due to the difference in the workflow when compared to the NxTAG-RPP. The ePlex RP is a random access, cartridge-based assay, and samples are run as soon as they are received in the laboratory, 24 h a day, 7 days a week. The NxTAG-RPP assay took an average of 5 h from nucleic acid extraction to analysis, and was not interfaced, while the ePlex RP takes about 2 h from setup to result and is interfaced.

References