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9/11/2023

THE EVALUATION OF THREE COMMERCIALLY AVAILABLE RESPIRATORY 4-PLEX PANELS

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Introduction

- Four-plex assays for the detection of SARS-CoV-2, respiratory syncytial virus (RSV), influenza A (FLU A) and influenza B (FLU B) have become increasingly valuable in the wake of the SARS-CoV-2 pandemic.
- With the advancement of technology in robotics and automated fluid handlers, sample-to-answer platforms are more common in laboratories.
- These platforms can free up valuable laboratory staff time and improve accuracy by reducing human error.

Materials

Table 1: Commercial assays used in study

Platform	Test
Abbott Alinity m	RESP-4-PLEX ASSAY #09N79-090
Qiagen NeuMoDx™	Flu A-B/RSV/SARS-CoV-2 Vantage Assay #300900
Cepheid® GeneXpert® System	Xpert® Xpress SARS-CoV-2/Flu/RSV #XP3COV2/FLU/RSV

- Residual portions of nasopharyngeal swab specimens, previously submitted and tested for respiratory viruses, were selected and retrieved from frozen archives.
- Clinical samples included those positive SARS-CoV-2, RSV, FLU A and FLU B, with high, medium, and low CT values. Twenty positive and 20 negative samples for each target were run on each instrument.
- Both RSV A and B subtypes were tested. The FLU samples included subtypes H1N1 and H3N2 for FLU A, and lineages Yamagata and Victoria for FLU B.
- Co-infection study samples were obtained from isolate stocks except RSV was obtained from positive clinical sample.

Methods

- Specimens were thawed and divided into three aliquots then refrozen to -80°C, to ensure an equal freeze-thaw regimen across the assays being tested.
- A 10-fold dilution series in GTH media was prepared for each virus for each assay to compare limits-of-detection (LOD).
- Mixed virus samples (12) were also tested in high/low ratios, with an approximate 5 log difference in concentration for this co-infection study.
- The LOD studies used SARS-CoV-2 (B.1.1.7 alpha), FLU A (pdmH1), FLU B (VIC) and RSV (A).

Clinical Specimen Comparison

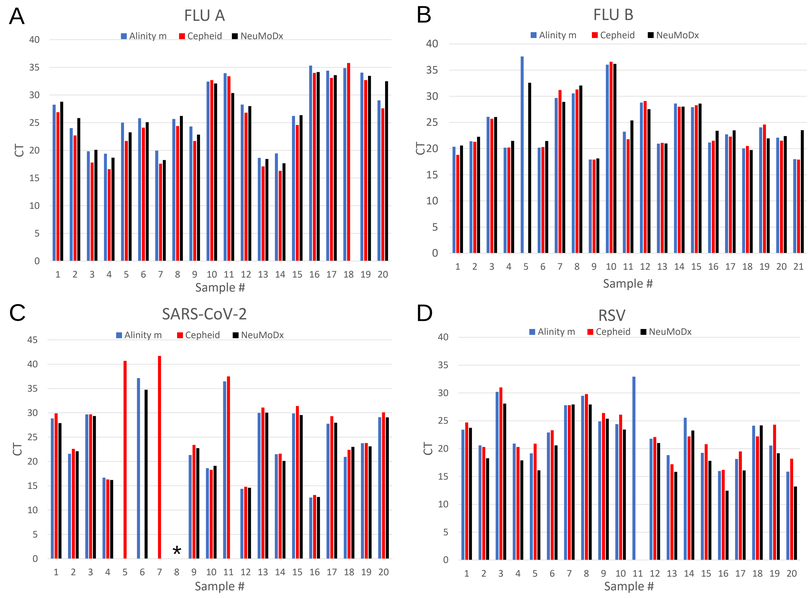

Figure 1: Results of positive samples tested in all three assays.
*Sample 8 resulted negative in all 3 assays for SARS-CoV-2.

Table 2: Percent positive over all three assays

Viral Target	Alinity m	NeoMoDx	Cepheid
FLU A	20/20 (100%)	19/20 (95%)	20/20 (100%)
FLU B	20/20 (100%)	20/20 (100%)	19/20 (95%)
RSV	20/20 (100%)	19/20 (95%)	19/20 (95%)
SARS-CoV-2	17/20 (85%)	16/20 (80%)	18/20 (90%)

Relative Sensitivity

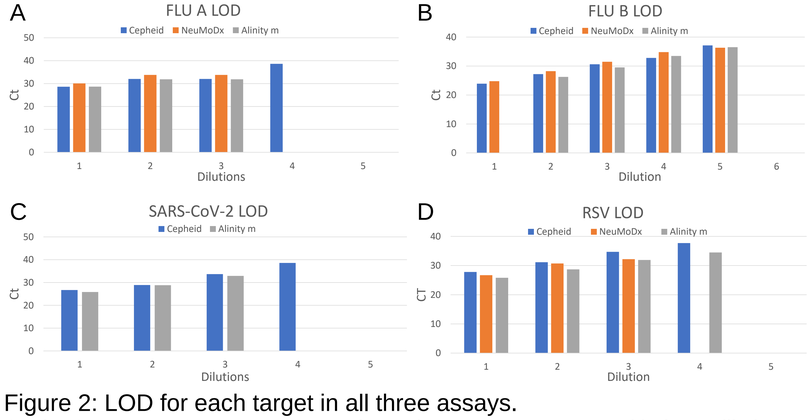

Figure 2: LOD for each target in all three assays.
C. The SARS-CoV-2 LOD was not performed on NeuMoDx assay with these dilutions. Successive dilutions were run on each assay' until results were negative.

Co-Infection

Table 3: Detection of viruses in mixed samples with high and low titers performed on the Abbott Alinity m.

CO-INFECTION	SARS-CoV-2		FLU A	
Sample 1	Strong	23.46 Ct	Weak	33.42 Ct
Sample2	Weak	38.57 Ct	Strong	18.13 Ct
CO-INFECTION	SARS-CoV-2		FLU B	
Sample 3	Strong	21.91 Ct	Weak	33.98 Ct
Sample 4	Weak	36.60 Ct	Strong	17.40 Ct
CO-INFECTION	SARS-CoV-2		RSV	
Sample 5	Strong	23.58 Ct	Weak	38.32 Ct
Sample 6	Weak	33.05 Ct	Strong	20.57 Ct
CO-INFECTION	FLU A		FLU B	
Sample 7	Strong	16.48 Ct	Weak	33.71 Ct
Sample 8	Weak	34.91 Ct	Strong	17.43 Ct
CO-INFECTION	FLU A		RSV	
Sample 9	Strong	17.31 Ct	Weak	34.06 Ct
Sample 10	Weak	33.05 Ct	Strong	20.37 Ct
CO-INFECTION	FLU B		RSV	
Sample 11	Strong	17.54 Ct	WeaK	37.35 Ct
Sample 12	Weak	33.69 Ct	Strong	21.12 Ct

Results

- Cepheid and Alinity m presented with less failed samples, requiring fewer repeats (data not shown).
- Cepheid, NeuMoDx and Alinity m detected 96%, 94%, and 97% of the clinical specimens accurately
- In the LOD studies, Cepheid® GeneXpert® System proved slightly more sensitive for FLU A detection while all three other targets were comparable across instruments.
- The co-infection study showed no loss of detection of low titer virus when mixed with a high titer virus on any platform.
- Validation was done prior to the Abbott recall of Alinity m Resp-4-Plex AMP Kit due to false positive results for FLU B and RSV targets. No negative samples showed any false positive results.

Conclusions

- Multiple respiratory viruses often present with similar clinical symptoms, and respiratory 4-plex assays provide a convenient method for rapid identification of the cause of most infections, without the significant expense of much larger respiratory panels.
- Alinity m and NeuMoDx™ have a higher throughput sample capability which affords increased lab efficiency but may not be ideal for laboratories with smaller testing volumes.
- All platforms tested in this study performed well, returning a high level of detection sensitivity and accuracy.
- Ease of use and rapid sample-to-answer platforms with sensitive detection make these systems highly valuable.