Evaluation and Comparison of Multiple Commercial Assays for the Detection of Influenza A, Influenza B and Respiratory Syncytial Virus (RSV)

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Introduction

Influenza A, B and RSV viruses are important causes of severe respiratory infections in children. Signs and symptoms of upper respiratory infections caused by these viruses are difficult to distinguish from infections caused by other respiratory pathogens. Early diagnosis of influenza and RSV infection can provide effective guidance for antiviral therapy in cases of influenza.

Goal of the study: To evaluate the analytic performance characteristics of the five commercial assays for the detection of influenza A/B and RSV. The five assays are: Nanosphere RV+, Focus ASR Direct and Extracted methods, Quidel and altona RVP assays.

Methods

- The evaluation study was performed on 40 respiratory specimens (20 positive and 20 negative) including Broncheoalveolar lavages (BAL), Nasopharyngeal (NP) swabs, NP aspirates, NP wash and endotracheal aspirates.
- Nanosphere RV+ and Focus ASR Direct method are direct sample to answer assays. For accuracy study, the samples were tested and compared to the reference method, Focus Simplexa™ Flu A/B & RSV Direct Assay.
- Focus ASR Extracted method, Quidel and altona RVP assays require RNA extraction prior to PCR setup. Total nucleic acids were extracted using the NucliSens™ easyMAG (bioMérieux). PCR were setup on each platform and the results were compared to the reference method, Focus Simplexa™ Flu A/B & RSV Assay.
- Precisions were performed with a positive and a negative patient sample pool. The samples were assayed in triplicate over three runs to test the intra- and inter-assay reproducibility. For real-time PCR, the mean, standard deviation (SD) and coefficient of variation (%CV) of the Cycle Threshold (Ct) for each target were calculated.
- Limit of detection (LOD) was conducted on the serial dilutions of positive Flu A/B/RSV control pool in 8 replicates (95% is the cutoff).
- Cross-reactivity was determined by testing against 10 other viruses.
- We also compared the overall turn-around-time (TAT), workflow, and ease of use for each platform.

Results

- The assay design and TAT for each platform were compared and listed on Table 1.
- The analytic performance characteristics of each platform were compared and the results were listed on Table 2.
- The sensitivity of each target were ranged from 95%-100% for the 5 platforms (Figure 1).
- The specificity was 100% on all 5 platforms.
- The analytical sensitivity (LOD) for each target were compared on Figure 2. In general, the extracted methods are more sensitive than direct methods.
- No cross-reactivity was detected on all platforms.

Acknowledgements

- We would like to thank Nanosphere Inc., Focus Diagnostics, Quidel Molecular and altona Diagnostics for providing the kits for the evaluation.
- We would like to thank the staff from Clinical Virology Laboratory and Microbiology Laboratory at Children's Hospital Los Angeles for their technical support.

Table 1. Assay Design Comparison

	Focus IVD Direct RVP	Nanosphere RV+	Focus ASR Direct RVP	Focus IVD Extracted RVP	Focus ASR Extracted RVP	Quidel RVP	altona RVP
Manufacturer	Focus Diagnostics	Nanosphere Inc	Focus Diagnostics	Focus Diagnostics	Focus Diagnostics	Quidel Molecular	altona Diagnostics
Reagent Name	Simplexa™ Flu A/B & RSV Direct	Verigene® Respiratory Virus Plus Nucleic Acid Test on the Verigene® System	Flu A/B & RSV	Simplexa™ Flu A/B & RSV	Flu A/B & RSV	Influenza A+B Assay & RSV + hMPV Assay	RealStar® Influenza S&T RT-PCR Kit 3.0 & RealStar® RSV RT-PCR Kit 1.0
Product Label	FDA-Cleared	FDA-Cleared	ASR	FDA-Cleared	ASR	FDA-Cleared	RUO
Main Targets	Flu A/B/RSV	Flu A/B/RSV	Flu A/B/RSV	Flu A/B/RSV	Flu A/B/RSV	Flu A/B/RSV/hMPV	Flu A/B/RSV
Subtype Differentiation	None	Flu A-H1/H3/2009 H1N1; RSV-A/B	None	None	None	Flu A/H1N1	RSV-A/B
Technology	Multiplex Real-Time PCR	Multiplex PCR/Hybridization	Multiplex Real-Time PCR	Multiplex Real-Time PCR	Multiplex Real-Time PCR	Multiplex Real-Time PCR	Multiplex Real-Time PCR
Instrument	3M Integrated Cycler	Nanosphere Processor/Reader	3M Integrated Cycler	3M Integrated Cycler	3M Integrated Cycler	ABI 7500	ABI 7500
Nucleic Acid Extraction	None	None	None	Yes	Yes	Yes	Yes
Max Sample Per Run	8	1	94 (96 including controls)	94 (96 including controls)	94 (96 including controls)	46 (48 including controls)	46 (48 including controls)
Assay Setup Time	~5 min	~5 min	~25 min	~15 min	~20 min	~20 min	~20 min
Instrument Time	1.1 hour	2.5 hour	1.1 hour	1.1 hour	1.1 hour	1.5 hour	1.5 hour
Total Turnaround Time	~1.4 hour	~2.8 hour	~1.8 hour	~2.5 hour	~ 2.6 hour	~3.0 hour	~3.0 hour
Comments	Reference Method	1 Sample per run	Time & temperature sensitive on sample treatment step	Reference Method	1 Multiplex reaction/sample	2 Multiplex reactions/sample	2 Multiplex reactions/sample

Table 2. Analytical Performance Comparison

	Focus IVD Direct RVP	Nanosphere RV+	Focus ASR Direct RVP	Focus IVD Extracted RVP	Focus ASR Extracted RVP	Quidel RVP	altona RVP
Sensitivity	90.0%-100.0%*	21/21 (100%)	20/21 (95.2%)	100.0%*	21/21 (100%)	20/21 (95.2%)	20/21 (95.2%)
Specificity	84.6%-99.9%*	20/20 (100%)	20/20 (100%)	96.0%-100.0%*	20/20 (100%)	20/20 (100%)	20/20 (100%)
Reproducibility/SD/%CV	99.2%-99.5%*	100%	100%/CV≤2.5%/SD≤±1.8	99.0%-100.0%*	100%/CV≤0.9%/SD≤±1.7	100%/CV≤1.7%/SD≤±1.7	100%/CV≤1.5%/SD≤±2.2
LOD (Flu A) $TCID_{50}$/mL	0.005-10.0*	0.1-10.0*	0.1	0.01-1.0*	0.02	0.02	0.02
LOD (Flu B) $TCID_{50}$/mL	2.0-20.0*	0.1-1.0*	0.5	5.0-10.0*	0.1	0.2	0.1
LOD (RSV) $TCID_{50}$/mL	1.0-3.0*	1.0-10.0*	5	1.0-5.0*	1	1	1
Cross-Reactivity	0/32 (0%)*	0/10 (0%)	0/10 (0%)	0/32 (0%)*	0/9(0%)	0/9 (0%)	0/9(0%)

* From manufacturer product insert.

Figure 1. Sensitivity Comparison

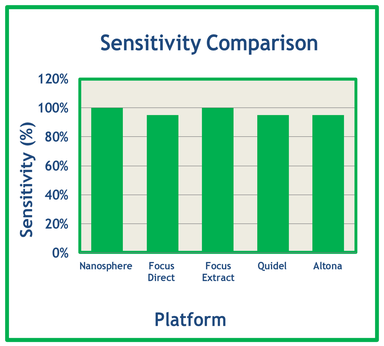
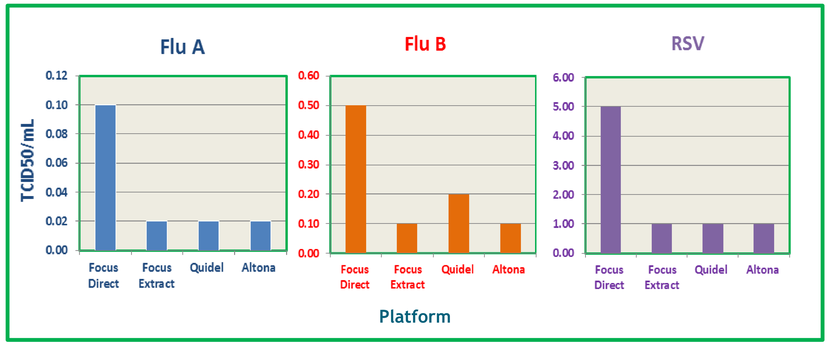


Figure 2. Analytical Sensitivity (Limit of Detection)



Conclusion

- All 5 assays are accurate and sensitive with TAT from 1.8 to 3.0 hours.
- Verigene RV+ and Focus ASR assays have system analysis function but Quidel and altona assays require operator's analysis for interpretation.
- Verigene RV+ and Focus direct panel are examples of sample-to-answer technologies that is conducive to "STAT" testing.
- Focus ASR extracted method, Quidel and altona assays require nucleic acid extraction. But they are high throughput, which makes them more suitable for batch testing to maximize the instrument capacity, minimize the control and reagent consumptions and reduce total TAT.
- The platform selector should consider multiple factors, including the laboratory operation conditions and the patient management requirements in addition to the assay analytical performance.