Address for Correspondence:
Molecular Diagnostic Laboratory, 2nd floor Research Tower
50 N Dunlap St. Memphis, TN 38103

EVALUATION OF SIMPLEXA™ FLU A/B & RSV DIRECT ASSAY FOR DETECTION OF INFLUENZA AND RESPIRATORY SYNCYTIAL VIRUS

Patel A, Infield J, Patel C, Smith C, Greene K, and Davis J
Molecular Diagnostics Laboratory, Le Bonheur Children's Hospital, Department of Pediatrics, University of Tennessee Health Science Center, and the Children's Foundation Research Center at Le Bonheur Children's Hospital, Memphis, TN

Revised Abstract

Background: Seasonal epidemics of Influenza (Flu) and respiratory syncytial virus (RSV) are responsible for significant morbidity and mortality worldwide. The ability to efficiently and accurately detect and differentiate respiratory viruses is paramount for effective treatment, infection control, and epidemiological surveillance. At present, nucleic acid amplification testing is preferred for rapid, sensitive, and accurate detection of viral respiratory infections. In this study we compared the performance characteristics of two FDA-cleared nucleic acid-based tests, Simplexa™ Flu A/B & RSV Direct assay (SIRD; Focus Diagnostics Inc., Cypress, CA), and Prodesse ProFlu+™ (Proflu+; Hologic, Bedford, MA) in detecting Flu A, B and RSV viruses from clinical nasopharyngeal (NP) swab specimens.
Methods: A total of 188 NP swab specimens (FLU A=90, FLU B=22, RSV=26, INVALID=50) were analyzed and compared by both SIRD and ProFlu+. Of the 188 NP swab specimens, 50 specimens were analyzed by SIRD assay which were invalid by ProFlu+ assay. The Simplexa™ Flu A/B & RSV Direct assay was performed from clinical specimens that have not undergone nucleic acid extraction on the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories. For Prodesse assay, the nucleic acid was extracted from 200 µl of specimen using the NucliSENS easyMAG (bioMerieux, Durham, NC) and the assay was performed on the SmartCycler (Cepheid, Sunnyvale, CA) according to the package insert.
Results: Out of 90 FLU A positive, 22 FLU B positive, 26 RSV positive by ProFlu+ Assay, the SIRD Assay detected 83 FLU A (5 negative, and 2 invalid), 21 FLU B (1 negative), and 26 RSV. In addition, of the 50 invalid results by ProFlu+ assay, the SIRD Assay gave 34 negative results and 16 invalid results. The observed average Ct with ProFlu+ assay for FLU A was 29.29 (20.7 – 37.9), FLU B = 25.54 (15.7 – 36.3), RSV = 24.13 (19.5 – 31.4) and with SIRD assay for FLU A = 32.51 (22.7 – 39.6), FLU B = 31.35 (23.2 – 36.3), RSV = 27.75 (21.3 – 35.1).
Conclusion: Typically most nucleic acid amplification testing requires extraction of nucleic acid from clinical specimens and has more sensitivity compared to amplification methods that require no extraction of nucleic acid from the primary clinical specimens. In this study we found good correlation between SIRD and ProFlu+ assay in detecting FLU and RSV viruses. The SIRD assay is very easy to set up and provides results rapidly since it integrates sample processing, PCR amplification, and target detection. This assay also can be useful in the laboratory's work flow during the busy respiratory seasons.

Background

Seasonal epidemics of Influenza (Flu) and respiratory syncytial virus (RSV) are responsible for significant morbidity and mortality worldwide. The ability to efficiently and accurately detect and differentiate respiratory viruses is paramount for effective treatment, infection control, and epidemiological surveillance. At present, nucleic acid amplification testing is preferred for rapid, sensitive, and accurate detection of viral respiratory infections. In this study we compared the performance characteristics of two FDA-cleared nucleic acid-based tests, Simplexa™ Flu A/B & RSV Direct assay (SIRD; Focus Diagnostics Inc., Cypress, CA), and Prodesse ProFlu+™ (ProFlu+; Hologic, Bedford, MA) in detecting Flu A, B and RSV viruses from clinical nasopharyngeal (NP) swab specimens.

Methods

A total of 188 NP swab specimens (FLU A=90, FLU B=22, RSV=26, INVALID=50) were analyzed and compared by both SIRD and ProFlu+. The SIRD assay was performed from clinical specimens that have not undergone nucleic acid extraction on the 3M Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories. For the Prodesse assay, the nucleic acid was extracted from 200 µl of specimen using the NucliSENS easyMAG (bioMerieux, Durham, NC) and the assay was performed on the SmartCycler (Cepheid, Sunnyvale, CA) according to the package insert.

Results

Accuracy and Sensitivity:

Out of 90 Influenza A positive, 22 Influenza B positive, 26 RSV positive swab samples detected by the Prodesse ProFlu+ Assay, the SIRD Assay detected 83 Influenza A (Fig. 1), 21 Influenza B (Fig. 2), and 26 RSV (Fig. 3) positive samples. In addition, of the 50 invalid results by ProFlu+ assay, the SIRD Assay gave 34 negative results and 16 invalid results. The observed average Ct with the ProFlu+ assay for FLU A was 29.29 (20.7 – 37.9), FLU B = 25.54 (15.7 – 36.3), RSV = 24.13 (19.5 – 31.4) and with SIRD assay for FLU A = 32.51 (22.7 – 39.6), FLU B = 31.35 (23.2 – 36.3), RSV = 27.75 (21.3 – 35.1). (Fig. 4)

Fig.1: Influenza A Accuracy

Flu A	Prodesse RT-PCR		
SIRD RT-PCR	Positive	Negative	Total
Positive	83	0	83
Negative	5	48	53
Total	88	48	136*

Sensitivity = 94.3% PPV = 100.0%
Specificity = 100.0% NPV = 90.6%

Fig.2: Influenza B Accuracy

Flu B	Prodesse RT-PCR		
SIRD RT-PCR	Positive	Negative	Total
Positive	21	0	21
Negative	1	116	117
Total	22	116	138

Sensitivity = 95.5% PPV = 100.0%
Specificity = 100.0% NPV = 99.1%

Fig.3: RSV Accuracy

RSV	Prodesse RT-PCR		
SIRD RT-PCR	Positive	Negative	Total
Positive	26	0	26
Negative	0	112	112
Total	26	112	138

Sensitivity = 100.0% PPV = 100.0%
Specificity = 100.0% NPV = 100.0%

Fig.4: Δ average ct between SIRD and Reference PCR

Average ct	Prodesse Assay	SIRD Assay
Flu A	29.9	32.51
Flu B	25.54	31.35
RSV	24.13	27.75

*2 swab samples detected positive for Influenza A by the Prodesse ProFlu+ Assay gave internal control failures using the SIRD Assay.

Reproducibility:

The SIRD Assay showed a high level of reproducibility using low and mid concentrations of Influenza A, B, and RSV for each run in triplicate over 3 days by 2 different operators. (Fig. 5)

Fig. 5: Average value of ct for reproducibility samples run in triplicate over 3 days using SIRD Assay

	Flu A Mid	Flu A Low	Flu B Mid	Flu B Low	RSV MID	RSV Low
Run 1	28.1	34.5	32.5	34.5	32.3	34.4
Run 2	27.9	34.7	32.2	35.4	30.3	33.9
Run 3	28.2	34.8	33.7	35.8*	30.1	33.7
Mean	28.0	34.7	32.8	35.0	30.9	34.0
SD	0.47	0.36	0.78	0.96	1.46	0.55
%CV	1.68	1.03	2.36	2.75	4.72	1.63

*One replicate was not detected for Influenza B by the Simplexa FluA/B& RSV Direct Assay on Run 3.

Stability:

Nasopharyngeal swab specimens were stored at varying temperatures (room temperature, 4°C, and -20°C) over the course of 5 days and tested using the SIRD Assay in triplicate by 2 different operators on Day 1 and Day 5. The SIRD Assay had a high precision for the detection of Influenza A/B and RSV at low and mid concentration levels over the 5 day period. (Fig. 6-8)

Fig. 6: Influenza A Stability Average ct

Flu A Low	Day 1	Day 5
RT	29.0	29.2
4°C	-	29.2
-20°C	-	29.3

Flu A Mid	Day 1	Day 5
RT	27.1	27.2
4°C	-	26.9
-20°C	-	27.4

Fig. 7: Influenza B Stability Average ct

Flu B Low	Day 1	Day 5
RT	35.9	35.2
4°C	-	35.9
-20°C	-	34.5

Flu B Low	Day 1	Day 5
RT	32.4	31.8
4°C	-	31.9
-20°C	-	30.7

Fig. 8: RSV Stability Average ct

RSV Low	Day 1	Day 5
RT	32.9	32.8
4°C	-	32.5
-20°C	-	32.9

RSV Mid	Day 1	Day 5
RT	29.0	29.2
4°C	-	29.1
-20°C	-	29.2

Conclusions

The SIRD Assay demonstrated a high level of reproducibility of Influenza A, Influenza B, and RSV at low and mid level viral concentrations both within and in-between runs on the Focus 3M integrated cycler with coefficient of variances ranging from 1.03% to 4.72% for each target.

The SIRD Assay performed well compared against the reference PCR method for Influenza A/B and RSV detection showing a sensitivity rate above 94.3% and NPV above 90.0% for each viral target.

The SIRD Assay demonstrated a high level of reproducibility for each viral target over a 5 day time period stored at varying temperatures of room temperature, 4°C, and -20°C.

The SIRD Assay demonstrated a lower average ct detection level when compared to the reference PCR method. The SIRD Assay for Influenza A showed ct detection levels 3 cycles later, Influenza B showed ct detection levels 6 cycles later, and RSV showed ct detection levels 3.5 cycles later. Despite the SIRD Assay demonstrating weaker ct detection levels for the three targets, there was no significant increase in the number of false negatives when compared to the reference PCR method.

Acknowledgements

We wish to thank Focus Diagnostics for their support and reagents.