Performance of a molecular diagnostic, MultiCode based, sample-to-answer assay for the simultaneous detection of Influenza A, B and Respiratory Syncytial Viruses

J.J.C Voermans, S. Deniz, M.P.G. Koopmans, A.A. van der Eijk, S.D Pas
Department of Viroscience, Erasmus MC, Rotterdam

Introduction

Rapid diagnostics is required in cases with respiratory failure for clinical decision making regarding isolation and antiviral therapy.[1,2]Techniques like immune-chromatographic test (ICT) and direct immunofluorescence assay (DFA) have lower sensitivities and specificities than molecular diagnostic assays, but have the advantage of quick turnaround times and ease-of-use. Here, we evaluated the performance of an automated, easy to use, sample-to-answer system, which preforms an Influenza A/B virus (fluA/fluB), respiratory syncytial virus (RSV) and sample processing control (SPC) multiplex RT-PCR of 1-12 samples within 2 hours.

Methods

The performance of the fluA/fluB/RSV assay on the ARIES (Luminex), a system using MultiCode technology (a probe-free real-time RT-PCR method with melting curve confirmation), was evaluated using published laboratory developed automated real-time RT-PCR assays (LDA) for fluA, fluB, RSV-A and RSV-B [3,4].

Analytical performance of the FluA/FluB/RSV assay (ARIES, Luminex):
-**Genotype inclusivity** :16 avian (H1-H16) and 33 human fluA strains, 3 fluB strains and the two RSV (A/B) strains.
-**Analytical specificity**: 40 high positive non-fluA/fluB/RSV-viruses
-**Analytical sensitivity**: 0.5 log dilution series of A/H1N1p2009 B/Yamagata, RSVA and RSVB compared to LDA assays.
-**Linearity**: 0.5 log dilution series of A/H1N1p2009 B/Yamagata, RSVA and RSVB compared to LDA assays.
-**Repeatability**: 35 replicates of a control positive for fuA, fluB and RSV in different runs.

Clinical performance: compared to both LDA + ICT (BinaxNOW influenza A/B and RSV test) + DFA using selected (pretreated), -80°C stored, respiratory tract samples from 2006 until 2015 (retrospective) and prospective testing of original respiratory tract samples from December 2015 onwards.

Results

Genotype inclusivity

*All fluA, fluB and RSVA/B strains tested for analytical performance evaluation were detected. External lysis with MPLC lysisbuffer (Roche) of avian and highly pathogenic fluA strains yielded correct results.
*No aspecific reactions with non fluA/fluB/RSV high positive controls were identified.

Analytical sensitivity

ARIES fluA/fluB/RSV assay was less sensitive for fluA (0.5 log), RSV-A (1 log), RSV-B (2 log) and for FluB (2.5 log) compared to LDA

Repeatability

Replicates of a positive process control (PPC, n=35, figure 1)

	fluA		RSV		SPC	fluB	
	Ct	Tm ©	Ct	Tm ©	Tm ©	Ct	Tm ©
Average	34.1	84.0	33.6	75.8	78.3	36.6	79.8
STDEV	0.9	1.7	1.1	0.1	0.3	1.1	0.1
%CV	2.6	2.0	3.2	0.2	0.4	3.0	0.1

Figure 1: Results repeatability

Concentration of fluB in PPC, was close to the ARIES limit of detection, and tested positive 32 out of 35 (91.4%). T- and F-tests gave no significant difference (>0.05).

Linearity

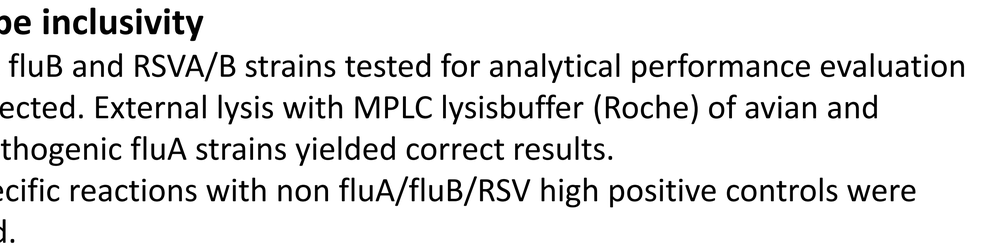

Figure 2: Results linearity

Robustness

1.8% of the cassettes failed during operation (pre-RUO and RUO).
1.1% of the cassettes failed during operation (RUO only).

Clinical performance

In total, 227 samples were included in the clinical performance evaluation.
Sample type distribution (figure 3):

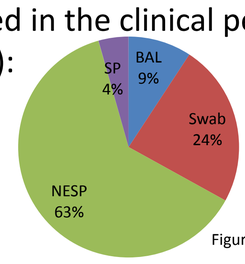

Figure 3: sample type distribution

17.9% tested positive for fluA, 14.2% for fluB and 42.7% for RSV, (RSV-A , 24.5% and RSV-B 20.4%) in both LDA and ARIES.

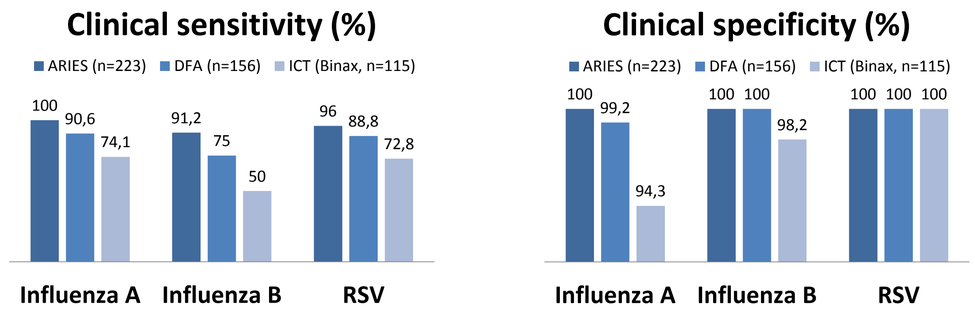

Figure 4: Clinical sensitivity and specificity

*Confirmed discrepant results were found in 7 samples (3 fluB and 4 RSV-A), which tested positive in LDA and negative in ARIES (3.1%, LDA Ct values 27.9 - 33.9).

*If compared to the DFA (n=156) and ICT (n=116), ARIES detected 17 (10.6%; 3 fluA, 5 fluB, 9 RSV) and 32 (28.1%; 7 fluA, 3 fluB, 22 RSV) more samples respectively, all confirmed by LDA (Ct range 16.5-31.6).

*ARIES has a higher clinical sensitivity and specificity compared to DFA and ICT (figure 4)

Conclusion

1. The ARIES influenza A/B/RSV assay is a specific and rapid molecular assay
2. Although analytically the ARIES is less sensitive for fluB and RSV-A and RSV-B than the LDA assays, the performance in clinical samples is comparable to LDA and better than those of the established rapid assays.

References:
1 Blaschke AJ et al, *Journal of the pediatric infectious diseases society, Vol3, No.2, (2014)112-118*
2 Dunn et al. Diagnostic microbiology and infectious disease 79 (2014) 10-13
3 Hoek RA et al. *Scand J Infect Dis. 45 (2013) 65-69*
4 Dewhurst-Maridor G et al. *Journal of Virological Methods 120 (2004) 41–49*

COI: The cassettes for this study were provided by Luminex Corp.

This study was approved by the local Medical ethical committee under MEC-2015-475

E-mail: j.voermans@erasmusmc.nl s.pas@erasmusmc.nl

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