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

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Introduction

Rapid diagnostics is required in cases with respiratory failure for clinical decision making regarding isolation and antiviral therapy. 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 rapid molecular assay for clinical decision making regarding isolation and antiviral treatment of 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.

- Analytical performance of the FluA/FluB/RSV assay (ARIES, Luminex):
  - Genotype inclusivity: 16 avian (H1N1, H1N2) 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, RSV-A and RSVB compared to LDA assays.
  - Linearity: 0.5 log dilution series of A/H1N1p2009 B/Yamagata, RSV-A and RSVB compared to LDA assays.
  - Repeatability: 35 replicates of a control positive for fluA, 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 RSV-A/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)

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

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

Robustness

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.

Conclusion

References:
2 Dunn et al. Diagnostic microbiology and infectious disease 79 (2014) 10-13

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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

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