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Timely diagnosis of respiratory tract infections: Evaluation of the performance of the Respifinder assay compared to the xTAG Respiratory Viral Panel assay

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ABSTRACT

Background: Respiratory tract infections are the most common cause of hospitalization in infants and young children and are typically caused by viral or, less commonly, bacterial pathogens.

Existing non-molecular diagnostic methods have several drawbacks such as limited sensitivity, long turn-a-round time and limited number of pathogens that can be detected.

Objectives: Nucleic acid amplification methods can increase sensitivity and enable the initiation of appropriate interventions without delay.

Broad-spectrum detection and identification circumvent the use of individual diagnostic DNA or RNA based assays. At present, several commercial assays are available for broad-spectrum detection.

Study design: We compared the performance of the xTAG Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics, Toronto, Canada) with that of the Respifinder (Pathofinder, Maastricht, Netherlands) for 9 external quality assurance (EQA) panels (QCMD, Scotland) consisting of a total of 106 EQA samples.

Results: Both the RVP and the Respifinder assay have an excellent specificity. Sensitivity was 33% and 78% for the RVP and the Respifinder assay, respectively. For both assays, sensitivity was low for weak positive samples.

Discussion: The results of our study seem to indicate a better sensitivity for the Respifinder. Analysis of patient samples is necessary to evaluate the clinical performance.

1. Background

Lower respiratory tract infections (LRTI) are a common cause of hospitalization and are caused by various bacterial or viral pathogens.[1] To date, more than 20 viruses have been shown to cause respiratory tract infections and every year new viruses are associated with LRTI.[2–7] Studies suggest that an early and accurate identification of a viral etiologic agent during emergency department evaluation or in hospitalized patients has been associated with a decrease in antibiotic use, a decreased number of additional tests performed, a decreased length of stay in the emergency department and a decreased length of hospital stay.[8–11]

Clinical diagnosis of a respiratory tract infection is difficult in patients with atypical illness characteristics and in low prevalence setting. Identification of the etiological agent can be useful if there is a need for a specific therapeutic approach, if hospital hygiene measures are necessary and for epidemiological reasons.[12]

Laboratory techniques such as antigen immunoassays, viral culture and serology have several shortcomings such as a limited sensitivity, long turnaround times and limited spectrum of detectable pathogens.[12,13] A combination of methods is often necessary to improve diagnosis. The use of nucleic acid amplification tests (NAAT) such as the polymerase chain reaction (PCR) enables same day detection of causal pathogens and therefore the prompt initiation of appropriate interventions. Multi parameter NAAT allow broad spectrum detection of pathogens, thereby saving time and work.[14] In addition, a study by Brunstein and colleagues reports preliminary clinical evidence that the diagnosis of co-infections is medically relevant and that an effective treatment for severe RTI requires the detection of all involved pathogens.[15]

2. Objectives

This study compares the performance of the xTAG Respiratory Viral Panel (RVP) assay (Luminex Molecular Diagnostics, Toronto, Canada) with the Respifinder assay (Respifinder) (Pathofinder, Maastricht, Netherlands) for the detection of viral respiratory pathogens.

The RVP assay comprises a multiplex reverse transcription polymerase chain reaction (RT-PCR), followed by a multiplex Target-Specific Primer Extension. The target-specific primers are

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chimeric primers juxtaposed to a Universal Tag sequence. This allows sorting on a Luminex xMAP instrument.[16]

The Respifinder assay is based on the multiplex ligation-dependent probe amplification (MLPA) technology. The MLPA reaction is preceded by a pre-amplification step to ensure the detection of DNA and RNA viruses with the same specificity and sensitivity as individual singleplex real-time RT-PCR.[17]

Both assays can detect and differentiate following respiratory viruses: influenza A (InfA) and B (InfB), respiratory syncytial virus A (RSVA) and B (RSVB), parainfluenza 1–4 (PIV-1 to PIV-4), coronavirus NL63 (CoV-NL63), coronavirus OC43 (CoV-OC43), coronavirus 229E (CoV-229E), human metapneumovirus (hMPV) and adenovirus (adeno). Additionally, in each assay a probe for InfA H5N1 is included. The RVP assay can also subtype for InfA H1 and InfA H3. The Respifinder assay can detect rhinovirus (rhino), which is also detected by the RVP, but this assay cannot differentiate rhinovirus from enterovirus (rhino-entero). Moreover, the detection of coronavirus HKU1 (CoV-HKU1) and coronavirus SARS (SARS-CoV) is also included in the RVP assay.

3. Study design

A total of 9 external quality assurance (EQA) panels (QCMD, Scotland) containing 106 EQA samples, of which 95 samples were expected to be positive for influenza, respiratory syncytial virus, parainfluenza, coronavirus, rhinovirus, human metapneumovirus and adenovirus were analyzed once. [MV.RS 2007/2008, ADV 2007, PINF 2006/2008, RV.CV 2007/2008, INF 2006/2008].

Extraction of nucleic acids (NA) was performed with easyMag (bioMérieux, Lyon, France), using the generic 2.0.1 protocol. An extraction and amplification control was included for every sample according to the manufacturer's instructions. Sample volume for extraction was 200 μl, elution volume was 50 μl for RVP and 100 μl for Respifinder.

All panels were analyzed with the RVP and Respifinder assay according to the manufacturer's instructions. Respectively 5 μl for RVP and 10 μl for Respifinder of NA were added to the PCR mixture resulting in an end concentration of 2% of the total sample concentration for both assays. Amplification, hybridization and ligation were performed on a PTC200 (Bio-Rad Laboratories, Hercules, CA, US). For RVP, data acquisition was performed on the LX200 (Luminex Molecular Diagnostics), using the TDAS RVP-1 software. For Respifinder, fragment analysis was performed on the CEQ 8000 genetic analysis system (Beckman Coulter, Brea, CA, US), followed by analysis with the Fragment Analysis software.

4. Results

For all negative samples, a negative result was obtained with both assays.

A positive result was found with the Respifinder assay in 74 (78%) of 95 positive samples. Most of the false negative samples (15 of 21) were weak positive, except for 1 adenovirus type 31 sample. For 1 weak positive sample an inconclusive result (extraction and amplification control negative) was obtained. Weak positive samples were samples with a stock dilution of 10^{-6}, *Ct*-value ≥35 by independent testing or a concentration ≤100 copies/ml. For 4 samples, a negative or an inconclusive result was obtained. These samples had an expected *Ct*-value of 33–34. Additionally, an adenovirus was detected in 3 RSV positive samples, which was confirmed with an adenovirus specific real-time PCR.[18] In 1 InfA H1 sample, the H5N1 variant was also detected.

The RVP was positive in 31 (33%) samples. All adenovirus, CoV-NL63, CoV-OC43, and CoV-229E samples were false negative. Furthermore, for PIV-1, 7 of 8 samples were false negative and 1 of 8 samples resulted in an inconclusive result (signal for viral target within the equivocal zone as mentioned in the kit insert). For InfA, 8 of 14 samples were false negative and 5 of 14 samples showed an inconclusive result. Almost all weak positive samples were false negative (25 of 30) or inconclusive (1 of 30).

The sensitivity for weak positive samples was 47% for Respifinder and 13% for RVP. Results are shown in Table 1 (Supplementary data in Table 2).

Table 1
Pathogen results after analysis of EQA samples with Respifinder and RVP.

Pathogen	Sensitivity (%)		Inconclusive[b] (%)	
	Respifinder	RVP	Respifinder	RVP
hMPV	70	30	0	0
RSVA	100	43	0	29
RSVB	100	60	0	0
Adeno type 1	100	0	0	0
Adeno type 3	100	0	0	0
Adeno type 4	100	0	0	0
Adeno type 31	0	0	0	0
PIV-1	63	0	25	13
PIV-2	100	100	0	0
PIV-3	100	100	0	0
PIV-4	83	83	0	0
Rhino 16	80	40	0	0
Rhino 72	75	100	0	0
Rhino 90	80	60	0	0
CoV-NL63	80	0	0	0
CoV-OC43	100	0	0	0
CoV-229E	50	0	0	0
InfA H1	43	14	0	29
InfA H3	71	0	14	60
InfB	83	50	0	0
Weak positive samples[a]	47	13	3	3
All	78	33	3	8

[a] Samples with an expected result: dilution of 10^{-6}, *Ct*-value ≥35 or concentration ≤100 copies/ml.
[b] Inconclusive result: for RVP: signal for viral target within the equivocal zone as mentioned in the kit insert, for Respifinder: extraction and amplification control negative.

5. Discussion

Both assays have an excellent specificity which was demonstrated by the concordant results for all negative samples.

RVP was positive in no more than 31 of 95 samples, which results in an overall sensitivity of 33%. The limited sensitivity can be explained by a limited sensitivity for adenovirus, CoV-NL63, CoV-OC43, CoV-229E, PIV-1, InfA and for weak positive samples. A positive result was found with the Respifinder assay in 74 of 95 samples, which is equivalent with an overall sensitivity of 78%. In general, the false negative results can be explained by the limited sensitivity for weak positive samples. The false positive result for InfA H5N1 could probably be explained by cross-hybridization of a variant of InfA H1N1 with the H5N1 probe. According to the kit insert, the diagnosis of a H5N1 infection may not be based on a positive finding with the Respifinder assay alone.

For weak positive samples, sensitivity was low for both assays. It should be noticed that less than 60% of the participants in the EQA round reported a positive result in 14 of 30 weak positive samples. Furthermore, the clinical relevance of these weak positive samples remains unclear. A recent study, published by Utokaparch et al.[19] suggests that the total viral load for children with LRTI is significantly increased compared to children with non-LRTI.

This study was performed on quality assurance samples only and the obtained results have to be confirmed with analysis of clinical samples. However, these analytical evaluation results can give an indication on the technical performance of both assays.[20]

A study on the clinical performance of RVP by Mahony et al.[21] seems to indicate that the RVP has a higher sensitivity (98.5%) compared to direct fluorescent-antibody assay (DFA) and culture (68.8%). The sensitivity of the Respifinder compared to cell culture was 100%, except for PIV-3 (80%).[17]

Data on the performance characteristics of other commercial multiplex assays show that these assays are more sensitive than viral culture.[22,23]

Only few studies compared the performance of commercially available multiplex assays. A study by Balada-Llasat et al.,[23] evaluating the performance of 3 commercial assays for the diagnosis of respiratory viral infections in adults showed that, when compared to culture, the RVP had a sensitivity and specificity of 100% and 91%, compared to MultiCode-PLx (EraGen Biosciences, Madison, WI, US) with 89% and 87% and Resplex II (Qiagen, Hilden, Germany) with 89% and 94%, respectively. However, the Resplex II offered the broadest virus detection range and the MultiCode-PLx offered the greater ease of use. Another study[22] seems to indicate that the Respifinder has a better sensitivity than the Seeplex RV12 detection kit (Seegen, Rockville, MD, US).

To conclude, multi parameter assays can be a useful tool for broad spectrum detection of respiratory pathogens, although time-to result could be improved. Negative results should be interpreted with care because of the limited sensitivity for some pathogens.

Conflict of interest statement

Funding: None. Competing interests: None declared. Ethical approval: Not required.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2011.08.017.

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