

Timely diagnosis of respiratory tract infections: evaluation of the performance of the Respifinder assay compared to the RVP assay.

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INTRODUCTION

Respiratory tract infections are the most common infectious diseases of humans worldwide, causing morbidity and mortality. Lower respiratory tract infections (LRTI) are a common cause of hospitalization. They may be caused by viral or bacterial pathogens (Fig. 1). Prolonged duration of hospitalization and over-prescription of antibiotics represent a high economic cost. Studies suggest that timely diagnosis of acute respiratory infections can lead to a significant decrease of antibiotic use or duration of antibiotic therapy, number of additional diagnostic tests performed, length of stay in the emergency department and duration of hospital stay¹. Diagnostic methods such as culture and serology are time-consuming and have several other drawbacks such as limited sensitivity, long turn-around time and a limited spectrum of detectable pathogens² (Table 1). Nucleic acid amplification methods can increase sensitivity and enable the initiation of appropriate interventions without delay. Novel, multiparameter PCR tests circumvent the use of individual diagnostic DNA or RNA based assays. We compared the performance of the xTAG Respiratory Viral Panel assay (RVP) (Luminex Molecular Diagnostics) with the Respifinder assay (Respifinder) (Pathofinder) for the detection of viral respiratory pathogens (Table 2).

Test method	Turn-around time	Advantages	Disadvantages
Non-IF antigen detection	15 - 30 min	- Rapid result - Ease of use - Detect non-viable virus - Good specificity for RSV and influenza (in season)	- Generally less sensitive than cell culture - Limited to RSV, influenza A and B - supplemental testing recommended if negative
IF antigen detection	30 - 90 min	- Rapid result - Better sensitivity than cell culture for RSV - Detect non-viable virus	- Generally less sensitive than cell culture, especially for adenovirus - Expertise required for reading
Conventional cell culture	3 - 10 days	- Broad range of detection - Increased sensitivity over rapid antigen methods - viral isolate available	- Limited to 8 viruses - Long time to detect some viruses - Less sensitive for RSVs as compared to antigen methods - Expertise required for reading CPE - Significant technical time
Rapid cell culture	24 - 72 h	- Shorter time to detection as compared to conventional culture - Detect viruses that replicate poorly in cell culture - Requires less expertise than reading CPE	- Not always as sensitive for detection of RSV, Adenovirus - Technical time to stain and read - Detection limited to viruses tested by pre-CPE staining - Isolates not always available

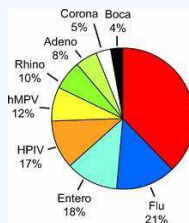


Fig. 1 Laboratory-confirmed common viruses causing serious respiratory infections requiring hospitalization or emergency room visitation in children/adults younger than 19 years of age³

RVP	Respifinder
INF A	INF A
INF A H1	INF A
INF A H5	INF A H5N1
INF B	INF B
RSV A	RSV A
RSV B	RSV B
PEV 1	PEV 1
PEV 2	PEV 2
PEV 3	PEV 3
PEV 4	PEV 4
Coronavirus NL63	Coronavirus NL63
Coronavirus OC43	Coronavirus OC43
Coronavirus HKU1	Coronavirus HKU1
Coronavirus 229E	Coronavirus 229E
Coronavirus SARS	Coronavirus SARS
HMPV	HMPV
Enterovirus	Rhinovirus
Adenovirus	Adenovirus

Table 2: Respiratory pathogens included in RVP and Respifinder^{4, 5}

Table 1: Comparison of non-molecular test methods for respiratory virus detection²

MATERIALS AND METHODS

A total of 106 EQC samples of 9 QCMD Quality Control panels (Scotland) were analysed, of which 95 samples were expected to be positive. [MV.RS 2007/2008, ADV 2007, PINF 2006/2008, RV.CV 2007/2008, INF 2006/2008]. Extraction was performed with easyMag (Biomérieux) using the generic 2.0.1 protocol. The panels were analysed with the RVP and Respifinder assays according to the manufacturer's instructions (Fig. 2, 3). Amplification, hybridization and ligation were performed on a PTC 200 (Bio-Rad). Data acquisition was performed with LX200 (Luminex), using the TDAS RVP-1 software, for RVP or with CEQ 8000 (Beckman), using the Fragment Analysis software, for Respifinder.

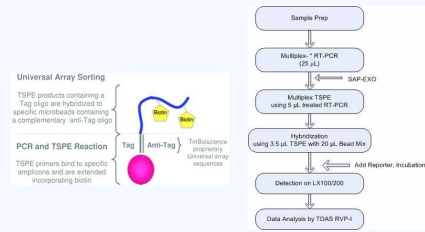


Fig. 2 Summary of xTAG RVP technique⁴

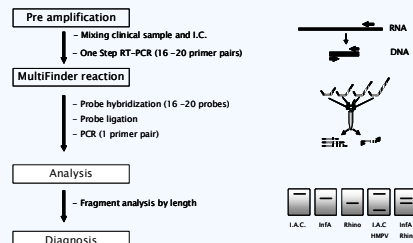
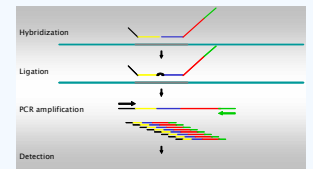


Fig. 3 Summary of Respifinder technique⁵



RESULTS

QCMD panel	Pathogen	Sample	Respifinder	RVP
MV.RS07 (stock dil)	HMPV-12	RS.MVRS-08.10 ¹¹	neg	neg
		RS.MVRS-08.10 ¹²	pos	pos
		RS.MVRS-08.10 ¹³	pos	pos
RSV-A	RS.MVRS-08.10 ¹⁴	RS.MVRS-08.10 ¹⁴	neg	neg
		RS.MVRS-08.10 ¹⁵	pos (weakly weak)	pos (weakly weak)
		RS.MVRS-08.10 ¹⁶	pos (weakly weak)	pos (weakly weak)
RSV-B	RS.MVRS-08.10 ¹⁷	RS.MVRS-08.10 ¹⁷	pos	pos
		RS.MVRS-08.10 ¹⁸	pos	pos
		RS.MVRS-08.10 ¹⁹	pos	pos
H5N1	RS.MVRS-08.10 ²⁰	RS.MVRS-08.10 ²⁰	pos	pos
		RS.MVRS-08.10 ²¹	pos	pos
		RS.MVRS-08.10 ²²	pos	pos
MV.RS.08 (stock dil)	HMPV-12	RS.MVRS-08.10 ²³	neg	neg
		RS.MVRS-08.10 ²⁴	pos	pos
		RS.MVRS-08.10 ²⁵	pos	pos
RSV-A	RS.MVRS-08.10 ²⁶	RS.MVRS-08.10 ²⁶	pos (strong pos)	pos (strong pos)
		RS.MVRS-08.10 ²⁷	pos (strong pos)	pos (strong pos)
		RS.MVRS-08.10 ²⁸	pos (strong pos)	pos (strong pos)
RSV-B	RS.MVRS-08.10 ²⁹	RS.MVRS-08.10 ²⁹	pos	pos
		RS.MVRS-08.10 ³⁰	pos	pos
		RS.MVRS-08.10 ³¹	pos	pos
H5N1	RS.MVRS-08.10 ³²	RS.MVRS-08.10 ³²	pos	pos
		RS.MVRS-08.10 ³³	pos	pos
		RS.MVRS-08.10 ³⁴	pos	pos

Table 3: Results for Respiratory Syncytial Virus and Metapneumovirus

QCMD panel	Pathogen	Sample	Respifinder	RVP
RV.CV07 (Ct value)	Rhinovirus 16	RV.CV07-09.38-40	neg	neg
		RV.CV07-04.33-34	pos	pos
		RV.CV07-08.29-30	pos	pos
Rhinovirus 72	RV.CV07-02.33-34	RV.CV07-02.33-34	pos	pos
		RV.CV07-14.29-30	pos	pos
		RV.CV07-12.29-30	pos	pos
Rhinovirus 90	RV.CV07-02.37-38	RV.CV07-02.37-38	pos	pos
		RV.CV07-14.33-34	pos	pos
		RV.CV07-05.30-32	pos	pos
Coronavirus NL63	RV.CV07-02.37-38	RV.CV07-02.37-38	pos	pos
		RV.CV07-02.37-38	pos	pos
		RV.CV07-02.37-38	pos	pos
Coronavirus 229E	RV.CV07-06.34-36	RV.CV07-06.34-36	pos	pos
		RV.CV07-06.34-36	pos	pos
		RV.CV07-06.34-36	pos	pos
RV.CV08 (stock dil)	Rhinovirus 16	RV.CV08-02.10 ¹¹	pos	pos
		RV.CV08-09.10 ¹¹	pos	pos
		RV.CV08-09.10 ¹¹	pos	pos
Rhinovirus 72	RV.CV08-11.10 ¹¹	RV.CV08-11.10 ¹¹	pos	pos
		RV.CV08-06.10 ¹¹	pos	pos
		RV.CV08-06.10 ¹¹	pos	pos
Rhinovirus 90	RV.CV08-10.10 ¹¹	RV.CV08-10.10 ¹¹	pos	pos
		RV.CV08-04.10 ¹¹	pos	pos
		RV.CV08-12.10 ¹¹	pos	pos
Coronavirus NL63	RV.CV08-07.10 ¹¹	RV.CV08-07.10 ¹¹	pos	pos
		RV.CV08-07.10 ¹¹	pos	pos
		RV.CV08-07.10 ¹¹	pos	pos
Coronavirus OC43	RV.CV08-03.10 ¹¹	RV.CV08-03.10 ¹¹	pos	pos
		RV.CV08-03.10 ¹¹	pos	pos
		RV.CV08-03.10 ¹¹	pos	pos
Neg	RV.CV08-08.10 ¹¹	RV.CV08-08.10 ¹¹	neg	neg
		RV.CV08-08.10 ¹¹	neg	neg
		RV.CV08-08.10 ¹¹	neg	neg

Table 4: Results for Rhinovirus and Coronavirus

QCMD panel	Pathogen	Sample	Respifinder	RVP
INF06 (stock dil)	Influenza A H1	INF06-10.10 ¹¹	neg	neg
		INF06-02.10 ¹¹	neg	neg
		INF06-07.10 ¹¹	pos	pos
Influenza A H3	INF06-08.10 ¹¹	INF06-08.10 ¹¹	pos	pos
		INF06-12.10 ¹¹	pos	pos
		INF06-04.10 ¹¹	pos	pos
Influenza B	INF06-09.10 ¹¹	INF06-09.10 ¹¹	pos	pos
		INF06-11.10 ¹¹	pos	pos
		INF06-03.10 ¹¹	pos	pos
neg	INF06-05.10 ¹¹	INF06-05.10 ¹¹	neg	neg
		INF06-05.10 ¹¹	neg	neg
		INF06-05.10 ¹¹	neg	neg
INF08 (Ct value)	Influenza A H1	INF08-10.36	neg	neg
		INF08-03.33	neg	neg
		INF08-07.33	pos	pos
Influenza A H3	INF08-05.34	INF08-05.34	pos	pos
		INF08-12.30	pos	pos
		INF08-09.34	pos	pos
Influenza B	INF08-09.34	INF08-09.34	pos	pos
		INF08-02.34	pos	pos
		INF08-11.33	pos	pos
neg	INF08-04.34	INF08-04.34	neg	neg
		INF08-04.34	neg	neg
		INF08-04.34	neg	neg

Table 5: Results for Influenza virus

QCMD panel	Pathogen	Sample	Respifinder	RVP
PINF06 (Ct value)	P influenza type 1	PINF06-07.34-35	pos	neg
		PINF06-04.31-33	pos	neg
		PINF06-05.31-33	pos	neg
P influenza type 2	PINF06-05.28-29	PINF06-05.28-29	pos	inconclusive
		PINF06-05.28-29	pos	pos
		PINF06-05.28-29	pos	pos
P influenza type 3	PINF06-06.30	PINF06-06.30	pos	pos
		PINF06-06.30	pos	pos
		PINF06-06.30	pos	pos
P influenza type 4	PINF06-06.34-35	PINF06-06.34-35	pos	pos
		PINF06-09.31-33	pos	pos
		PINF06-10.28-29	pos	pos
Neg	PINF06-03.31	PINF06-03.31	neg	neg
		PINF06-03.31	neg	neg
		PINF06-03.31	neg	neg
PINF08 (Ct value)	P influenza type 1	PINF08-07.34	pos	neg
		PINF08-07.34	pos	neg
		PINF08-07.34	pos	neg
P influenza type 2	PINF08-08.33	PINF08-08.33	pos	pos
		PINF08-08.33	pos	pos
		PINF08-08.33	pos	pos
P influenza type 3	PINF08-09.31	PINF08-09.31	pos	pos
		PINF08-09.31	pos	pos
		PINF08-09.31	pos	pos
P influenza type 4	PINF08-10.35	PINF08-10.35	pos	pos
		PINF08-10.35	pos	pos
		PINF08-10.35	pos	pos
Neg	PINF08-08.31	PINF08-08.31	neg	neg
		PINF08-08.31	neg	neg
		PINF08-08.31	neg	neg

Table 6: Results for Parainfluenza

QCMD panel	Pathogen	Sample	Respifinder	RVP
ADV07 (copies/ml)	Adeno type 1	ADV07-11.100	pos	neg
		ADV07-04.10000	pos	neg
		ADV07-08.100000	pos	neg
Adeno type 3	ADV07-07.500	ADV07-07.500	pos	neg
		ADV07-01.5000	pos	neg
		ADV07-03.50000	pos	neg
Adeno type 4	ADV07-12.250	ADV07-12.250	pos	neg
		ADV07-09.2500	pos	neg
		ADV07-02.25000	pos	neg
Adeno type 31	ADV07-05.1000	ADV07-05.1000	neg	neg
		ADV07-06.2000	neg	neg
		ADV07-10	neg	neg

Table 7: Results for Adenovirus

DISCUSSION

RVP was positive in only 31/95 samples. For 8 samples an inconclusive result was obtained. Additionally, all Adenovirus, Coronavirus NL63, Coronavirus OC43 and Coronavirus 229E samples were false negative with RVP. A positive result was found with the Respifinder assay in 75/95 samples. All false negative samples were weak positive, except for the 2 adenovirus type 31 samples. For 3 weak positive samples an inconclusive result was obtained. In 3 RSV positive samples, an adenovirus was also detected, which was confirmed with an adenovirus specific real-time PCR. Another RSV positive sample showed the presence of Bordetella pertussis. This needs to be confirmed. In one Influenza A H1 sample the H5N1 variant was also detected. This can be explained by cross-hybridization of a new variant of Influenza H1N1 with the H5N1 probe. The H5N1 probe will be removed from the Respifinder panel by Pathofinder.

CONCLUSION

Multiparameter PCR tests for the detection of respiratory pathogens such as RVP assay and Respifinder assay can contribute greatly to a fast and reliable diagnosis of LRTIs. Both the RVP and Respifinder assay have an excellent specificity. All Adenovirus, Coronavirus NL63, Coronavirus OC43 and Coronavirus 229E samples were false negative with RVP. Sensitivity was 32.6 % and 79 % for the RVP assay and Respifinder assay respectively. For weak positive samples, sensitivity was low for both assays. The clinical relevance of these weak positive is unclear. This study was performed on QC samples. Results have to be confirmed by analysis of clinical samples.

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