

Tm Bioscience ID-Tag RVP allows for detection of up to 19 viral respiratory pathogens in a single specimen

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Introduction

Over 80% of all respiratory tract infections are caused by viral pathogens. The clinical presentation of patients affected by different viral pathogens can be similar, making diagnosis difficult. Moreover, many viruses are not detected by routine methods.

Antigen-based assays, although rapid and inexpensive, lack sensitivity. Viral culture is more sensitive than rapid methods, but the major drawback is the length of time required for the organism to grow in appropriate cell lines. Nucleic acid amplification does not require viral growth and is more sensitive than rapid assays and culture. However, there are only a few commercially available assays that can detect viral pathogens in a multiplex format.

This study describes the evaluation of a new multiplex platform, ID-Tag RVP (Tm Bioscience Corp, Toronto, Ontario), for the detection of respiratory viruses present in our pediatric population. This qualitative assay simultaneously detects 19 common respiratory viruses in a single specimen and offers the potential to significantly improve the sensitivity and turnaround time of respiratory viral detection.

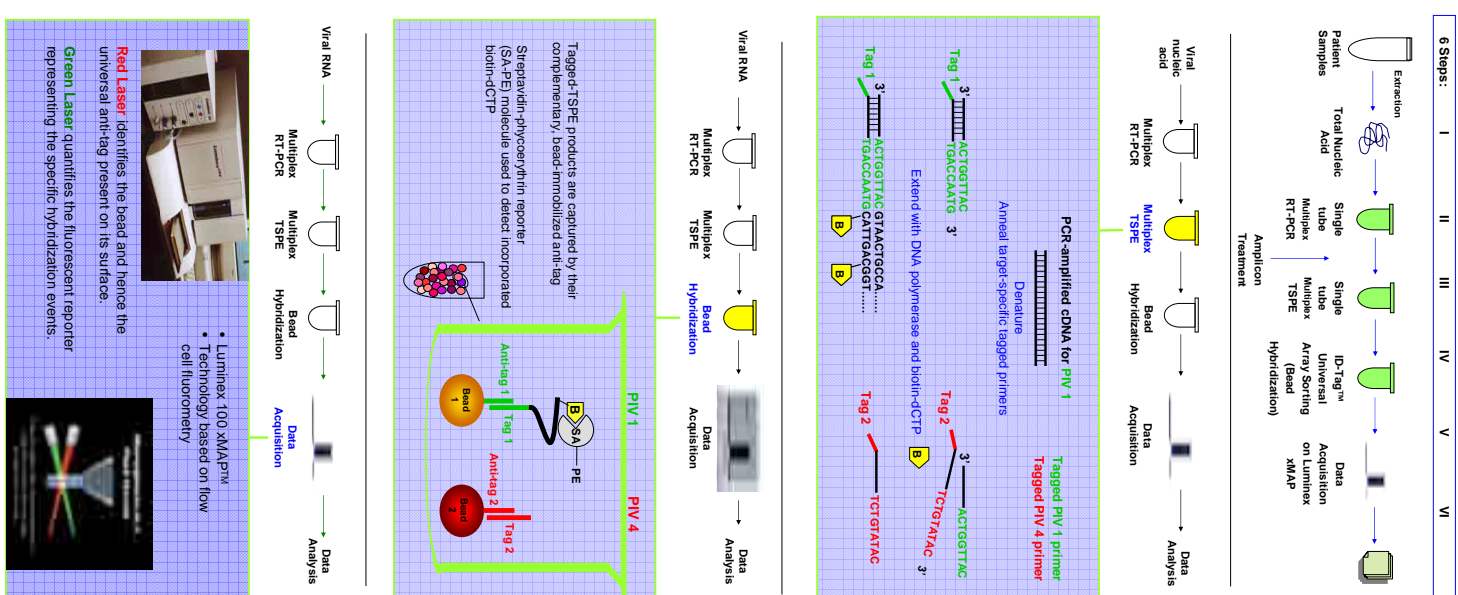
Viral pathogens detected by ID-Tag RVP

Virus	Type/Subtype
Influenza A (FluA)	H1
	H3
	H5
	Non-specific Influenza A
Influenza B (FluB)	A
Respiratory Syncytial Virus (RSV)	B
Coronavirus	229E
	OC43
	NL63
	HKU1
Parainfluenza Virus (PIV)	1
	2
	3
	4
Human Metapneumovirus (hMPV)	
Enterovirus/Rhinovirus (EV/Rhino)	
Adenovirus	

Materials and Methods

- > **Patent samples:** Specimens (nasal wash or nasopharyngeal swab) were collected in M4 viral transport media during respiratory viral season (January to March 2007). A total of 1,031 specimens that were negative by the rapid viral screen (Bimax NOW RSV, Flu A/B) were submitted for DFA/culture and analyzed using the MultiCode-Plx RVP assay. Eight hundred and eleven samples were processed immediately and 244 samples were extracted and stored at -80°C until processing (max. one month).
- > **Viral nucleic acid extraction:** Two hundred µL of sample was spiked with 20 µL of the appropriate dilution (per package insert) of NS2 (extraction control) and extracted on the automated extractor EasyMAG (BioMérieux, Inc., Durham, NC). Sample was eluted in a final volume of 55 µL.
- > **ID-Tag RVP assay:** Single tube multiplex RT-PCR, multiplex target-specific extension (TSPE) and bead hybridization were performed following the manufacturer's recommendation.
- > **Detection and Data analysis:** Data acquisition was performed on x-MAP LumineX 100 instrument with the IS2.3 software. The data generated was analyzed by the Tag-H Data Analysis Software RVP-I (TDAS RVP-I).

ID-Tag Technology Overview



Results

- > DFA/Culture identified a viral target in 307 of the 1,031 (30%) specimens. The turnaround time for culture was 1 (FluA) to 10 (Rhinovirus) days with an average of 3 days.
- > The ID-Tag RVP assay identified viral nucleic acid in 721 of the 1,031 (70%) specimens.
- > The positivity rate in the 811 fresh and 220 frozen samples was comparable (70 and 72% respectively).
- > The most commonly found virus was EV/Rhinovirus, followed by FluA, RSV, hMPV, adenovirus, FluB, coronavirus, and PIV. The assay does not distinguish EV from Rhinovirus.
- > There were a total of 105 samples with co-infections (4 of them contained 3 different viral pathogens), representing 10% of the specimens analyzed.

Assay	Positive	Negative	No call	Co-infections
DFA/Culture	307 (30%)	724 (70%)	NA	1 (0.1%)
ID-Tag RVP	721 (70%)	283 (27%)	27 (3%)	105 (10%)

Virus	#Pos by DFA or Culture	#Pos by ID-Tag	#Pos detected by ID-Tag but not DFA/Culture	% Pos not detected by DFA/Culture
hMPV	0	86	86	100%
Coronavirus	0	27	27	100%
EV/Rhino	27	301	274	91%
FluB	26	47	21	45%
Adenovirus	38	59	21	36%
FluA	126	192	66	34%
PIV	18	24	6	25%
RSV	72	93	21	23%

Sensitivity and Specificity Using DFA/Culture as a Gold Standard

FluA	ID-Tag Pos	ID-Tag Neg	SENS	SPEC
DFA/Culture Pos	118	8	94%	96%
DFA/Culture Neg	74	824	92%	
EV/Rhinovirus	ID-Tag Pos	ID-Tag Neg	SENS	100%
Culture Pos	28	0	100%	
DFA/Culture Neg	273	711	72%	
RSV	ID-Tag Pos	ID-Tag Neg	SENS	94%
DFA/Culture Pos	68	4	96%	
DFA/Culture Neg	25	930	97%	

Sens when ID-Tag Pos is true Positive

Adenovirus	ID-Tag Pos	ID-Tag Neg	SENS	SPEC
DFA/Culture Pos	32	6	84%	91%
DFA/Culture Neg	26	960	97%	
PIV	ID-Tag Pos	ID-Tag Neg	SENS	94%
DFA/Culture Pos	17	1	96%	
DFA/Culture Neg	7	1005	99%	
FluB	ID-Tag Pos	ID-Tag Neg	Sens	100%
DFA/Culture Pos	26	0	98%	100%
DFA/Culture Neg	18	984	98%	

The discrepant samples will be analyzed by a third party laboratory, and a confirmation of the ID-Tag RVP data may result in an adjustment of the sensitivity and specificity rate.

Summary

We analyzed 1,031 clinical specimens from pediatric patients that were suspected to have a viral respiratory infection and were negative by a rapid screen (Bimax). All samples that were submitted for DFA and culture were analyzed by the ID-Tag RVP assay. The comparable positivity rate in fresh and frozen samples suggests good stability of the extracted viral nucleic acid at -80°C for the period stored. ID-Tag RVP detected EV/Rhinovirus in 29% of specimens, while culture detected EV/Rhinovirus in only 2.6% of specimens. The increase in sensitivity for the RVP as compared to current practice was also significant for FluB. Pathogens that are not detected by our current standard of care, hMPV and coronavirus, had an incidence of 8% and 3%, respectively. The ID-Tag RVP assay had a sensitivity of >84%, and a specificity of >72% when compared to DFA/culture. The sensitivity increases to >91% when compared to any positive.

Conclusions

- > The ID-Tag RVP is a high throughput, multiplex assay that detects respiratory viral pathogens with an increased sensitivity compared to DFA and culture.
- > The RVP is more rapid than culture, allows for subtyping of certain viruses, and enables detection of co-infections.
- > The inclusion of an extraction control helps to identify potential extraction failures and PCR inhibition.
- > While the high detection rate of EV/Rhinovirus by the ID-Tag assay is notable, little is known about its clinical significance.
- > This system offers accurate and timely identification of respiratory viral pathogens. Implementation of the ID-Tag platform will positively impact both infection control practices and patient care.

Acknowledgments

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