

SIMULTANEOUS DETECTION OF 17 RESPIRATORY VIRUSES AND SUBTYPES WITH THE LUMINEX DIAGNOSTICS® RESPIRATORY VIRUS PANEL KIT



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BACKGROUND AND OBJECTIVE

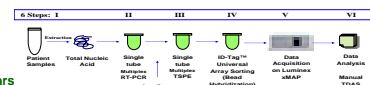
Many different viruses cause lower respiratory tract disease but their symptoms overlap considerably, especially in children. Children also have more frequent and severe respiratory tract viral disease than adults and can deteriorate quickly if not managed appropriately. Rapid laboratory detection of multiple respiratory viruses in the pediatric population is therefore important medically, fosters responsible antibiotic stewardship, and can improve fiscal outcomes. The current threat of a H5N1 influenza A pandemic and the recent SARS scare underscore the need to rapidly distinguish common respiratory viruses from these pathogens. The ID Tag™ Respiratory Virus Panel (RVP, Luminex Diagnostics®, formerly TM BioSciences®, Toronto, Canada) is a multiplex PCR kit that can simultaneously detect 17 common respiratory viruses and subtypes in a single working shift. We evaluated the performance of RVP compared to immunofluorescence (IF) on direct specimens sent to our laboratory for detection of common respiratory viruses from children 0-18 years of age.

METHODS

SPECIMENS AND STORAGE: 212 nasopharyngeal washes (NW) singly-positive or negative for RSV, influenza A, influenza B, parainfluenza, adenovirus (Chemicon) or human metapneumovirus (Diagnostic Hybrids) by cytospin direct IF were evaluated, some of which had also been cultured and yielded enterovirus or rhinovirus. Approximately 80% of the specimens had been stored at -70 °C since testing in late 2006-early 2007.

RVP: Total nucleic acids were automatically extracted by the Qiagen EZ1 Virus Minikit 2.0 and then frozen at -70°C until testing 1-3 days later by RVP. Results were read on the Luminex and automatically interpreted as positive (mean fluorescence intensity or MFI>300), "no-call" (MFI 101-299), or negative (MFI < 100).

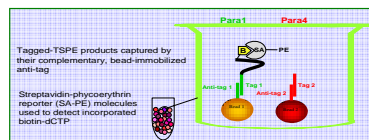
ID Tag™ RVP Overview



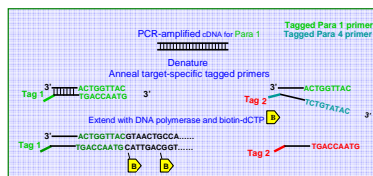
II. Setup & Multiplex PCR (3 hrs)

Viruses	Comment	Results
Influenza A	H1, H3	HI not scored
Influenza B		1
RSV	A, B	2
Coronaviruses	NL63, HKU1, 229E, OC43	SARS available outside the US
Parainfluenza	1, 2, 3, 4	4
HMPV		1
Enterovirus	No differentiation	1
Adenovirus		1
Controls	MS-2 bacteriophage	Internal (antigen) control
	Lambda DNA	Run control
		Total Analysis: 18 (21)

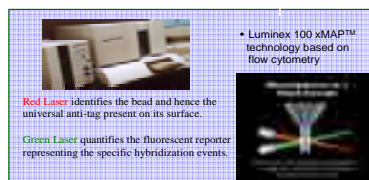
IV: Bead Hybridization + Reporter Addition (1 hr)



III: Amplicon Treatment & Multiplex TSPE (2.5 hrs)



V & VI: Detection and Analysis (30 minutes)



RESULTS

RVP COMPARES FAVORABLY WITH IF AND ENTEROVIRUS & RHINOVIRUS CULTURE

IF or Cult Pos	INITIAL RESULTS – FROZEN SPECIMENS					RESOLVED RESULTS					
	Tested	Pos	Type	No Call	Sensitivity	Resolved by	Tested	Pos	Type	No Call	Sensitivity
RSV	25	23	A: 8 B: 15	1	92%	Testing fresh specimens	16	16	A: 13 B: 3	0	100%
Influenza A	21	14	H1: 1 H3: 12 No call: 1	1	67%	Testing fresh specimens	19	19	H1: 9 H3: 10	0	100%
Adenovirus	19	14		5	74%	< cutoff to 200	19	19		0	100%
HMPV	22	21			95%	Retest, in-house PCR	21	21			100%
Influenza B	14	13			95%						
Parainfluenza	25	25	Para1: 4 Para2: 2 Para3: 19 Para4: 0		100%						
Enterov/Rhino	9	9			100%						
TOTAL	135	119			88%	TOTAL	75	75			100%

RVP DETECTS MORE CO-INFECTIONS THAN IF

IF or Cult Pos	No. Tested	No. Pos	CO-INFECTING VIRUS DETECTED BY RVP					
			Enterov/Rhino	HMPV	Para3	NL63	RSVA	Para2
Influenza B	14	3 (21%)	2		1			
Influenza A	40	6 (15%)	3	2			1	
HMPV	22	3 (14%)	2		1			
RSV	41	5 (12%)	3				1	1
Adenovirus	19	1 (5%)					1	
Parainfluenza	25	1 (4%)	1					
Enterov/Rhino	9	0						
TOTAL	251	19 (8%)	11 (58%)	2	2	2	1	1

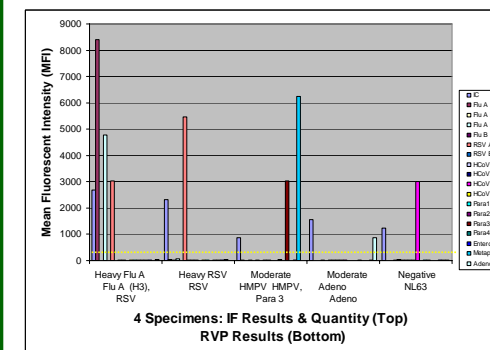
RVP DETECTS MANY VIRUSES IN IF-NEGATIVE SPECIMENS

No. IF-Neg NW	POSITIVE RVP RESULT					
	No. Pos.	Enterov/Rhino	HMPV	Flu A	Adeno	NL63
42	11 (26%)	6 (55%)	2	1	1	1

RVP IS ROBUST

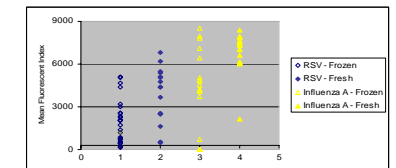
- The internal control failed to amplify in only 1/212 (<1%) NW, although many specimens contained visible blood or were cloudy.

TYPICAL RVP RESULTS



RVP IS MOST SENSITIVE WITH FRESH SPECIMENS

Storage	Virus Pos by IF	No. Tested	No. Pos	No Call	Negative	RVP RESULT		Sensitivity
						Median MFI (Range)	Fresh MFI > Frozen	
Fresh	RSV	16	16	0	0	4393 (329-6802)	2.2x	100%
	Influenza A	19	19	0	0	7317 (2128-9397)	1.5x	100%
	TOTAL	35	35	0	0			100%
Frozen	RSV	25	23	2	0	2032 (150-5101)		92%
	Influenza A	21	14	1	0	4831 (1-9834)		67%
	TOTAL	46	37	3	0			89%



CONCLUSIONS

- RVP is a promising new assay for the simultaneous detection in a day of 17 respiratory viruses and subtypes infecting children.
- RVP appears to be as sensitive as IF for detection of most common respiratory viruses, and perhaps as sensitive as culture for the enterovirus/rhinovirus group. Adenoviruses are also detected if the cutoff is lowered to 200, but additional studies are required before recommending this change.
- RVP detects viruses in many IF-negative specimens and dual infections, even when one virus is present in higher quantity than another. The enterovirus/rhinovirus group was common in many of these samples. The newly-described NL-63 coronavirus was also found, but 229E, OC43, or HKU1 were not identified.
- Sub-type was determined by RVP in all but one influenza A-positive specimen, although correlation with conventional sub-typing was not assessed.
- Freshly-collected NW are recommended for RVP over those stored frozen for prolonged periods of time
- RVP may be a suitable replacement for viral culture in most circumstances and should be further assessed for its purpose
- Multiplexed respiratory virus molecular tests like RVP will likely have a significant and positive impact on healthcare outcomes for children. The structure and function of virology laboratories of the future may be significantly affected as well.