Performance Evaluation of a Multiplex Respiratory Pathogen Panel with Bronchoalveolar Lavage Specimens

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Introduction

Background: Multiplex molecular panels have established clinical utility for the diagnosis of respiratory pathogens. Currently, specimen sources for FDA-cleared methods are limited to nasopharyngeal swabs. The study goal was to establish performance of the Verigene™ FlexPanel Respiratory Pathogen Panel [VFP] (Luminex Corp. Austin, TX) in a non-FDA approved specimen type consisting of bronchoalveolar lavage (BAL). Sensitivity and specificity of VFP in BAL was verified. VFP assay concordance was compared to 2 viral respiratory panels, and viral culture. Data on prevalence of respiratory pathogens detected by VFP in BAL was collected during December 2016 – March 2017.

Methods: BAL specimens were obtained from patient samples submitted to the Mayo Clinic Florida molecular virology laboratory for immunocompromsed host (ICH) testing. BAL specimens were subjected to viral and bacterial culture by standard methods within 72 hours of initial receipt. All specimens were tested within 48 hours of initial collection for influenza A, B, and RSV with the Prodesse ProFlu+(Hologic, San Diego, CA) [PROD] panel prior to 1/3/2017, or VFP after 1/3/2017. Frozen BAL specimens used for crossover viral panel testing were stored for <12 months at -20° C. BAL specimens were spun at 3,000 rpm for 10 minutes prior to VFP/PROD testing. Supernant (200 µl) was tested with VFP without modification of pre-set instrument parameters. Precision and accuracy studies utilized previously characterized patient specimens (influenza A:H1), a commercially available respiratory viral control panel (NATtrol Respiratory Verification Panel, Zeptometrix Corp. Franklin, MA) [VCP], and a *Bordetella pertussis* culture suspension (*B. pertussis* A639, Zeptometrix Corp. Franklin, MA).

Pathogen prevalence data in BAL was generated as part of follow-up testing of ICH. Twenty specimens tested by VFP were sent to a collaborating laboratory (Mayo Clinic Arizona) for comparison with the Biofire Film Array (BFA) respiratory panel (Biofire Diagnostics LLC, Salt Lake City, UT). Statistical analysis and kappa coefficient of concordance was determined using GraphPad Quick Calc software (www.graphpad.com).

Results: Sensitivity, specificity and precision with RNA (Influenza A:H1) and DNA targets (*B. pertussis* A639) was 100%. Accuracy of VFP utilizing VCP spiked 1:10 into culture-negative BAL was 100% (N=121). A failure rate of 4.26% (4/94) was noted, primarily due to instrument issues. 3/4 failed runs corrected upon repeat; one influenza A:H3 result failed to subtype upon retesting. (data not shown)

Of 50 specimens tested by PROD, VC, and VFP for influenza A, B, and RSV, 48% (24/50; 4 positive, 20 negative) agreed by all assays. 98% (49/50) results agreed between PROD and VFP, 48% (24/50) agreed between VC and VFP. VFP detected 8 rhinovirus, 4 dual infections, 1 hMPV, 1 adenovirus, and 1 *Bordetella* sp. not detected by PROD or VC. Of 21 VFP specimens tested by BFA, there was 85.7% (18/21) concordance. VFP detected 1 rhinovirus and 1 parainfluenza 2 not detected by BFA. In one specimen adenovirus was detected with VFP, coronavirus OC43 by BFA.

Preliminary analysis of ICH BAL specimens collected Dec. 2016 - end of Feb. 2017 tested with VFP yielded an overall disease prevalence of 21.90% (38/173). Rhinovirus was detected in 36.0% of 25 lung transplant patients.

Conclusions: VFP demonstrated excellent accuracy, and good concordance with PROD and BFA. No pre-analytical or analytical issues were noted with use of BAL specimens. VFP consistently detected more analytes than viral culture. In a prevalence survey rhinovirus was most often identified in BAL of lung transplant patients. The high degree of accuracy and rapid turnaround time of VFP in BAL specimens is a notable improvement in pathogen detection in ICH.

Specific Aims

1. **Analytical performance of BAL specimens with the Verigene FlexPanel respiratory pathogen panel (VFP)**
2. **Comparison of VFP with other molecular panels and viral culture for detection of Influenza A, B, and RSV**
3. **Prevalence of respiratory viruses in BAL specimens from immunocompromised hosts collected during the 2017 respiratory season**

Table 1

Comparison of VFP, PROD, and VC for Detection of Influenza A, B, and RSV in BAL Specimens

	VFP	PROD	VC	VFP/PROD Kappa (95% CI)	VFP/VC Kappa (95% CI)
Influenza A	7 (4:H1, 3:H3)	7	3	1.0 (1.0-1.0)	0.563 (0.194-0.993)
Influenza B	2	2	1	1.0 (1.0-1.0)	0.658 (0.033-1.0)
RSV	13* (7:A, 6:B)	14	0	0.949 (0.851-1.0)	0.0 (0.0-0.0)
Negative	21	27	37^	0.960 (0.881-1.0)	0.151 (-0.07-0.372)
Other Viruses	7**	N/A	3 CMV, 2 HSV‡		
Total	50	50	50		

* 4 dual infections: RSV/Rhinovirus
** (4) Rhinovirus, (1) Human Metapneumovirus, (1) Adenovirus, (1) *B. parapertussis/ bronchoseptica*
^ 6 negative specimens were toxic in cell culture
‡ (1) dual HSV/Influenza A, (1) dual HSV/CMV

Table 2

VFP Analytic Targets

Influenza A	Influenza B	Respiratory Syncytial Virus type A
Influenza A (subtype H1)	Parainfluenza type 1	Respiratory Syncytial Virus type B
Influenza A (subtype H3)	Parainfluenza type 2	*Bordetella pertussis*
Adenovirus	Parainfluenza type 3	*Bordetella parapertussis/ bronchoseptica*
Human Metapneumovirus	Parainfluenza type 4	*Bordetella holmesii*
Rhinovirus		

Table 3

Comparison of VFP and BFA in BAL Specimens

	VFP	BFA
Influenza A	4	4
Influenza B	2	2
RSV	4	4
Adenovirus	1	Coronavirus
HMPV	1	1
Parainfluenza	3	2
Rhinovirus	2	1
Negative	5	5
Total	21	21

Kappa (95%CI) all analytes: 0.674 (0.35 - 0.997)

Table 4

Prevalence of Respiratory Viruses Detected by VFP in BAL Specimens Collected Jan. 1, 2017 – March 31, 2017

Analyte	Total Detected	Tranplant Type		No Transplant	% Prevalence (N=264)	Duplicate Tests
		Lung	Other*			
Rhinovirus	13	9	1	3	4.92	9
Influenza A	4	3	0	1	1.51	0
Influenza B	2	1	0	1	0.75	0
RSV Type A	0	0	0	0	0	0
RSV Type B	5	3	1	1	1.89	1
HMPV	5	4	1	0	1.89	0
Adenovirus	2	2	0	0	0.76	3
PIV Type 1	0	0	0	0	0	0
PIV Type 2	2	2	0	0	0.76	1
PIV Type 3	3	0	2	1	1.14	1
PIV Type 4	1	1	0	0	0.38	0
B. parapertussis/ bronchoseptica[a]	1	1	0	0	0.38	0
B. pertussis	0	0	0	0	0	0
B. holmesii	0	0	0	0	0	0
Dual infections**	1	1	0	0	0.38	0
Total Positives	39	27	5	7	**14.8**	15

N= 290; 264 with duplicate tests removed
* Heart, lung, kidney
** Rhinovirus/Parainfluenza type 3
[a] Previous Rhinovirus infection

Conclusions

1. **Although not FDA-approved for BAL specimens, VFP demonstrated no pre-analytical or analytical issues with this specimen type.**
2. **Performance of VFP in BAL specimens was excellent compared to PROD and BFA. VC compared poorly to molecular methods.**
3. **Rhinovirus was most prevalent among lung transplant recipients. Overall prevalence of respiratory pathogens was 14.8% during the period January – March 2017.**

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