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Evaluation of the BioFire FilmArray Respiratory Panel and the GenMark eSensor Respiratory Viral Panel on Lower Respiratory Tract Specimens

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We evaluated the performance characteristics of the FilmArray respiratory panel and the eSensor respiratory viral panel on clinical and spiked lower respiratory tract specimens (LRTS). The overall agreement between the two methods was 89.5% (51/57). The lower limit of detection of both assays for all targets in LRTS was comparable to that for nasopharyngeal swab specimens.

Lower respiratory tract infections, especially pneumonia, can be particularly severe and a cause of high morbidity and mortality in cancer and hematopoietic stem cell transplant (HSCT) patients (1). In HSCT recipients, progression of respiratory viral infections from the upper to the lower respiratory tract occurs most frequently with respiratory syncytial virus (RSV), human metapneumovirus (hMPV), parainfluenza viruses (PIV), and influenza viruses (2, 3). Two of the several multiplexed molecular assays cleared by the Food and Drug Administration (FDA) for the detection of respiratory pathogens include the FilmArray respiratory panel (FA RP) test (BioFire Diagnostics, Salt Lake City, UT) and the eSensor respiratory virus panel (eS RVP) test (GenMark Diagnostics, Carlsbad, CA). However, both assays are cleared only for testing on nasopharyngeal swab (NPS) specimens. A few studies evaluating both the FA RP and the eS RVP have included a small number of other specimen types, including bronchoalveolar lavage (BAL) fluids, bronchial washes (BW), sputum (SPT), throat swabs, and tracheal aspirates (4–8). In this study, we compare the performances of both the FA RP and the eS RVP exclusively with lower respiratory tract specimens (BAL fluids, BW, and SPT) from cancer patients with symptoms of pneumonia. In addition to accuracy, the lower limit of detection (LOD) of the assays for each target in BAL fluids was compared to the LOD for NPS specimens.

This was a retrospective study performed with specimens collected from July 2010 to March 2013 and stored at −80°C following testing by direct fluorescent antibody assay and viral culture as previously described (4). A total of 52 samples (31 BAL fluid, 17 BW, and 4 SPT) from 45 patients were included: 32 consecutive positive samples and 20 randomly selected negative specimens. Patients' ages ranged from 25 to 89 years old, with 25 liquid-tumor patients (12 with leukemia and 13 with lymphoma) and 20 solid-tumor patients (12 with lung cancer and 8 with other types of cancer). The most common symptoms included radiological evidence of lung abnormality (infiltrates or nodules) and/or fever and cough. The study was granted a waiver of the HIPPA authorization and informed consent by the Memorial Sloan-Kettering Cancer Center (MSKCC) institutional review board.

To increase the number of positive specimens tested, BAL fluids ($n = 14$) negative for all 20 targets detected by the FA RP were combined, split into 4 pools, and spiked with organisms from the Zeptometrix respiratory panel (Zeptometrix, Buffalo, NY). This panel includes all the targets listed in Table 1. The concentration of each viral stock solution varied from a cycle threshold of 22 to 28 as measured by the vendor internal real-time PCR (Zeptometrix, personal communications), which may be higher than the concentration found in clinical specimens. A clinical isolate was used for coronavirus HKU1. Targets were diluted in negative BAL fluids in pools of 4 or 5 targets. Four stock solutions were prepared at a ratio of 1/25 (i.e., 10 μl of target into 250 μl of negative BAL fluids). The four prepared pools are listed in Table 1. Each pool was tested 20 times to determine accuracy.

The LOD was determined by 10-fold serial dilution (10, 10^{-1}, 10^{-2}, and 10^{-3}) of each pool described above. For comparison, the LOD in negative NPS specimens prepared in the same manner and with the same source of isolates was also determined. All testing was performed in triplicate for each dilution. Intra- and interassay precision was determined for both assays, and the percent coefficient of variation (CV) was calculated. For the FA RP, the intra-assay precision was determined by testing the same specimen (i.e., pool 1) multiple times on the same instrument. Interassay precision was determined by testing the same specimen on multiple instruments ($n = 7$) over a 2-month period. For the eSensor RVP, intra-assay reproducibility was determined by testing the same specimen on the same XT-8 tower, while the interassay reproducibility was determined by testing the same specimen on multiple XT-8 towers ($n = 4$) over a 2-month period.

The FA RP was performed as previously described for NPS specimens but using 300 μl of raw specimen instead of NPS specimens (4). Mucoid specimens were pretreated prior to testing using the LBM Snotbuster system (Copan Diagnostics, Murrieta, CA), which picks up the specimen with a sputum dipper and transfers it to 0.5 ml of SL solution (dithiothreitol [DTT] and phosphate buffer solution). Three hundred microliters of the treated specimen was then tested using the FA RP. Pretreatment provided results for specimens which originally yielded an invalid result. The eS RVP was performed as previously described for NPS

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TABLE 1 BAL stock solutions[a]

Pathogen	Source	Pool 1	Pool 2	Pool 3	Pool 4
Parainfluenza virus 4	Zeptometrix	X			
Influenza virus A H3	Zeptometrix	X			
Influenza virus A 2009 H1	Zeptometrix	X			
Influenza virus B	Zeptometrix	X			
Parainfluenza virus 1	Zeptometrix		X		
Parainfluenza virus 2	Zeptometrix		X		
Parainfluenza virus 3	Zeptometrix		X		
Influenza virus A H1	Zeptometrix		X		
Human metapneumovirus	Zeptometrix		X		
Rhinovirus/ enterovirus	Zeptometrix			X	
Coronavirus HKU1	Clinical isolate			X	
Coronavirus 229E	Zeptometrix			X	
Coronavirus OC43	Zeptometrix			X	
Coronavirus NL63	Zeptometrix			X	
Adenovirus	Zeptometrix				X
Respiratory syncytial virus	Zeptometrix				X
Bordetella pertussis	Zeptometrix				X
Mycoplasma pneumoniae	Zeptometrix				X
Chlamydophila pneumoniae	Zeptometrix				X

[a] Each pool contains an equal volume of each target with an "X" in the column (e.g., pool 3 contains all 4 coronaviruses and rhinovirus).

TABLE 2 Comparison of viral culture, FA RP, and eS RVP pathogen detection in lower respiratory tract specimens[a]

Target	No. of viruses detected by method		
	Culture	FA RP	eS RVP
Adenovirus	1	1	2
RSV	7	9	8
Rhinovirus	9	11	11
PIV-1	2	2	2
PIV-3	5	7	7
hMPV	5	4	4
IA H3	1	1	2
IB	2	2	2
Total no. of viruses detected	32	36[b]	38[c]
Total no. of negative specimens	20	17	18

[a] FA RP, FilmArray respiratory panel; eS RVP, eSensor respiratory viral panel; RSV, respiratory syncytial virus; PIV, parainfluenza virus; hMPV, human metapneumovirus; IA, influenza A virus; IB, influenza B virus.
[b] Two specimens positive by FA RP only: RSV and rhinovirus.
[c] Four specimens positive by eS RVP only: adenovirus C, RSVA/B, IA H3, and rhinovirus (all coinfections).

specimens but using 200 μl of raw specimen instead of NPS specimens (7).

The reference standard was established as a consensus result, in which a true positive was defined as a sample testing positive by at least 2 of the methods (FA RP, eS-RVP, or viral culture). Statistical analysis was performed using Fisher's exact test. A P value of <0.05 was considered significant.

The FA RP and the eS RVP detected all but one of the 32 viruses detected by viral culture and additionally detected 4 and 6 viruses, respectively, missed by viral culture (Table 2). The overall sensitivities of viral culture, the FA RP, and the eS RVP for targets detected were 89.5% (34/38), 100% (34/34), and 100% (34/34), respectively, while their specificities were 90.5% (19/21), 90.5% (19/21), and 82.6% (19/23), respectively. The agreement between the FA RP and the eS RVP assays was 89.5% (51/57; $\kappa = 0.77$, good). The differences between culture and each of the multiplex assays was statistically significant ($P < 0.0001$), and the difference between the FA RP and the eS RVP was statistically significant ($P < 0.0001$).

Additional rhinoviruses not detected by culture were detected by both molecular assays. In immunocompromised hosts, severe complications, including pneumonia, can occur from infection with rhinovirus. Two recent studies have shown that in HSCT recipients, rhinovirus was a significant cause of viral pneumonia and the severity could be similar to that of the 2009 H1N1 pandemic influenza A (9).

The eS RVP detected 5 coinfections: RSV A/RSV B/rhinovirus, RSV A/hMPV, influenza virus B/influenza virus A H3, rhinovirus/adenovirus C, and rhinovirus/PIV-3 (Table 2). The FA RP detected 1 coinfection, rhinovirus/PIV-3. The significance of dual infections is unclear and seems to depend on which viruses are present, with RSV coinfections often reported to cause more severe diseases (10, 11). Both RSV A and B subgroups may circulate during a given respiratory season, and coinfections likely occur, although rarely reported, probably due in part to the lack of discrimination of current testing methods (12). Dual infections with influenza viruses, although rare, have also been reported, and the clinical presentation does not seem to be different from that for patients infected with a single influenza virus type (13, 14).

The eS RVP provides typing information on adenovirus (adenovirus B/E and adenovirus C). Following completion of this study, a second version of the FA RP was released (FA RP v. 1.7). The new panel has an additional adenovirus assay to increase the sensitivity for adenovirus detection. In our study, the number of true clinical specimens positive for adenovirus was low ($n = 2$), and the FA RP missed the one adenovirus C virus detected by the eS RVP, even on repeat testing with the FA RP v. 1.7. The presence of adenovirus C was confirmed by a laboratory-developed test using adenovirus MultiCode analyte-specific reagents (ASR) (data not shown). The FA RP package insert does state that the assay has reduced sensitivity for adenovirus serotypes 2 and 6, which belong to adenovirus species C. A recent article evaluating the FA RP v. 1.7 reported increased sensitivity of the assay for adenovirus detection, from 42.7% to 83.3% (15).

The LOD of the FA RP was lower in BAL fluids than in NPS for all targets except for PIV-4, for which the LOD was similar (data not shown), suggesting that all targets should be detected in BAL fluids with sensitivity similar or better than that in NPS specimens. The same conclusion was reached for eS RVP, since the LOD was lower (~10-fold) than that of the FA RP for all targets except hMPV, for which the LOD was equal (data not shown). For the FA RP, the percent agreement for all replicates of each target on each instrument was 100% except for *Mycoplasma pneumoniae*, for which the percent agreement was 84.4%. The percent CV for the T_m ranged from 0.17 to 0.49%. All T_m values fell within ranges as set by the manufacturer for NPS specimens. There were no differ-

ences in percent CV between the interassay and intra-assay reproducibility data (data not shown). For the eS RVP, the percent agreement for all replicates of each target on each instrument was 100%. The percent CV for the interassay reproducibility of the electrical signal ranged from 8.0 to 40% for each target, while the percent CV for the intra-assay reproducibility ranged from 4 to 30%. All percent CV were within the ranges established by the manufacturers for NPS specimens (data not shown).

Two previous studies have evaluated the performance of the BioFire FA RP on a relatively large number of BAL fluids (>50) (5, 8). Rand et al. showed an overall increased rate of detection over that of viral culture, as high as 50%; however, no distinction was made between the performance of the assay in lower respiratory tract specimens and that in NPS specimens (8). Hammond et al. showed, similar to our result, an increase in the number of viruses detected in BAL fluids by the FA RP, although the positivity rate was lower than that with NPS specimens (5). Their patient population was similar to ours, with a high number of immunocompromised patients. This is the first report of the performance of the eS RVP on a large number of lower respiratory tract specimens.

The FA RP and the eS RVP have significantly different workflows. The FA RP is a low-throughput, sample-to-result system, with minimal manipulation and a laboratory turnaround time (TAT) of about 65 min. The eS RVP has several steps prior to detection on the XT-8 platform, including a nucleic acid extraction step and amplification and hybridization steps, all of which can be batched for a laboratory TAT of at least 6 h. Implementation of the eS RVP requires adequate laboratory space to separate each step of the process and prevent amplicon contamination, which can occur following either the amplification or hybridization step. Therefore, the choice and implementation of either platform will depend on each laboratory test volume, workflow, and available space.

Our study is unique in that it not only confirms the accuracy of two FDA-cleared multiplexed assays on lower respiratory tract specimens using a high number of positive clinical specimens from immunocompromised patients but also compares their LOD in different specimen types. The results of our study are in agreement with those of two previous studies evaluating the FA RP on a large number of BAL specimens (5, 8). Limitations of the study include its retrospective nature and the limited diversity of the true clinical samples, which prompted us to use spiked specimens to completely assess the performance of the two assays and the lack of specimens positive for bacterial pathogens. In addition, the use of a reference standard that includes the tests being evaluated might have resulted in overestimation of the tests' sensitivities.

In conclusion, the performance of both the FA RP and the eS RVP was excellent with lower respiratory tract specimens, and their use should improve diagnosis of viral pneumonia.

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