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FilmArray respiratory panel assay: An effective method for detecting viral and atypical bacterial pathogens in bronchoscopy specimens

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ABSTRACT

The BioFire FilmArray Respiratory Panel (FA RP) is a rapid multiplexed molecular assay approved for detection of viral and atypical bacterial pathogens in nasopharyngeal specimens. This study aimed to evaluate the performance of the BioFire FilmArray Respiratory Panel v1.7 on bronchoscopy specimens. We tested 133 bronchial specimens (87 archived and 46 prospectively collected) with the FA RP and compared the results to the Luminex NxTAG Respiratory Pathogen Panel (NxTAG RPP). After discordant analysis, 123 specimens gave concordant results using the FA RP and the NxTAG RPP for an overall agreement of 93.9% (kappa = 0.88 [95% CI 0.80–0.96]), a positive percent agreement of 93.7% (95% CI 83.7–97.7) and a negative percent agreement of 94.1% (95% CI 84.9–98.1). In conclusion, the BioFire FilmArray RP performed reliably to detect a broad range of respiratory pathogens in bronchoscopy specimens.

1. Introduction

Respiratory viruses cause a range of clinical syndromes from mild, self-limited upper respiratory tract (URT) infection to complicated lower respiratory tract (LRT) infection, especially in patients with immunosuppression and/or chronic lung disease (Chemaly et al. 2006; Garbino et al. 2004; Kim et al. 2007). Diagnosis of suspected LRT infection in immunocompromised hosts is particularly challenging due to the broad range of viral, bacterial and other infectious and non-infectious etiologies presenting in a similar fashion (Bajaj and Tombach 2017). The rapid detection of infectious causes can lead to diagnostic clarity, targeted and timely therapy and implementation of infection control practices to limit transmission (Kim et al. 2007).

Recently several molecular diagnostic platforms with panels that detect an extensive range of respiratory pathogens have been introduced (Hanson and Couturier 2016; Ramanan et al. 2018). These multiplex assays significantly increase diagnostic yield (i.e. the number and range of organisms detected) by detecting potential pathogens not routinely identified by traditional methods (Hanson and Couturier 2016; Ko and Drews 2017). The BioFire FilmArray Respiratory Panel (FA RP) is one such fully automated method that simultaneously detects 17 respiratory viruses and 3 bacterial targets (Poritz et al. 2011). The FA RP was chosen for this study for its comprehensive list of targets, rapid turnaround time and ease of use. However, the FA RP is currently only approved for nasopharyngeal (NP) specimens with limited data available on its performance using bronchoscopy specimens.

LRT specimens obtained by bronchoscopy are often needed to diagnose suspected LRT infection in critically ill and immunocompromised patients (Brownback et al. 2014). FA RP performed on bronchoscopy specimens has shown to increase diagnostic yield in patients who previously tested negative on NP specimens (Azadeh et al. 2018; Lachant et al. 2017).

The aim of this study was to evaluate the ability of the FA RP to detect respiratory pathogens in bronchoscopy specimens (bronchoalveolar lavages [BAL], bronchial aspirates [BAS] and bronchial washes [BW]) when compared to respiratory pathogen detection by Luminex NxTAG Respiratory Pathogen Panel (NxTAG RPP).

2. Material and methods

2.1. Clinical specimens

This study was done at a diagnostic microbiology laboratory servicing an acute, tertiary care center in Vancouver, Canada and included both archived and prospectively collected bronchoscopy specimens. Archived, positive and negative convenience specimens included BAL and BW collected between December 2015 and November 2018. The initial clinical testing on archived specimens was performed at the time of

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collection at a reference laboratory (BC Center for Disease Control Public Health Laboratory) using the NxTAG RPP. The positive archived specimens for this study were selected to reflect a wide range of targets detected by FA RP. Testing by FA RP on archived specimens was done during September 2016 and February 2019. A few archived BW positive for *Mycoplasma pneumoniae (M. pneumoniae)* had been initially tested at the same reference laboratory using a lab developed multiplexed PCR (LD PCR) for *M. pneumoniae*, *Legionella pneumophila* (*L. pneumophila*) and *Chlamydophila pneumoniae* (*C. pneumoniae*) based on the protocol by Welti et al. (Welti et al. 2003). These specimens were subsequently tested by FA RP and NxTAG RPP.

Prospectively collected bronchoscopy specimens (BALs and BAS) were collected between December 2016 and May 2017. These specimens were randomly selected from patients with suspected acute respiratory tract infection from specific hospital locations, chosen because they typically house immunocompromised patients and patients with underlying chronic lung disease (bone marrow transplant unit, respiratory/thoracic unit, intensive care unit). Prospectively collected specimens were tested in parallel using the FA RP in the clinical laboratory and the NxTAG RPP in the reference laboratory.

Archived specimens were stored at −70 °C for long term storage and prospectively collected specimens were kept at 4 °C for storage less than one week.

2.2. FilmArray respiratory panel testing

The BioFire FA RP version 1.7 (Biomerieux, St-Laurent, Canada) detects the following viral and bacterial pathogens: adenovirus (AdV); human coronavirus (hCoV) 229E, HKU1, NL63 and OC43; influenza A (Inf A) subtypes H1, H1–2009 and H3; influenza B (Inf B); human metapneumovirus (hMpV); parainfluenza virus (PIV) type 1, type 2, type 3 and type 4; respiratory syncytial virus (RSV); rhinovirus/enterovirus (hRV/EV); *Bordetella pertussis (B. pertussis)*; *C. pneumoniae* and *M. pneumoniae*. The assay was performed according to the manufacturer's instructions. All testing was performed on neat undiluted bronchoscopy specimens without pre-treatment of mucoid specimens. Briefly, 300 μL of sample were mixed with sample buffer and injected into a test pouch containing all necessary reagents for nucleic extraction, PCR amplification and detection of the respective targets. The test pouch was inserted into the BioFire FilmArray 2.0 instrument and was run using the provided software.

2.3. Luminex NxTAG respiratory pathogen panel testing

The Luminex NxTAG RPP (Luminex Molecular Diagnostics, Toronto, Canada) detects the following viral and bacterial pathogens: AdV, human bocavirus (BoV), hCoV (229E, NL63, OC43 and HKU1) Inf A virus (subtypes H1, 2009 H1N1, H3), Inf B virus, hMpV, PIV (types 1, 2, 3, and 4), RSV (types A and B), hRV/EV, *L. pneumophila, C. pneumoniae*, and *M. pneumoniae*. The test was performed at a reference laboratory and was previously validated for use with bronchoscopy specimens (Jassem et al. 2016).

Nucleic acid extraction was performed from 200 μL of specimen on the MagMAX Express-96 Deep Well Magnetic Particle Processor using the MagMAX-96 Viral RNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instructions.

Nucleic acids (35 μL) were amplified and hybridized on the Eppendorf Mastercycler pro PCR System (Fisher Scientific, Waltham, MA) with thermocycling conditions set by the manufacturer. Data acquisition was performed on the MAGPIX instrument according to manufacturer's instructions.

2.4. Contrived specimens for Bordetella pertussis

To assess detection of *B. pertussis*, spiked specimens were generated. BAL that had previously tested negative for all targets by the FA RP were pooled. A 0.5 McFarland standard suspension of a *B. pertussis* clinical isolate was diluted with the pooled negative BAL to a concentration of 3750 CFU/mL – the detection limit of the FA RP for this target as given by the manufacturer (FA RP version 1.7 package insert). FA RP testing was performed as described using 300 μL of the diluted suspensions.

2.5. Interpretation of results

When FA RP results were in agreement with the initial NxTAG RPP no further testing was done. When FA RP and initial NxTAG RPP results were discordant, a repeat NxTAG RPP test was done. Discordant *M. pneumoniae* results were re-tested by the LD PCR for *M. pneumoniae*, *L. pneumophila* and *C. pneumoniae* described above. Consensus was defined as a minimum of 2 out of 3 results being in agreement (FA RP, initial NxTAG RPP and repeat NxTAG RPP/LD PCR).

2.6. Statistical methods

The positive percent agreement (PPA), negative percent agreement (NPA) and 95% confidence intervals (CI) were calculated using the software at http://vassarstats.net/clin1.html. Overall agreement between the FA RP assay and either NxTAG RPP or consensus result was measured by the kappa statistic (https://www.graphpad.com/quickcalcs/kappa2). Differences between test performances were assessed using McNemar's 2-tailed *P* values (https://www.graphpad.com/quickcalcs/mcNemar1). All websites were accessed in February 2019.

3. Results

3.1. Archived specimens

A total of 87 archived specimens (BAL $n = 83$, BW $n = 4$) from 76 adult patients were selected for the initial evaluation of the FA RP. The specimens were from patients with a mean age of 53 (standard deviation [SD] = 14.8, age unknown for 4 patients) at the following hospital locations: outpatient bronchoscopy suite ($n = 33$), intensive care unit ($n = 12$), respiratory/thoracic unit ($n = 10$), respiratory ambulatory unit ($n = 7$), bone marrow transplant unit ($n = 4$), medicine unit ($n = 4$), tuberculosis unit ($n = 1$), pre-admitting center ($n = 1$) and unknown ($n = 15$).

The majority of the specimens ($n = 83$) had been initially tested by NxTAG RPP and subsequently by FA RP. Four *M. pneumoniae* positive BW had been initially tested by a LD PCR that detects *M. pneumoniae*, *L. pneumophila* and *C. pneumoniae* and were tested by both FA RP and NxTAG RPP for the study.

Of the 87 archived specimens, there were 50 specimens with one target detected by either method, 5 specimens with 2 targets detected by either method and one specimen with 3 targets detected by one method (NxTAG RPP). One *M. pneumoniae* positive BW was very mucoid and repeatedly failed testing by the FA RP. This specimen was excluded from the analysis, leaving 86 archived specimens.

Concordant results between the FA RP and NxTAG RPP were obtained for 71 specimens results (overall agreement = 82.7%, kappa = 0.65 [95% CI 0.48–0.81]) with 41 concordant positive and 30 concordant negative results detected. Thirty-eight specimens tested positive for 1 pathogen by both FA RP and NxTAG RPP (hCoV 229E $n = 2$, hCoV NL63 $n = 3$, hCoV HKU1 $n = 3$, hCoV OC43 $n = 3$, Inf A $n = 4$, Inf B $n = 4$, PIV 1 $n = 1$, PIV 3 $n = 6$, PIV 4 $n = 2$, hRV/EV $n = 4$, RSV $n = 3$, *M. pneumoniae* $n = 3$). In 3 specimens 2 pathogens (RSV + hRV/EV; hCoV NL63 + hRV/EV; hRV/EV + PIV 2) were detected by both methods. For influenza A we observed 3 concordant positive specimens for Inf A/H1–2009. One specimen was positive for Inf A without a subtype by FA RP and positive for Inf A/H1–2009 by NxTAG RPP. This was considered a concordant result.

Discordant results were obtained in 15 specimens (Table 1). In one, NxTAG RPP detected BoV and human hRV/EV while the FA RP result was

negative. Since BoV is not a FA RP target, this result was disregarded and only the hRV/EV result was considered discordant.

Specimens with discordant viral targets were re-tested by NxTAG RPP to determine if degradation of the analyte had occurred during storage of these archived samples that would account for the discordant results. Details of the discordant analysis are provided in table 1. One specimen discordant for PIV 4(FA RP negative, NxTAG RPP positive) could not be repeated due to insufficient sample and was excluded from analysis leaving 85 archived samples for analysis. After resolution of discordant results for the archived specimens the overall PPA and NPA were 93.6% (95% CI 81.4–98.3) and 89.5% (95% CI 74.3–96.6) respectively (Table 3). A perfect (kappa = 1.0) or very good agreement (kappa >0.8) was found for all detected targets except hRV/EV and Inf A. The agreement between the 2 tests for hRV/EV (kappa = 0.77) and Inf A (kappa = 0.74) was considered to be good.

3.2. Prospectively collected specimens

A total of 46 prospectively collected specimens were obtained from 36 adult patients with a mean age of 56 (SD = 14.6) from the following hospital units: intensive care unit ($n = 22$), respiratory/thoracic unit ($n = 13$), bone marrow transplant unit ($n = 11$). Included were 37 BAL and 9 BAS that were tested by FA RP and LMX NxTAG RPP in parallel.

Results from both test methods were concordant in 43 samples (overall agreement = 93.5%, kappa = 0.87 [95% CI 0.73–1.0]); 13 specimens were concordant positive for one viral pathogen (AdV $n = 3$, hMpV $n = 4$, RSV $n = 2$, hCoV NL63 $n = 1$, hCoV OC43 $n = 1$, hRV/EV $n = 1$, Inf A H3 $n = 1$) and 30 samples tested negative by both methods. No coinfections were observed in this group.

Three discordant AdV results were noted (Table 1). Two specimens were collected from the same patient 1 day apart. After repeat NxTAG RPP and discordant analysis all 3 specimens were considered positive for AdV.

For prospectively collected specimens, the positive and negative percent agreement between the FA RP and the NxTAG RPP was 100% (kappa = 1.0) for all targets detected, except AdV. The FA RP failed to detect 1 AdV positive specimen, resulting in a PPA and NPA for AdV of 83.3% (95% CI 36.5–99.1) and 100% (95% CI 89.1–100), respectively. The positivity rate for prospectively collected specimens was 32.6%.

3.3. Overall performance of FA RP

For archived ($n = 85$) and prospectively collected ($n = 46$) study specimens combined, the initial agreement between the 2 platforms was 86.4% (kappa = 0.73 [95% CI 0.61–0.84]). After discordant analysis, concordance between FA RP and Nx TAG RPP was demonstrated for 123 of the 131 included specimens, resulting in an overall agreement of 93.9% (kappa = 0.88 [95% CI 0.80–0.96]). In total, FA RP results were not confirmed for 8 specimens after discordant analysis, 4 considered false positives and 4 considered false negatives (Table 2). The PPA and NPA for all targets in archived and prospectively collected specimens combined were 93.7% (95% CI 83.7–97.7) and 94.1% (95% CI 84.9–98.1), respectively (Table 3). Overall, there was no significant difference in the performance of the FA RP and NxTAG RPP (McNemar P = 0.72).

For single targets, the PPA was 100% for all targets detected (Table 2), except AdV (PPA = 83.3% [95% CI 36.5–99.1]), hRV/EV (PPA = 81.8% [95% CI 47.8–69.8]) and Inf A (PPA = 83.3% [95% CI 36.5–99.1]). NPA was high (NPA > 98%) for all targets (Table 2). The greatest variability was observed for hRV/EV and Inf A, with 4 and 2 discordant results respectively, noted after discordant analysis.

As B. pertussis is not included in the NxTAG RPP and positive clinical specimens were not available, contrived positive specimens were tested by FA RP. All 4 samples spiked with a *B. pertussis* isolate at the limit of detection, tested positive for the organism (data not shown). For *C. pneumoniae* no clinical specimens or isolates were available. The diagnostic performance for these 2 targets could not be evaluated.

4. Discussion

The results of this study demonstrate that the BioFire FA RP can reliably detect a broad range of respiratory pathogens when performed using specimens collected by bronchoscopy. Thus far, the majority of studies evaluating the performance of the FA RP were done using NP swabs (Andersson et al. 2014; Babady et al. 2018; Butt et al. 2014; Hayden et al. 2012; Kaku et al. 2018; Loeffelholz et al. 2011; Pierce et al. 2012; Renaud et al. 2012; Van Wesenbeeck et al. 2013). Only a few studies have included LRT specimens, such as BAL and BAS, in addition to URT samples. Azadeh et al. found that testing of BAL specimens with the FA RP increased the diagnostic yield in immunocompromised patients with an initial negative NP swab (Azadeh et al. 2018). In the

Table 1
Analysis of discordant results in archived and prospectively collected specimens.

Discordant target	FA RP result	Initial NxTAG RPP result	Repeat NxTAG RPP	Consensus result for discordant target
Archived specimens				
hCoV 229E	Negative	hCoV 229E	Negative	Negative
hCoV NL63	Negative	hCoV NL63	Negative	Negative
hCoV NL63, hRV/EV	hCoV 229E	hCoV 229E, hCoV NL63, hRV/EV	hCoV 229E + hRV/EV	hCoV NL63: Negative, hRV/EV: Positive
hMPV	hMPV	Negative	hMPV	Positive
Inf A H3	Inf A H3, PIV 3	PIV 3	PIV 3	Negative
Inf A H3	Negative	Inf A H3	Inf A H3	Positive
Inf B	Negative	Inf B	Negative	Negative
Inf B	Inf B	Negative	Negative	Negative
hRV/EV	Negative	hRV/EV	Negative	Negative
hRV/EV	Negative	hRV/EV	hRV/EV	Positive
hRV/EV	hRV/EV	Negative	hRV/EV	Positive
hRV/EV	hRV/EV	Negative	Negative	Negative
hRV/EV	hRV/EV	Negative	Negative	Negative
M. pn[a]	PIV 4, M. pn[a]	PIV 4	M. pn[a]	Positive
PIV 4	Negative	PIV 4	nd[b]	Excluded
Prospective specimens				
AdV	Negative[c]	AdV	AdV	Positive
AdV	AdV[c]	Negative	AdV	Positive
AdV	AdV	Negative	AdV	Positive

[a] *M. pneumoniae*, this sample was re-tested by LD PCR.
[b] Not done: insufficient sample for discordant analysis.
[c] Two samples from same patient, collected 1 day apart.

Table 2
Performance of FA RP compared to NxTAG RPP or consensus method (CM) for targets detected in archived and prospectively collected specimens after discordant analysis.

	No of results for FA RP/CM				PPA[a]		NPA[a]		kappa	
Target	+/+	+/−	−/+	−/−	%	95% CI	%	95% CI		95% CI
Adenovirus	5		1	125	83.3	36.5–99.1	100	96.3–100	0.91	0.72–1.0
Coronavirus 229E	3			128	100	31.0–100	100	96.4–100	1.0	1.0–1.0
Coronavirus HKU1	3			128	100	31.0–100	100	96.4–100	1.0	1.0–1.0
Coronavirus NL63	5			126	100	46.3–100	100	96.3–100	1.0	1.0–1.0
Coronavirus OC43	4			127	100	39.6–100	100	96.3–100	1.0	1.0–1.0
Metapneumovirus	5			126	100	31.0–100	100	96.3–100	1.0	1.0–1.0
Influenza A[b]	5	1	1	124	83.3	36.5–99.1	99	95.0–99.9	0.83	0.59–1.0
Influenza B	4	1		126	100	39.6–100	99.2	95.0–99.9	0.89	0.66–1.0
Parainfluenza Virus 1	1			130	100	5.5–100	100	96.4–100	1.0	1.0–1.0
Parainfluenza Virus 2	1			130	100	5.5–100	100	96.4–100	1.0	1.0–1.0
Parainfluenza Virus 3	7			124	100	56.1–100	100	96.3–100	1.0	1.0–1.0
Parainfluenza Virus 4	3			128	100	31.0–100	100	96.4–100	1.0	1.0–1.0
Respiratory Syncytial Virus	6			125	100	51.7–100	100	96.3–100	1.0	1.0–1.0
Rhinovirus/Enterovirus	9	2	2	118	81.8	47.8–69.8	98.3	93.5–99.7	0.80	0.61–0.99
Mycoplasma pneumoniae	4			127	100	39.6–100	100	96.3–100	1.0	1.0–1.0

[a] PPA: positive percent agreement; NPA: negative percent agreement.
[b] Inf A/H1–2009, $n = 3$; Inf A H3, $n = 3$; Inf A no subtype, $n = 1$.

most extensive study to date, Ruggiero et al. evaluated the performance of the FA RP on retrospective clinical and spiked LRT specimens and showed that the FA RP performed very well on these specimen types. Additionally, they determined that the limit of detection (LOD) of FA RP on LRT specimens was either lower or very similar to the LOD on NP swabs for all targets (Ruggiero et al. 2014). However, all of these studies were done on archived samples only and were limited by the small number of LRT samples included and low coverage of FA RP targets in the clinical samples tested. To our knowledge, this is the largest study to date that investigated performance of the FA RP on archived and prospectively collected bronchoscopy specimens.

We found a high positivity rate of 33% in prospectively collected bronchoscopy specimens from immunocompromised and hospitalized patients during influenza season. This finding is consistent with studies by Azadeh et al. where a similarly high positivity rate was noted for the FA RP when testing BAL in immunocompromised hosts (Azadeh et al. 2015; Azadeh et al. 2018). While syndromic molecular testing can increase sensitivity for detection of respiratory pathogens, these tests cannot distinguish between colonized and infected patients; therefore it is important to perform these tests only in patients with appropriate clinical indication. Furthermore, results need to be interpreted with caution in immunosuppressed patients where prolonged shedding is known to occur (Charlton et al. 2019).

Our study showed very good agreement between the BioFire FA RP and the Luminex NxTAG RPP for the detection of respiratory pathogens on bronchoscopy specimens. Other studies confirmed high agreement between the FA RP and various versions of the Luminex respiratory panel on NP swabs (Chan et al. 2017; Chen et al. 2016; Popowitch et al. 2013; Tang et al. 2016). A few studies, which have included NP swabs and a limited number of BW and BAL, found that FA RP detected more viruses than the Luminex method (Babady et al. 2012; Rand et al. 2011). It was not mentioned if there was a difference in test performance between BAL and NP swabs. In this study, there was no significant difference in the number of viruses detected by either system. To our knowledge a direct comparison of FA RP and NxTAG RPP has not been done on lower respiratory specimens.

The performance of the FA RP on archived and prospective specimens overall was similar, although a higher number of discordant results were noted in the archived specimens which resulted in a slightly decreased NPA when compared to prospective study specimens, possibly an effect of prolonged sample storage and multiple freeze/thaw cycles (Murphy and Bustin 2009; Shao et al. 2012).

The greatest number of discordant results was noted for the AdV, hRV/EV and Inf A targets. Possible explanations for the discordant results were low viral loads, sample degradation as a result of prolonged storage and reagent competition in specimens with multiple targets detected. Unfortunately, quantitative indicators are not provided by the assessed platforms and consequently it was difficult to determine the target concentration in the samples.

Hammond et al. found a higher number of specimens positive for hRV/EV by FA RP when compared to direct fluorescent antibody testing and real time PCR, which the authors attributed to low analyte concentrations in the samples and a slightly higher sensitivity of the FA RP assay (Hammond et al. 2012).

Previous studies have shown decreased sensitivity of AdV detection by FA RP (Couturier et al. 2013; Pierce et al. 2012; Popowitch et al. 2013). Couturier et al. noted that the LOD of FA RP compared to their lab developed tests was much higher (>2.5 log difference) for AdV than other viral targets (Couturier et al. 2013). However, these studies used an earlier version of FA RP with decreased sensitivity of AdV due to limited coverage of some AdV serotypes [FilmArray v 1.6 package insert]. The FA RP assay has since been revised and the modified FA RP version 1.7 has demonstrated increased sensitivity for AdV and improved AdV serotype coverage (Andersson et al. 2014; Doern et al. 2013). With the updated FA RP version 1.7 used in this study we did not observe any significant differences in the performance for AdV between the 2 multiplex assays. Only 1 of 3 AdV results remained discordant and was considered to be a false negative by FA RP.

Table 3
Overall performance of the FA RP in relation to NxTAG RPP or consensus method (CM) after discordant analysis.

	no of results for FA RP/CM				PPA[a]		NPA[a]		kappa		McNemar
specimens	+/+	+/−	−/+	−/−	%	95% CI	%	95% CI		95% CI	P value
retrospective ($n = 85$)	44	4	3	34	93.6	81.4–98.3	89.5	74.3–96.6	0.83	0.72–0.95	1.0
prospective ($n = 46$)	15	0	1	30	93.8	67.7–99.7	100	85.9–100	0.95	0.86–1.0	1.0
archived + prospective ($n = 131$)	59	4	4	64	93.7	83.7–97.7	94.1	84.9–98.1	0.88	0.80–0.96	0.72

[a] PPA: positive percent agreement; NPA: negative percent agreement.

While we noted 2 discordant results for Inf A H3, the overall performance of the FA RP for influenza A had good agreement with NxTAG RPP. Due to the low number of various influenza subtypes it is difficult to draw any conclusion on influenza A subtype performance.

Limitations of our study include the low number of positive samples for some pathogens detected by FA RP and the use of archived specimens with possible loss of target during prolonged storage. The FA RP results were evaluated against NxTAG RPP or a consensus method, thus the observed performance characteristics of the FA RP might be biased in favor of NxTAG RPP. As there are limitations associated with the selection of archived specimens, prospectively collected specimens have been included in this study to mitigate these effects. Furthermore, the FA RP provides only a qualitative detection of target presence and the discordant analysis is limited by the lack of any quantitative information. Due to the unavailability of a true gold standard the results of this study reflect the agreement between 2 multiplex platforms. Ideally the results of the FA RP would have been compared to single-plex PCR for each target.

In summary, the BioFire FA RP v.1.7 reliably detects respiratory pathogens in bronchoscopy specimens. The use of the FA RP to test bronchoscopy specimens in our hospital setting will enable a more complete approach to the diagnosis of LRT infections in our most vulnerable patients.

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Declaration of Competing Interest

Biomerieux Canada supplied the BioFire equipment and FA RP kits, but otherwise had no role in study design, implementation and interpretation of results or manuscript preparation.

References

Andersson ME, Olofsson S, Lindh M. Comparison of the FilmArray assay and in-house real-time PCR for detection of respiratory infection. Scand J Infect Dis 2014;46:897–901.

Azadeh N, Sakata KK, Brighton AM, Vikram HR, Grys TE. FilmArray respiratory panel assay: comparison of nasopharyngeal swabs and Bronchoalveolar lavage samples. J Clin Microbiol 2015;53:3784–7.

Azadeh N, Sakata KK, Saeed A, Mullon JJ, Grys TE, Limper AH, et al. Comparison of respiratory pathogen detection in upper versus lower respiratory tract samples using the BioFire FilmArray respiratory panel in the immunocompromised host. Can Respir J 2018;2018, 2685723.

Babady NE, Mead P, Stiles J, Brennan C, Li H, Shuptar S, et al. Comparison of the Luminex xTAG RVP fast assay and the Idaho technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. J Clin Microbiol 2012;50:2282–8.

Babady NE, England MR, Jurcic Smith KL, He T, Wijetunge DS, Tang YW, et al. Multicenter evaluation of the ePlex respiratory pathogen panel for the detection of viral and bacterial respiratory tract pathogens in nasopharyngeal swabs. J Clin Microbiol 2018;56.

Bajaj SK, Tombach B. Respiratory infections in immunocompromised patients: lung findings using chest computed tomography. Radiology of Infectious Diseases 2017;4:29–37.

Brownback KR, Thomas LA, Simpson SQ. Role of bronchoalveolar lavage in the diagnosis of pulmonary infiltrates in immunocompromised patients. Curr Opin Infect Dis 2014;27:322–8.

Butt SA, Maceira VP, McCallen ME, Stellrecht KA. Comparison of three commercial RT-PCR systems for the detection of respiratory viruses. J Clin Virol 2014;61:406–10.

Chan KH, To KKW, Li PTW, Wong TL, Zhang R, Chik KKH, et al. Evaluation of NxTAG respiratory pathogen panel and comparison with xTAG respiratory viral panel fast v2 and film Array respiratory panel for detecting respiratory pathogens in nasopharyngeal aspirates and swine/avian-origin influenza a subtypes in culture isolates. Adv Virol 2017;2017, 1324276.

Charlton CL, Babady E, Ginocchio CC, Hatchette TF, Jerris RC, Li Y, et al. Practical guidance for clinical microbiology laboratories: viruses causing acute respiratory tract infections. Clin Microbiol Rev 2019;32.

Chemaly RF, Ghosh S, Bodey GP, Rohatgi N, Safdar A, Keating MJ, et al. Respiratory viral infections in adults with hematologic malignancies and human stem cell transplantation recipients: a retrospective study at a major cancer center. Medicine 2006;85:278–87.

Chen JH, Lam HY, Yip CC, Wong SC, Chan JF, Ma ES, et al. Clinical evaluation of the new high-throughput Luminex NxTAG respiratory pathogen panel assay for multiplex respiratory pathogen detection. J Clin Microbiol 2016;54:1820–5.

Couturier MR, Barney T, Alger G, Hymas WC, Stevenson JB, Hillyard D, et al. Evaluation of the FilmArray(R) respiratory panel for clinical use in a large children's hospital. J Clin Lab Anal 2013;27:148–54.

Doern CD, Lacey D, Huang R, Haag C. Evaluation and implementation of FilmArray version 1.7 for improved detection of adenovirus respiratory tract infection. J Clin Microbiol 2013;51:4036–9.

Garbino J, Gerbase MW, Wunderli W, Deffernez C, Thomas Y, Rochat T, et al. Lower respiratory viral illnesses: improved diagnosis by molecular methods and clinical impact. Am J Respir Crit Care Med 2004;170:1197–203.

Hammond SP, Gagne LS, Stock SR, Marty FM, Gelman RS, Marasco WA, et al. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. J Clin Microbiol 2012;50:3216–21.

Hanson KE, Couturier MR. Multiplexed molecular diagnostics for respiratory, gastrointestinal, and central nervous system infections. Clin Infect Dis 2016;63:1361–7.

Hayden RT, Gu Z, Rodriguez A, Tanioka L, Ying C, Morgenstern M, et al. Comparison of two broadly multiplexed PCR systems for viral detection in clinical respiratory tract specimens from immunocompromised children. J Clin Virol 2012;53:308–13.

Jassem R, Chow R, Azana K, Gunadasa K, Paccagnella A, McNabb A, et al. Evaluation of the Luminex MAGPIX NxTAG respiratory pathogen panel. JAMMI 2016;1. https://doi.org/10.3138/jammi.1.1.13.

Kaku N, Hashiguchi K, Iwanaga Y, Akamatsu N, Matsuda J, Kosai K, et al. Evaluation of FilmArray respiratory panel multiplex polymerase chain reaction assay for detection of pathogens in adult outpatients with acute respiratory tract infection. J Infect Chemother 2018. https://doi.org/10.1016/j.jiac.2018.05.006.

Kim YJ, Boeckh M, Englund JA. Community respiratory virus infections in immunocompromised patients: hematopoietic stem cell and solid organ transplant recipients, and individuals with human immunodeficiency virus infection. Semin Respir Crit Care Med 2007;28:222–42.

Ko F, Drews SJ. The impact of commercial rapid respiratory virus diagnostic tests on patient outcomes and health system utilization. Expert Rev Mol Diagn 2017;17:917–31.

Lachant DJ, Croft DP, McGrane Minton H, Prasad P, Kottmann RM. Nasopharyngeal viral PCR in immunosuppressed patients and its association with virus detection in bronchoalveolar lavage by PCR. Respirology 2017;22:1205–11.

Loeffelholz MJ, Pong DL, Pyles RB, Xiong Y, Miller AL, Bufton KK, et al. Comparison of the FilmArray respiratory panel and Prodesse real-time PCR assays for detection of respiratory pathogens. J Clin Microbiol 2011;49:4083–8.

Murphy J, Bustin SA. Reliability of real-time reverse-transcription PCR in clinical diagnostics: gold standard or substandard? Expert Rev Mol Diagn 2009;9:187–97.

Pierce VM, Elkan M, Leet M, McGowan KL, Hodinka RL. Comparison of the Idaho technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. J Clin Microbiol 2012;50:364–71.

Popowitch EB, O'Neill SS, Miller MB. Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. J Clin Microbiol 2013;51:1528–33.

Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. PLoS One 2011;6, e26047.

Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. 2018. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev 31 e00024–17.

Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 2011;49:2449–53.

Renaud C, Crowley J, Jerome KR, Kuypers J. Comparison of FilmArray respiratory panel and laboratory-developed real-time reverse transcription-polymerase chain reaction assays for respiratory virus detection. Diagn Microbiol Infect Dis 2012;74:379–83.

Ruggiero P, McMillen T, Tang YW, Babady NE. Evaluation of the BioFire FilmArray respiratory panel and the GenMark eSensor respiratory viral panel on lower respiratory tract specimens. J Clin Microbiol 2014;52:288–90.

Shao W, Khin S, Kopp WC. Characterization of effect of repeated freeze and thaw cycles on stability of genomic DNA using pulsed field gel electrophoresis. Biopreserv Biobank 2012;10:4–11.

Tang YW, Gonsalves S, Sun JY, Stiles J, Gilhuley KA, Mikhlina A, et al. Clinical evaluation of the Luminex NxTAG respiratory pathogen panel. J Clin Microbiol 2016;54:1912–4.

Van Wesenbeeck L, Meeuws H, Van Immerseel A, Ispas G, Schmidt K, Houspie L, et al. Comparison of the FilmArray RP, Verigene RV+, and Prodesse ProFLU+/FAST+ multiplex platforms for detection of influenza viruses in clinical samples from the 2011–2012 influenza season in Belgium. J Clin Microbiol 2013;51:2977–85.

Welti M, Jaton K, Altwegg M, Sahli R, Wenger A, Bille J. Development of a multiplex real-time quantitative PCR assay to detect chlamydia pneumoniae, legionella pneumophila and mycoplasma pneumoniae in respiratory tract secretions. Diagn Microbiol Infect Dis 2003;45:85–95.