Multicenter Evaluation of the BioFire FilmArray Respiratory Panel 2 for the Detection of Viruses and Bacteria in Nasopharyngeal Swab Samples

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ABSTRACT The FilmArray® Respiratory Panel 2 (RP2) is a multiplex *in vitro* diagnostic test for the simultaneous and rapid (~45 minutes) detection of 22 pathogens directly from nasopharyngeal swab (NPS) samples. It contains updated (and in some instances redesigned) assays that improve upon the FilmArray® Respiratory Panel (RP; version 1.7), with a faster run time. The organisms identified are adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A H1, influenza A H1-2009, influenza A H3, influenza B, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, respiratory syncytial virus, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. Two new targets are included in the FilmArray RP2: Middle East respiratory syndrome coronavirus, and *Bordetella parapertussis*. This study provides data from a multicenter evaluation of 1612 prospectively collected NPS samples with performance compared to FilmArray RP or PCR and sequencing. The overall percent agreement between FilmArray RP2 and the comparator testing was 99.2%. The RP2 demonstrated a positive percent agreement of 91.7% or greater for detection of all but three analytes: coronavirus OC43, *B. parapertussis*, and *B. pertussis*. The FilmArray RP2 also demonstrated a negative percent agreement of ≥93.8% for all analytes. Of note, the adenovirus assay detects all genotypes with a demonstrated increase in sensitivity. The FilmArray RP2 represents a significant improvement over FilmArray RP with a substantially shorter run time that could aid in diagnosis of respiratory infections in a variety of clinical scenarios.

INTRODUCTION

Upper respiratory infections are common and contribute significantly to morbidity and mortality. They are also one of the leading reasons for healthcare visits thus resulting in significant healthcare costs (1, 2). Because the symptoms related to infections with many of the causative agents are very similar, definitive diagnosis requires laboratory testing. Toward that end, the concept of syndromic testing has been widely adopted with testing for multiple agents of respiratory infection at the same time with a single test. By using these syndromic diagnostics, proper antimicrobial stewardship may be better achieved by allowing antimicrobial or antiviral therapy to be given in a timely and appropriate manner (3, 4). Most importantly, it may prevent the unnecessary use of antibiotics in the face of a viral diagnosis. Additionally, studies have demonstrated that rapid diagnosis of respiratory infections can lead to decreased length of stay, better antimicrobial stewardship and better patient cohorting to prevent nosocomial infections.(3, 5-8)

The FilmArray Respiratory Panel (RP) was first introduced as a syndromic multiplex molecular test in 2011 for detection of 15 viruses; additional viral analytes and bacteria were made available with a software update in 2012 following FDA clearance for these new indications. Adenovirus inclusivity was improved with the addition of new primers following an additional FDA clearance in 2013 (version 1.7; v1.7). All FilmArray RP references henceforth in this manuscript are to the current commercially available version of the device as of the publication of this manuscript: FilmArray Respiratory Panel v1.7.

In order to ensure that a molecular diagnostic assay remains clinically relevant, and particularly for syndromic assays, it is important to periodically update the test to incorporate

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new sequence information and to accommodate emerging or previously unrecognized strains or pathogens. To this end, BioFire Diagnostics (BioFire) has updated the FilmArray RP product again by adding new assays to broaden the test's detection capabilities (particularly for adenoviruses), modifying a subset of assays to reflect newly available genetic sequences of currently included analytes, improving chemistry to enhance sensitivity overall, and for the inclusion of new analytes. The new test also has a decreased run time (~45 minutes vs ~65 minutes). The organisms detected by FilmArray RP2 include all of those identified by the FilmArray RP: adenovirus, coronavirus 229E (CoV-229E), coronavirus HKU1 (CoV-HKU1), coronavirus NL63 (CoV-NL63), coronavirus OC43 (CoV-OC43), human metapneumovirus (hMPV), human rhinovirus/enterovirus (HRV/EV), influenza A (FluA), influenza A H1 (FluA H1), influenza A H1-2009 (FluA H1-2009), influenza A H3 (FluA H3), influenza B (FluB), parainfluenza virus 1 (PIV1), parainfluenza virus 2 (PIV2), parainfluenza virus 3 (PIV3), parainfluenza virus 4 (PIV4), respiratory syncytial virus (RSV), *Bordetella pertussis* (detection of *ptxP*), *Chlamydia pneumoniae* (previously named *Chlamydophila pneumoniae*), and *Mycoplasma pneumoniae*. Two new targets are included: Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and *Bordetella parapertussis* (detection of IS*1001*). Note that results for MERS-CoV are masked in the FilmArray RP2 product that is FDA-cleared for the U.S. market. This analyte is reported in the FilmArray® Respiratory Panel 2*plus* (RP2*plus*) product, which is sold outside the U.S. for testing individuals demonstrating signs/symptoms of respiratory infection, and has been cleared by the U.S. FDA with a modified intended use to aid in the differential diagnosis of MERS-CoV infections only in cases meeting MERS-CoV clinical and/or epidemiological criteria. The FilmArray RP2 is identical to the current FilmArray RP with respect to specimen type, handling, testing workflow, pouch controls, and analysis software.

In the current study, data are presented for a prospective multicenter clinical evaluation of the performance of the FilmArray RP2 in residual nasopharyngeal swab (NPS) specimens collected in viral transport media (VTM). Performance is compared to the FilmArray RP for 20 of 22 analytes (all those in common between the two tests) as well as PCR followed by bidirectional sequencing for *B. parapertussis*. MERS-CoV was not circulating in the U.S. during the time of the study; therefore all specimens were assumed to be negative and no comparator testing was performed for this analyte.

MATERIALS AND METHODS

Clinical Specimens. The study was conducted at three geographically distinct U.S. sites (Nationwide Children's Hospital – Columbus, OH, Loyola University Medical Center – Maywood, IL, and Primary Children's Hospital – Salt Lake City, UT) over a period of approximately six months (January – March and September – November 2016). Between January and March 2016, specimens were collected and immediately frozen for later testing. Between September and November 2016, specimens were collected and tested fresh. Specimens meeting the following inclusion criteria were selected: specimen was an NPS collected in VTM with adequate residual volume (≥1.5ml), specimen was tested with FilmArray RP as Standard Of Care (SOC), and the specimen was held at room temperature for less than or equal to 4 hours or 4°C for less than or equal to three days before enrollment. A waiver of the informed consent requirement was obtained from the Institutional Review Boards (IRBs) at each study site for the use of residual NPS specimens. Clinical and demographic data were collected including hospitalization status at the time of specimen collection, the results of the clinician-ordered SOC FilmArray RP test, date of specimen collection, subject sex, and subject age at time of collection.

FilmArray RP2 Testing. Approximately 300 µl of specimen was subject to FilmArray RP2 testing according to manufacturer's instructions(9). All sample processing occurred in a biosafety cabinet with operators wearing gloves and other appropriate personal protective equipment. One sample was processed at a time and cleaning of work areas was done in accordance with manufacturer's instructions(9). The FilmArray RP2 test consists of automated nucleic acid extraction, reverse transcription, nucleic acid amplification, and results analysis in approximately 45 minutes per run (i.e. per specimen). The FilmArray® Software performs automated result analysis with each target in a valid run reported as 'Detected' or 'Not Detected'. If either internal control fails, the software automatically provides a result of 'Invalid' for all panel analytes.There are 22 targets as shown in TABLE 1, two of which are new to the FilmArray RP2. This study was conducted with an IUO version of the FilmArray RP2 that is identical to the final FDA-cleared/CE-IVD marked version. Note: Results for MERS-CoV are reported in this manuscript, but are only available for the FilmArray RP2*plus* version of the product.

Comparator Testing. Comparator testing consisted of SOC FilmArray RP testing performed at the source laboratory for all analytes in common between FilmArray RP and FilmArray RP2 (all analytes except MERS-CoV and *B. parapertussis*). All specimens were assumed negative for MERS-CoV, as it was not circulating in the U.S. during the time of enrollment for the study.

For *B. parapertussis*, two PCR assays targeting IS*1001* (the same target identified by FilmArray RP2) followed by bidirectional sequencing were used as the comparator method. Nucleic acid was extracted from specimens using a MagNA Pure LC 2.0 automated system with

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the Total Nucleic Acid Isolation – High Performance Kit (Roche Diagnostics, Indianapolis, IN). Both real-time PCR comparator assays were validated and found to have an LoD that was equivalent to the FilmArray RP2 assay. Testing was performed at BioFire in a blinded manner. Comparator assays were only considered positive when a bidirectional sequencing result of adequate quality was found to match a sequence for the expected analyte with an E-value of 1.0E-30 or lower when compared to the Genbank nucleotide database [Basic Local Alignment Search Tool (BLASTn) with default settings]. A specimen was considered to be "positive" with a sequence-confirmed result from either assay.

Results and Discrepant Analysis. A FilmArray RP2 result was considered a true positive (TP) or true negative (TN) only when it agreed with the result from the comparator method. Discrepant analysis ensued when results were discordant, i.e. false positive (FP) or false negative (FN) results. When sufficient specimen volume was available, discordant specimens were investigated using a combination of re-testing with FilmArray RP2 or comparator methods as well as testing with additional, independent molecular assays. For additional analysis of adenovirus targets, specimens were also tested with a combination of PCR assays targeting the *DBP*, *penton*, and *pol* genes (combined with bidirectional sequence analysis) (9) and the results of standard of care testing at one of the study sites (Nationwide Children's Hospital; NCH) using an adenovirus laboratory developed test (LDT) PCR targeting the hexon gene as described previously (10-12). Note that the performance data for positive percent agreement (PPA) and negative percent agreement (NPA) presented in this manuscript consist of unresolved data as presented in the package insert for the FDA-cleared test; discrepancy investigation is provided but was not used to recalculate performance data.

Statistical Analysis. The exact binomial two-sided 95% confidence intervals (95% CI) were calculated for performance measures according to the Wilson score method.

RESULTS

Demographics. A total of 1612 prospective study specimens collected from geographically/demographically diverse subject populations were analyzed in this study. Overall, the study included specimens from more male than female subjects (54%, 867/1612 and 46%, 745/1612, respectively). Most specimens were from pediatric subjects: 55% of the specimens were from children aged 5 and under, 21% were from those aged 6-21, 17% were from adults over the age of 50, and 8% were from adults aged 22-49. The majority of the specimens were obtained from hospitalized subjects and those visiting the emergency department (40%, 640/1612 and 40%, 643/1612, respectively), and 20% were obtained from subjects seen in an outpatient setting (329/1612).

FilmArray RP2 test performance. A total of 1623 specimens met the inclusion criteria and were initially tested in the clinical evaluation. The overall success rate on the initial test of these specimens was 99.3% (1611/1623); 12 tests were unsuccessful (one due to an incomplete test, one due to an instrument error, and 10 due to control failures). Eleven of these specimens were successfully retested. In addition another 10 specimens were later excluded for protocol reasons, resulting in a total of 1612 specimens included in the data analysis.

Summary of FilmArray RP2 findings. The FilmArray RP2 detected at least one analyte in 1020 of the 1612 specimens tested, yielding an overall positivity rate of 63.3% as shown in TABLE 2. The highest detection rate was seen in young children (≤ 5 years of age). The relative prevalence of each analyte among the positive specimens detected by the FilmArray RP2 is

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presented in TABLE 3. The most prevalent organisms detected during this study were HRV/EV, RSV, adenovirus, and FluA, which were found in 502 (31.1%), 199 (12.3%), 118 (7.3%), and 81 (5.0%) respectively. If taken together, coronaviruses (CoV-229E, HKU1, NL63, and OC43) were the third most prevalent target with 159 (9.9%) detections. For FluA H1 and the MERS-CoV targets, no positive analyte detections occurred in this prospective sample set. All other analytes were detected in less than 79 (< 4.9%) specimens.

The summary of performance characteristics for individual FilmArray RP2 targets is presented in TABLE 4. PPA and NPA were calculated with respect to the comparator methods along with 95% CI. The FilmArray RP2 demonstrated a PPA of 91.7% or greater for all but three analytes. Nine of 22 analytes demonstrated a PPA of 100%: CoV-HKU1, CoV-NL63, FluA, FluA H1-2009, FluA H3, FluB, PIV1, PIV4, and *C. pneumoniae*. Eight other targets demonstrated PPA of < 100%, but ≥ 90.0%: adenovirus, CoV-229E, hMPV, HRV/EV, PIV2, PIV3, RSV, and *M. pneumoniae*. For FluA H1 and MERS-CoV, no PPA could be calculated. The three analytes demonstrating a PPA < 90.0% were CoV-OC43 (80.5%), *B. parapertussis* (85.7%), and *B. pertussis* (66.7%). Additionally, nine analytes demonstrated a lower bound of the two-sided 95% CI < 80.0% due to few or no observations in the study. Overall, the FilmArray RP2 demonstrated a NPA of ≥ 93.5% for all analytes, with lower bounds of the two-sided 95% CI of ≥ 91.9%.

Comparator Analysis and Discrepancy Investigation. There were a total of 33,843 analyzable FilmArray RP2 organism results for the 1612 specimens. The overall percent agreement between FilmArray RP2 and the comparator testing was 99.2% (33,586/33,843). There were 1329 detected organism results with the FilmArray RP2; the comparator methods were positive for 1138 analytes. The overall PPA with respect to the

comparator method was 97.1% (1105/1138). There were 32,481 not detected results with the FilmArray RP2; the comparator methods were negative for 32,705 analytes. The overall NPA with respect to the comparator method was 99.3% (32,481/32,705).

Using comparator testing as truth, there were 224 FP detections and 33 FN detections overall; additional discrepancy analysis was performed for these 257 samples. For the 114 FP cases (51%) along with the 14 FN cases (42%) there was supportive evidence for the FilmArray RP2 result, bringing the adjudicated overall concordance for the positive and negative results to 98.5% and 99.7% respectively. A summary of the discrepancy investigation is presented in TABLE 5.

For the viral analytes, FilmArray RP2 detected a total of 1286 viral analytes. Using the comparator as truth, the overall PPA and NPA are 97.3% (1069/1099) and 99.2% (26,079/26,296) respectively. The results for several analytes of significant interest are further detailed below.

For adenovirus, a significant increase in detections was observed in comparison to FilmArray RP with a total of 118 detections, of which 48 (40.7%) were FP. FP specimens with sufficient volume were retested with the FilmArray RP to see if the original result had been an anomaly. When possible, specimens were also tested with a combination of PCR/sequencing assays targeting the *DBP* (N=38), *penton* (N=25), and *pol* (N=16) genes and the results of the NCH LDT assay (N=11). Combined, these investigations found additional evidence of adenovirus presence in 40 of the 48 FP specimens (TABLE 6). All 40 of these specimens had late amplification on the FilmArray RP2 test suggestive of low levels of analyte in these specimens. The four FP specimens for which the FilmArray RP retest was positive also had late amplification suggestive of a low level of analyte.

There were also 4 FN results for adenovirus. Additional discrepant analysis for these specimens included retesting with FilmArray RP2, a combination of PCR assays as above, and any available NCH LDT results for adenovirus. Combined, these investigations found additional evidence of adenovirus presence in three of the four FN specimens. Analysis of the FN specimen for which the FilmArray RP2 retest was positive indicated late amplification suggesting low analyte levels. All FN were adenovirus species C based on sequence analysis. A comprehensive summary of the adenovirus discordant analysis is provided in TABLE 6.

Among the coronaviruses, all but one of the four targets demonstrated good performance with PPA ≥91% and NPA ≥99.1%. The exception was CoV-OC43, which demonstrated a PPA of 80.5%. The majority of FN specimens observed were due to a known cross-reactivity in the comparator method (see package insert; https://www.online-ifu.com/ITI0040): a FilmArray RP Detected result for Coronavirus OC43 due to cross-reactivity with CoV-HKU1 is suspected whenever FilmArray RP reports detections for both CoV-HKU1 and CoV-OC43. This cross reactivity has been corrected by redesign of the CoV-OC43 assay for FilmArray RP2. Six of eight FilmArray RP2 FN specimens were TP for CoV-HKU1, i.e. co-detections reported by FilmArray RP and suggestive of this known cross-reactivity. As stated previously no MERS-CoV was detected in the cohort. Of note is a NPA of 100%, indicating a lack of cross-reactivity with other coronaviruses. Data for some archived MERS-CoV specimens and contrived MERS-CoV samples are provided in the manufacturer's package insert for FilmArray RP2*plus* (9).

The FluA targets showed no FP or FN detections; however there were no positive detections for FluA H1 during the study period which was predominated by FluA H1-2009. For FluB there were two FP detections which were confirmed on further investigation as TP. Influenza A, Influenza A H1, Influenza A H1-2009, and Influenza A H3 results were excluded

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from analyses for three specimens due to initial results of "Influenza A equivocal" or "Influenza A no subtype detected" from either FilmArray RP2 or FilmArray RP testing and insufficient specimen volume for re-testing.

Detections for HRV/EV were numerous with a total of 502, the highest of all detections in the trial. There were 77 FP results and 11 FN results. Specimens with sufficient volume were retested with FilmArray RP or FilmArray RP2. When possible, specimens were also tested with a combination of five PCR assays targeting the *5'UTR* gene. For the FP samples, 29 were positive with a FilmArray RP retest; late amplification for 28 of the 29 specimens were suggestive of low levels of analyte. Four more were positive with PCR assays. For the FN samples, four specimens were positive with FilmArray RP2 on repeat testing and one was positive with PCR assays. Three of the four FN specimen for which the FilmArray RP2 retest was positive had late amplification suggestive of low levels of analyte.

RSV detections totaled 199 making it the second most common analyte. Eight of 24 FP specimens were observed to contain RSV by independent molecular methods or retesting with FilmArray RP. These may have been missed by the SOC FilmArray RP test due to an estimated hundredfold difference in LoD between FilmArray RP and FilmArray RP2 (9).

Using the comparator as truth, the overall PPA and NPA are 92.3% (36/39) and 99.9% (6402/6409) respectively for all bacterial targets. The number of detections for each bacterial target was low (≤ 6) with the exception of *M. pneumoniae* (N=28) (TABLE.4). The two bacterial analytes demonstrating a PPA < 90.0% were both low prevalence: *B. parapertussis* (N = 6), and *B. pertussis* (N = 3).

The bacterial targets tended to be single analyte detections (*B. pertussis* 3/3, *C. pneumoniae* 5/6, and *M. pneumoniae* 21/28) with no co-pathogen present. For *B. parapertussis*,

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all six detections were in the context of a co-detection with one or more viruses. No sample had two bacterial targets detected. Discordant analysis for the bacterial targets in shown in TABLE 5.

DISCUSSION

This study of the FilmArray RP2 demonstrated the performance of the test in a large prospective study of 1612 residual NPS samples with 33,843 results generated. These data are significant as this is a substantial change compared to RP and the test will be adopted for use in a large number of clinical laboratories. The number of positive detections was relatively high for most organisms; notable exceptions were MERS-CoV and FluA H1, which were not circulating in the study populations during the study period. The FilmArray testing system was shown to be reliable with very few failures (99.3% success on the initial test attempt) and rapid with results available in approximately 45 minutes, which is shorter than FilmArray RP (approximately 65 minutes run time). The data presented here along with testing of archived positive NPS in VTM specimens and contrived specimens (not shown) (9) were used as part of the regulatory submissions for the FilmArray RP2 and RP2*plus*, which received 510(k) clearance in the U.S. (RP2) and CE/IVD marking in the EU (RP2*plus*) in June 2017. FilmArray RP2*plus* received *de novo* clearance in the U.S. in November, 2017.

Periodically updating testing that has been implemented is an important concept. The College of American Pathologists covers this for lab-developed testing in its Microbiology checklist stating that laboratories should have written policies and procedures to evaluate nucleic acid tests for compatibility with currently circulating microbial strains (13). For testing cleared by the FDA, FilmArray RP2 represents the fourth iteration of the multiplex panel since its introduction in 2011, providing an update of the primer probes based on a reexamination of known sequences for the majority of the pathogens and adjustment of the assay conditions to maximize performance. As noted there were a significant number of detections by RP2 that were not detected by RP (n=224). The overall design goal for RP2 was to increase sensitivity for all

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analytes relative to RP, and this may account for a significant number of the observed FP detections. This is supported by LoD studies reported in the product inserts (9, 14) and the discordant analysis performed in this study. The increased inclusivity/sensitivity and decreased time to result to 45 minutes for the FilmArray RP2 may lead to improvements in outcomes such as length of stay or proper stewardship and warrant further study.

Viruses are a common cause of upper respiratory infections in both adult and pediatric population and this was also seen in our study cohort. Viral detections were notably higher than those of the bacterial targets (1286 viral vs. 43 bacterial detections). The FilmArray RP2 showed an increased positive detection rate for all viral targets in comparison to FilmArray RP (217 more detections with 107 supported by additional discrepancy investigation) with the exception of Coronavirus OC43 (TABLE 5) reflecting the increased sensitivity and inclusivity of FilmArray RP2.

The most common viral analyte was HRV/EV with a total of 502 detections versus 436 with FilmArray RP. The increased HRV/EV detections may or may not be associated with true disease causation as the majority (61.2%, see Supplemental TABLE 1) were in the context of co-detection with other viral targets. Rhinovirus has been reported as a common detection among asymptomatic individuals with rates varying from 8 to 50% depending on the study (15-17). While FilmArray RP2 was updated to broaden inclusivity, there was no change to specificity for HRV/EV so that there are still cross reactions with enteroviruses, hence the rhinovirus/enterovirus designation.

One of the more extensive modifications occurred for the detection of adenovirus. Previous studies by Leber et al. demonstrated a lack of sensitivity with FilmArray RP for adenovirus types A, D, and F (12) despite an earlier redesign on the FilmArray RP in 2013 (18).

The redesign of the FilmArray RP2 specifically targeted all genotypes to include A-F genotypes, not only those typically associated with respiratory infections (types B, C, and E). In our cohort, genotypes A, B, C, D, and F were demonstrated to be detected by FilmArray RP2. Detection of all genotypes is important particularly in the immunocompromised where the finding of adenovirus of any genotype in the NPS in VTM may precede systemic infections (11, 12). In addition, the identification of species F in respiratory specimens has been reported in patients with respiratory illness (19) as well as in 2.3% of pediatric patient samples which were obtained after routine adenoidectomy/tonsillectomy (20).

There were relatively low numbers of bacterial detections with the FilmArray RP2 overall (N= 43). The reasons for this are likely due to true disease prevalence differences during the study period. Also, as seen in our data (Supplemental TABLE 1), the co-detection of bacteria and viruses is not common, particularly with *B. pertussis* as has been previously reported (21, 22). The target gene for *B. pertussis* in both FilmArray RP and FilmArray RP2 is the toxin promoter region. This single copy gene is known to be more specific than the more commonly used insertion sequences 481 (IS*481*) gene that is a multicopy target present in several *Bordetella* species. While having greater specificity, the toxin gene target may be less sensitive as has been reported (23). The diagnosis of pertussis-like illness is improved with the inclusion of the insertion sequence element 1001 target for *B. parapertussis* in FilmArray RP2. *B. parapertussis* is known to cause a pertussis-like illness and can co-circulate with other *Bordetella* species (24, 25). The prevalence of *B. parapertussis* is uncertain as it is not a reportable disease like *B. pertussis*, and is not tested for as commonly (26, 27). *M. pneumoniae* was the most common of the bacterial analytes with 28 detections, more than with FilmArray

RP. However, it should be noted that use of an NPS specimen for detection of *M. pneumoniae* may be suboptimal particularly when diagnosing lower respiratory tract infection (28, 29).

Overall the percentage of discrepant results was low (0.76%, N=257, TABLE 5), suggesting that the previous version of the FilmArray RP had relatively robust performance. Discrepancy analysis using FilmArray RP retests and PCR and bidirectional sequencing confirmed 114 of 224 FP (51%); strong evidence that FilmArray RP2 has increased sensitivity compared to FilmArray RP. There are some limitations for this study. This was a prospective study; however, some samples were frozen at ≤ -70°C prior to testing. However, data indicated that the frozen storage did not significantly affect performance (9). The study period bridges one calendar year (2016) and includes only two partial respiratory seasons so variations in circulating strains, particularly FluA, are limited. The comparator method for 20 of the targets was FilmArray RP. Data concerning FilmArray RP2 performance compared to other amplified platforms or culture is not provided and will await other studies. Finally, the lack of detections for MERS-CoV and FluA H1 in the prospective study limited data on the performance for these targets.

A significant redesign for the FilmArray RP2 has demonstrated excellent sensitivity and specificity in this multicenter clinical trial. This is an important step both for individual improvements in pathogen detection and as recognition by the manufacturer that continuous improvements with monitoring and inclusion of new or emerging strains or species is important. Both have been incorporated into the design of FilmArray RP2, improving its performance for the detection of infectious agents involved in respiratory infections. These changes include both new targets (MERS-CoV and *B. parapertussis*), improvements to existing targets, and decreased

time to result. These improvements, combined with the simplicity of the FilmArray RP2 testing process and a shorter time to result, make it a significant improvement in diagnostic testing.

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476 **TABLE 1** Analytes Detected by the FilmArray RP2

Analyte	Change relative to RP[a]
Viruses	
Adenovirus	Updated primers[b], additional assays
Coronavirus 229E	Updated primers
Coronavirus HKU1	*Not Modified*
Coronavirus NL63	*Not Modified*
Coronavirus OC43	Updated primers
Human Metapneumovirus	Updated primers
Human Rhinovirus/Enterovirus	Updated primers
Influenza A	Updated primers
Influenza A H1	Updated primers
Influenza A H1-2009	*Not Modified*
Influenza A H3	Updated primers
Influenza B	*Not Modified*
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	New
Parainfluenza Virus 1	Updated primers
Parainfluenza Virus 2	Updated primers
Parainfluenza Virus 3	Updated primers
Parainfluenza Virus 4	Updated primers
Respiratory Syncytial Virus	Updated primers
Bacteria	
Bordetella parapertussis (IS*1001*)	New
Bordetella pertussis (*ptxP*)	*Not Modified*
Chlamydia pneumoniae	*Not Modified*
Mycoplasma pneumoniae	Updated primers

477 [a] General pouch chemistry improvements led to increased sensitivity overall
478 [b] Assay modified for broader inclusivity.
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481 **TABLE 2** Positivity Rate for FilmArray RP2 Panel: For all Samples and By Age Groupings

All Samples (n=1612)		
	No.	% of Total
Negative Samples	592	36.7
Positive Samples	1020	63.3
Single Detections	775	48.1
Co-Detections	245	15.2
Positivity by Age Grouping		
	No.	% of Total
≤5 years (n=885)	698	78.9
6-21 years (n=331)	196	59.2
22-49 years (n=128)	53	41.4
50+ years (n=268)	73	27.2

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486 **TABLE 3** Prevalence of Detected Analytes Stratified by Age Group and Number
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FilmArray Result	Overall (N=1612)		≤5 yr (N=885)		6 – 21yr (N=331)		22 - 49 yr (N=128)		≥50 yr (N=268)	
	#	%	#	%	#	%	#	%	#	%
Viruses										
Adenovirus	118	7.3	96	10.8	18	5.4	2	1.6	2	0.7
Coronavirus 229E	16	1.0	3	0.3	7	2.1	1	0.8	5	1.9
Coronavirus HKU1	55	3.4	37	4.2	9	2.7	2	1.6	7	2.6
Coronavirus NL63	50	3.1	41	4.6	6	1.8	2	1.6	1	0.4
Coronavirus OC43	38	2.4	28	3.2	7	2.1	0	0	3	1.1
Human Metapneumovirus	81	5.0	60	6.8	12	3.6	3	2.3	6	2.2
Human Rhinovirus/Enterovirus	502	31.1	379	42.8	88	26.6	16	12.5	19	7.1
Influenza A	78	4.8	29	3.3	20	6.0	13	10.2	16	6.0
Influenza A H1	0	0	0	0	0	0	0	0	0	0
Influenza A H1-2009	74	4.6	26	2.9	19	5.7	13	10.2	16	6.0
Influenza A H3	4	0.2	3	0.3	1	0.3	0	0	0	0
Influenza B	16	1.0	7	0.8	7	2.1	1	0.8	1	0.4
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0	0	0	0	0	0	0	0	0	0
Parainfluenza Virus 1	10	0.6	9	1.0	0	0	1	0.8	0	0
Parainfluenza Virus 2	54	3.3	39	4.4	10	3.0	1	0.8	4	1.5
Parainfluenza Virus 3	53	3.3	44	5.0	6	1.8	2	1.6	1	0.4
Parainfluenza Virus 4	16	1.0	13	1.5	1	0.3	0	0	2	0.7
Respiratory Syncytial Virus	199	12.3	168	19.0	10	3.0	8	6.3	13	4.9
Bacteria										
Bordetella parapertussis (IS*1001*)	6	0.4	4	0.5	2	0.6	0	0	0	0
Bordetella pertussis (*ptxP*)	3	0.2	0	0	3	0.9	0	0	0	0
Chlamydia pneumoniae	6	0.4	1	0.1	4	1.2	1	0.8	0	0
Mycoplasma pneumoniae	28	1.7	10	1.1	14	4.2	3	2.3	1	0.4

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492 **TABLE 4** Performance summary and characteristics of the FilmArray RP2 versus those of the
493 comparator assays[a]

Analyte	Positive Percent Agreement[b]			Negative Percent Agreement[b]		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses						
Adenovirus	70/74	94.6	86.9-97.9	1490/1538	96.9	95.9-97.6
Coronavirus 229E	11/12	91.7	64.6-98.5	1595/1600	99.7	99.3-99.9
Coronavirus HKU1	43/43	100	91.8-100	1557/1569	99.2	98.7-99.6
Coronavirus NL63	40/40	100	91.2-100	1562/1572	99.4	98.8-99.7
Coronavirus OC43	33/41	80.5	66.0-89.8	1566/1571	99.7	99.3-99.9
Human Metapneumovirus	73/75	97.3	90.8-99.3	1529/1537	99.5	99.0-99.7
Human Rhinovirus/Enterovirus	425/436	97.5	95.5-98.6	1099/1176	93.5	91.9-94.7
Influenza A	78/78	100	95.3-100	1531/1531	100	99.7-100
Influenza A H1	0/0	-	-	1609/1609	100	99.8-100
Influenza A H1-2009	74/74	100	95.1-100	1535/1535	100	99.8-100
Influenza A H3	4/4	100	51.0-100	1605/1605	100	99.8-100
Influenza B	14/14	100	78.5-100	1596/1598	99.9	99.5-100
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0/0	-	-	1612/1612	100	99.8-100
Parainfluenza Virus 1	9/9	100	70.1-100	1602/1603	99.9	99.6-100
Parainfluenza Virus 2	46/47	97.9	88.9-99.6	1557/1565	99.5	99.0-99.7
Parainfluenza Virus 3	43/45	95.6	85.2-98.8	1557/1567	99.4	98.8-99.7
Parainfluenza Virus 4	9/9	100	70.1-100	1596/1603	99.6	99.1-99.8
Respiratory Syncytial Virus	175/176	99.4	96.9-99.9	1412/1436	98.3	97.5-98.9
Bacteria						
Bordetella parapertussis (IS*1001*)	6/7	85.7	48.7-97.4	1605/1605	100	99.8-100
Bordetella pertussis (*ptxP*)	2/3	66.7	20.8-93.9	1608/1609	99.9	99.6-100
Chlamydia pneumoniae	5/5	100	56.6-100	1606/1607	99.9	99.6-100
Mycoplasma pneumoniae	23/24	95.8	79.8-99.3	1583/1588	99.7	99.3-99.9

494 [a]These data are presented based on comparator assay only and do not reflect any discordant
495 analysis.

[b]The terms PPA and NPA are used instead of sensitivity and specificity to indicate that a non-gold standard comparator (e.g. PCR) was used for the analysis.

498 **TABLE 5** Results of Discrepant Investigation for FilmArray RP2

Result Disposition based on initial testing versus comparator	False Negatives			False Positives		
	Original Result	Discrepant Investigation Outcome:		Original Result	Discrepant Investigation Outcome:	
Analyte	Total	RP2 confirmed[a] (TN)	RP2 unconfirmed (FN)	Total	RP2 confirmed[a] (TP)	RP2 unconfirmed (FP)
Viruses						
Adenovirus	4	1	3	48	40	8
Coronavirus 229E	1	1	0	5	0	5
Coronavirus HKU1	0	-	-	12	3	9
Coronavirus NL63	0	-	-	10	3	7
Coronavirus OC43	8	2	6[b]	5	2	3
Human Metapneumovirus	2	2	0	8	6	2
Human Rhinovirus/Enterovirus	11	6	5	77	33	44
Influenza A	0	-	-	0	-	-
Influenza A H1	0	-	-	0	-	-
Influenza A H1-2009	0	-	-	0	-	-
Influenza A H3	0	-	-	0	-	-
Influenza B	0	-	-	2	2	0
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)	0	-	-	0	-	-
Parainfluenza Virus 1	0	-	-	1	0	1
Parainfluenza Virus 2	1	1	0	8	5	3
Parainfluenza Virus 3	2	0	2	10	4	6
Parainfluenza Virus 4	0	-	-	7	1	6
Respiratory Syncytial Virus	1	1	0	24	8	16
Bacteria						
Bordetella parapertussis (IS*1001*)	1	0	1	0	-	-
Bordetella pertussis (*ptxP*)	1	0	1	1	1	0
Chlamydia pneumoniae	0	-	-	1	1	0
Mycoplasma pneumoniae	1	0	1	5	5	0
Total	33	14	19	224	114	110

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500 [a]RP2 confirmed, the results of discrepant analysis supported the original FilmArray RP2 result as true

501 negative or true positive. RP2 unconfirmed, the results of discrepant analysis did not support the original

FilmArray RP2 result and result considered false negative or false positive. TN, true negative, FN, false negative; TP, true positive; FP, false positive.

[b]Six FN specimens were all TP for HKU1 due to a known cross reactivity in the comparator method(9)

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509 **TABLE 6** Summary of Species Determinations for all Adenovirus Positive samples.
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	Original RP2 result characterization compared to RP[a]		
Adenovirus species	Number of True Positives	Number of False Negatives	Number of False Positives[b]
A	0	0	2
B	20	0	7
C	47	3	17[c]
D	0	0	1
E	0	0	0
F	0	0	11[c]
Unable to Speciate	3	1	11
Total	**70**	**4**	**48**

511 [a]True positives= positive with RP and RP2; False negatives = RP positive, RP2 negative; False
512 positives= RP negative, RP2 positive .
513 [b] For specimens yielding a species identification (n=40), adenovirus was considered confirmed
514 (3 FN missed by RP2 and 37 FP missed by RP).
515 [c]One specimen indicated a co-infection with adenovirus species C and F
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