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# Comparison of the FilmArray Respiratory Panel and Prodesse Real-Time PCR Assays for Detection of Respiratory Pathogens▽†

M. J. Loeffelholz,[1]* D. L. Pong,[2] R. B. Pyles,[3] Y. Xiong,[4] A. L. Miller,[3] K. K. Bufton,[1] and T. Chonmaitree[1,4]

*Departments of Pathology,[1] Microbiology and Immunology,[3] and Pediatrics,[4] School of Medicine,[2] University of Texas Medical Branch, Galveston, Texas 77555*

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**We compared the diagnostic performance and overall respiratory pathogen detection rate of the premarket version of the FilmArray Respiratory Panel (RP) multiplex PCR assay (Idaho Technology, Inc., Salt Lake City, UT) with those of the Food and Drug Administration (FDA)-cleared Prodesse ProFlu+, ProFAST+, ProParaflu+, Pro hMPV+, and ProAdeno+ real-time PCR assays (Gen-Probe, San Diego, CA). The assays were performed on a panel of 192 nasopharyngeal-secretion specimens collected from 81 children under 1 year of age with upper respiratory tract symptoms. To resolve discordant results and confirm pathogens detected only by the larger FilmArray panel, we performed laboratory-developed real-time PCR assays. Among viruses detectable by both commercial assays (adenovirus, human metapneumovirus, influenza A virus, influenza B virus, parainfluenza viruses 1 to 3, and respiratory syncytial virus), the FilmArray and Prodesse assays showed good overall agreement (181/192 [94.3%]; kappa = 0.87; 95% CI, 0.79 to 0.94). FilmArray RP detected more parainfluenza viruses 1 and 3 than ProParaflu+ (18 versus 13) while ProAdeno+ detected more adenoviruses (11 versus 6), but these differences were not statistically significant. Additionally, FilmArray RP detected 138 pathogens (confirmed as true positives) not included in the Prodesse assays (rhinovirus [RV]/enterovirus [EV], 118; bocavirus, 8; coronavirus, 7; parainfluenza virus 4, 4; *Mycoplasma pneumoniae*, 1). FilmArray RP was cleared by the FDA following the completion of this study. The FDA-cleared version includes the following targets: adenovirus, coronaviruses HKU1 and NL63, human metapneumovirus (hMPV), influenza A virus (to type level only), influenza A H1 seasonal virus, influenza A H3 seasonal virus, influenza A virus H1-2009, influenza B virus, parainfluenza viruses 1 to 4, respiratory syncytial virus (RSV), and RV/EV (no differentiation). The larger panel in the FilmArray RP assay allowed the detection of additional respiratory pathogens compared to the Prodesse assays. In this population of young children with upper respiratory tract infection, RV/EV accounted for the majority of the additional pathogens detected by FilmArray RP.**

Respiratory virus diagnosis is an integral part of patient management. Accurate and rapid diagnosis can affect patient management and help prevent secondary spread of infection, prevent the use of unnecessary antibiotics, reduce costs related to unnecessary investigations, facilitate more timely (and hence more effective) use of antiviral drugs for influenza, and shorten hospital stays (6). Nucleic acid amplification tests, such as PCR, are well established as the most sensitive methods for the detection of respiratory viruses (10, 12, 17) and can provide a result in as little as an hour (23). Multiplex PCR assays are well suited for the detection of respiratory viruses, given that symptoms often are similar and that multiple viruses often cocirculate. There are several commercially available multiplex PCR assays for the detection of respiratory viruses, the performance characteristics of which have been evaluated and reported in the literature (1, 5, 20, 22, 23). Several multiplex PCR assays for respiratory viruses have been cleared by the U.S. Food and Drug Administration (FDA). The FilmArray Respiratory Panel (RP) (Idaho Technology, Inc., Salt Lake City, UT) is a highly multiplexed automated PCR assay that integrates specimen processing, nucleic acid amplification, and detection into a pouch. The premarket version of the FilmArray RP detects 17 respiratory viruses (adenovirus; bocavirus; coronaviruses 229E, HKU1, OC43, and NL63; human metapneumovirus [hMPV]; influenza A H1 seasonal virus; influenza A H3 seasonal virus; influenza A virus H1-2009; influenza B virus; parainfluenza viruses 1 to 4; respiratory syncytial virus [RSV]; and rhinovirus [RV]/enterovirus [EV]) plus three bacteria (*Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*). The FilmArray pouch contains all the reagents required for nucleic acid extraction, reverse transcription (RT)-PCR and PCR, and detection in a freeze-dried format. While the FilmArray assay has been compared to another highly multiplexed PCR assay (xTAG) (23), to our knowledge it has not been compared to the complete panel of FDA-cleared Prodesse real-time PCR assays: ProFlu+, ProFAST+, ProParaflu+, Pro hMPV+, and ProAdeno+ (Gen-Probe, Inc., San Diego, CA), which detect a total of 10 common respiratory viruses (adenovirus, hMPV, influenza A H1 seasonal virus, influenza A H3 seasonal virus, influenza A virus H1-2009, influenza B virus, parainfluenza viruses 1 to 3, and RSV).

The aim of this study was to compare the premarket version of the FilmArray RP with the FDA-cleared Prodesse real-time PCR assays ProFlu+, ProFAST+, ProParaflu+, ProhMPV+,

* Corresponding author. Mailing address: Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0740. Phone: (409) 747-2484. Fax: (409) 772-5683. E-mail: mjloeffe@utmb.edu.

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and ProAdeno+. We determined the diagnostic accuracy and overall pathogen yields in a panel of 192 nasopharyngeal secretion specimens (NPSs) collected from 81 children under 1 year of age with upper respiratory tract symptoms. In addition, we compared labor requirements and throughput, as well as instrument and reagent costs.

Following the completion of this study, the manufacturer of the FilmArray RP received clearance from the FDA to market the assay in the United States. The FDA-cleared assay includes the following targets: adenovirus, coronaviruses HKU1 and NL63, hMPV, influenza A virus (to type level only), influenza A H1 seasonal virus, influenza A H3 seasonal virus, influenza A virus H1-2009, influenza B virus, parainfluenza viruses 1 to 4, RSV, and RV/EV (no differentiation).

(The results of this study were presented in part at the 2011 Clinical Virology Symposium, Daytona Beach, FL.)

## MATERIALS AND METHODS

**Patient specimens.** NPSs ($n = 192$) collected from 81 children under 1 year of age were included in this study (mean, 2.4 NPSs/child; range, 1 to 8/child). Healthy children were enrolled at birth in a prospective study approved by the University of Texas Medical Branch Institutional Review Board. NPSs were collected within 7 days of the onset of upper respiratory tract symptoms. Specimens were collected between November 2008 and February 2011. Of the 192 NPSs, 159 (83%) were collected from children less than 6 months of age. NPS specimens were collected by vacuum suction using a suction catheter with a mucus trap (Mucaid; Laboratoires Pharmaceutiques Vygon, Écouen, France). The tubing was rinsed with 1 ml of phosphate-buffered saline (PBS). The specimen was then vortexed, the volume was measured, and aliquots were frozen at −70°C. Prior to PCR testing, NPSs were thawed and diluted in an equal volume of sterile 0.7% saline.

**FilmArray RP assay.** The FilmArray assay was performed according to the manufacturer's instructions, except that NPSs were used rather than the recommended nasopharyngeal swab specimens. In brief, 1 ml of water provided by the manufacturer was injected into the FilmArray pouch to rehydrate the reagents. Three hundred microliters of NPS was mixed with 500 μl of sample buffer, 300 μl of which (equivalent to approximately 112 μl of the original diluted NPS as described above) was then injected into the pouch. The pouch was then placed in the FilmArray instrument, and a preprogrammed run was initiated. Specimen processing in the FilmArray pouch involves mechanical lysis using zirconium beads and nucleic acid capture and purification using metallic beads. PCR is nested; the first stage is a highly multiplexed PCR, and the second stage consists of individual PCR mixtures containing a cyanine dye. Results are generated using amplification and melting curve data. The pouch contains all reagents required for specimen extraction, first-stage multiplex PCR, and individual second-stage real-time PCRs. The FilmArray RP includes two internal controls: an RNA process control that is added to the patient specimen and controls for every step inside the pouch, from lysis through extraction, reverse transcription, both PCR stages, and detection, and a DNA template control that is present in selected second-stage PCRs.

**Prodesse real-time PCR assays.** The Prodesse real-time PCR assays ProFlu+, ProFAST+, ProParaflu+, ProhMPV+, and ProAdeno+ were performed according to the manufacturer's instructions, except that NPSs were used in place of the recommended nasopharyngeal swab specimens. In brief, 50 μl of NPS was added to 130 μl of virus transport medium (Multitrans; Starplex Scientific, Inc., Etobicoke, Ontario, Canada). Following addition of 20 μl of Prodesse Universal Internal Control (Gen-Probe), specimens were processed using the easyMAG nucleic acid extractor (55-μl elution volume; bioMérieux, Inc., Durham, NC). Five microliters of specimen extract (equivalent to 1.25 μl of the original diluted NPS) was then added to 20 μl of each Prodesse master mixture and amplified using a SmartCycler (Cepheid, Sunnyvale, CA) under conditions specified by the manufacturer. Samples positive for influenza A virus in the ProFlu+ RT-PCR were also amplified using the ProFAST+ assay to identify the virus subtype. In addition to patient specimens, each Prodesse PCR run consisted of one negative extraction control, one positive extraction control, and one amplification control. Patient specimens and extraction controls also contained the Universal Internal Control.

**Laboratory-developed PCR assays (LDAs). (i) Adenovirus; bocavirus; coronaviruses 229E, NL63, and OC43; EV; hMPV; parainfluenza viruses 1 and 3; RSV; and RV.** Nucleic acids were extracted from 200 μl NPSs using MagMax Total Nucleic Acid isolation kits (Ambion/Applied Biosystems, Austin, TX) and a Biosprint 96 extraction platform (Qiagen, Valencia, CA). After extraction, the elution volume (200 μl) was diluted 1:1 with nuclease-free 0.1 mM EDTA.

cDNA was synthesized from extracted RNA using an iScript synthesis kit (Bio-Rad, Hercules, CA). Reaction mixtures (40 μl) were assembled in 96-well PCR plates through the addition of 8 μl iScript reaction mixture, 2 μl reverse transcriptase, and 30 μl of extracted RNA (equivalent to 1.5 μl of the original diluted NPS). RT was completed with a Bio-Rad C1000 thermocycler using the following protocol: (i) 1.5 min at 25°C, (ii) 42°C for 30 min, (iii) 85°C for 5 min, and (iv) indefinite hold at 4°C. The generated cDNA was analyzed immediately and then stored at −20°C.

cDNA and DNA templates were evaluated using quantitative PCR (qPCR) assays with primers amplifying adenovirus; bocavirus; coronaviruses 229E, OC43, and NL63; EV; hMPV; parainfluenza viruses 1 and 3; RSV; and RV targets (2, 4, 7–9, 11, 16, 21). A separate qPCR for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to evaluate both RNA and DNA quality (3). TaqMan probes (Sigma-Aldrich, St. Louis, MO; Integrated DNA Technologies, Coralville, IA) were used to track the specific amplification in each reaction. Each 25-μl reaction mixture contained 12.5 μl iQ supermix (Bio-Rad), 1 μl each of a 5 μM stock of forward and reverse primer, and 0.5 μl of a 7.5 μM stock of a fluorescently labeled probe for each qPCR target, 3 μl of cDNA or DNA template, and nuclease-free water. qPCR was completed in a C1000 thermocycler equipped with a CFX reaction module (Bio-Rad). Fluorescent signal data were collected at the end of each annealing/extension step. The cycling parameters for each assay were based on the references provided and were empirically confirmed using cloned amplicons. Starting quantity values were extrapolated from standard curves generated in parallel for each run. Cloned amplicons of known concentration were used to create a 10-fold dilution series run in parallel with each assay as described previously (19). Positive (spiked template of known concentration) and negative controls were included at every stage of the assays. All steps of the procedures were performed using a unidirectional work flow, including dedicated equipment and laboratory space. The limit of detection (a 100% hit rate) was ≤15 copies per PCR for all viruses except EV and RSV, for which it was 100 copies per PCR.

**(ii) Coronavirus HKU1, *M. pneumoniae*, and parainfluenza virus 4.** PCR and thermocycling conditions were developed at Idaho Technology, Inc., and are described in Table S1 in the supplemental material. NPSs were extracted as described above for Prodesse PCRs. Samples were coded and tested in blinded fashion. The specimen volume added to PCR mixtures was equivalent to 2.5 μl of the original diluted NPS. Each PCR run consisted of two positive controls of known concentration and up to six negative controls. The limits of detection of coronavirus HKU1, *M. pneumoniae*, and parainfluenza virus 4 LDAs were log 4.4 copies, 0.75 color-changing units, and 7 50% tissue culture infective doses ($TCID_{50}$) per PCR, respectively. The analytical specificity of the LDAs was confirmed by testing a large panel of upper respiratory tract pathogens and normal flora.

**Nucleotide sequencing.** Automated sequencing of the adenovirus polymerase and hexon genes was performed at the laboratory of Macrogen, Inc. (Rockville, MD), using the BigDye 3.1 terminator kit (Applied Biosystems, Carlsbad, CA) and an Applied Biosystems Model 3730xl DNA analyzer.

**Statistics.** Agreement between assays was measured using the kappa statistic. McNemar's test was used to compare proportions in 2-by-2 contingency tables. A $P$ value of ≤0.05 was considered significant.

## RESULTS

**Detection of viruses targeted by both FilmArray RP and Prodesse assays.** The performances of the FilmArray RP and Prodesse assays for individual viruses detectable by both assays were not significantly different (Table 1), although the performance for adenovirus approached statistical significance ($P = 0.0625$; McNemar's test). Of the 11 adenoviruses detected by Prodesse, 5 were false negative by FilmArray RP. Additionally, there were two, three, and one apparent false-positive FilmArray RP results for parainfluenza virus 1, parainfluenza virus 3, and RSV, respectively. In total, for viruses detectable by both assays, the FilmArray RP and Prodesse assays showed

TABLE 1. Comparison of positive and negative results by FilmArray RP and Prodesse assays[a] for viruses detectable by both assays

| Virus | No. of specimens with results: | | | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Kappa (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| | $PRO^-/FA^-$ | $PRO^+/FA^+$ | $PRO^+/FA^-$ | $PRO^-/FA^+$ | | | | | |
| Adenovirus | 181 | 6 | 5 | 0 | 54.5 | 100 | 100 | 97.3 | 0.69 (0.43–0.96) |
| Influenza A virus H1-2009 | 191 | 1 | 0 | 0 | 100 | 100 | 100 | 100 | 1 |
| Influenza B virus | 191 | 1 | 0 | 0 | 100 | 100 | 100 | 100 | 1 |
| Metapneumovirus | 180 | 12 | 0 | 0 | 100 | 100 | 100 | 100 | 1 |
| Parainfluenza virus 1 | 187 | 3 | 0 | 2 | 100 | 98.9 | 60.0 | 100 | 0.75 (0.39–1) |
| Parainfluenza virus 3 | 179 | 10 | 0 | 3 | 100 | 98.4 | 76.9 | 100 | 0.86 (0.71–1) |
| RSV | 181 | 10 | 0 | 1 | 100 | 99.5 | 90.9 | 100 | 0.95 (0.85–1) |

[a] Prodesse assays were considered the gold standard for comparison. Viruses targeted by both FilmArray RP and Prodesse assays, but not detected during this study, were as follows: influenza A H1 seasonal virus, influenza A H3 seasonal virus, and parainfluenza virus 2. PRO, Prodesse; FA, FilmArray RP; PPV, positive predictive value; NPV, negative predictive value.

good agreement (181/192 [94.3%]; kappa = 0.87; 95% confidence interval [CI], 0.79 to 0.94). Of the 11 specimens with discordant results, 6 were confirmed positive by an LDA (Table 2). Because of the observed difference in the performances of the tests for adenoviruses and the lower sensitivity of FilmArray RP for adenovirus serotypes 2 and 6 (FilmArray RP package insert, version 1, May 2011), nucleotide sequencing was performed to determine the adenovirus serotype in specimens positive by the Prodesse ProAdeno+ assay. Amplification of polymerase and hexon genes was successfully achieved for 9 of 11 specimens. GenBank BLAST yielded adenovirus polymerase accession numbers DQ105654, EU192322, EU192323, EU192325, and EU192327 and hexon accession numbers AB433289, EF429128, EU867453, EU867472, EU867486, EU867493, FJ943603, and FJ943622. Among the specimens positive for adenovirus by FilmArray RP, two were species C, serotype 1; two were species C, serotype 5; and one was species B, serotype 3. Among the specimens that were false negative by FilmArray RP, two were species C, serotype 2, and two (from the same child) were species F, serotype 41.

**Detection of additional pathogens by FilmArray RP.** FilmArray RP detected 155 pathogens not included in the Prodesse assays (bocavirus, 12; coronavirus, 9; *M. pneumoniae*, 1; parainfluenza virus 4, 4; RV/EV, 129) (Table 3). A total of 138 (89.0%) of the 155 pathogens were confirmed by LDAs: 8/12 (66.7%) bocaviruses, 7/9 (77.8%) coronaviruses, 1/1 *M. pneumoniae*, 4/4 parainfluenza virus 4, and 118/129 (91.5%) RV/EV. Comparison of FilmArray RP and LDAs for bocavirus; coronaviruses 229E, NL63, and OC43; and RV/EV showed satisfactory agreement (kappa $\geq$ 0.7) for all viruses except coronavirus OC43 (Table 3). FilmArray RP and LDAs for coronavirus HKU1, *M. pneumoniae*, and parainfluenza virus 4 were not compared because LDAs were performed (blinded) on a small subset of specimens.

TABLE 2. Resolution of FilmArray RP discordant specimens; viruses detectable by both FilmArray RP and Prodesse assays

| Virus | Result[a] | No. | No. positive by LDA |
|---|---|---|---|
| Adenovirus | $PRO^+/FA^-$ | 5 | 5 |
| Parainfluenza virus 1 | $PRO^-/FA^+$ | 2 | 0 |
| Parainfluenza virus 3 | $PRO^-/FA^+$ | 3 | 2 |
| RSV | $PRO^-/FA^+$ | 1 | 1 |

[a] PRO, Prodesse; FA, FilmArray RP.

Overall, 151 of 192 (78.6%) specimens were confirmed positive for a respiratory pathogen by FilmArray RP and 45 of 192 (23.4%) were positive by the Prodesse panel. FilmArray RP and Prodesse detected a total of 222 (confirmed) and 48 pathogens, respectively. Considering only viruses common to both commercial assay panels, FilmArray RP and Prodesse detected 44 (confirmed) and 48, respectively.

**Detection of coinfections.** Coinfections are listed in Table 4. Of 28 confirmed coinfections detected by FilmArray RP, 25 involved RV/EV. Of eight total confirmed bocaviruses detected by FilmArray RP, seven were detected with other viruses. Correspondingly, the Prodesse panel of assays, which does not detect RV/EV or bocavirus, detected many fewer coinfections.

**Frequency of invalid or failed results.** Of 192 FilmArray RP pouches, 2 (1.0%) failed to draw a vacuum and were discarded. Repeat testing using new pouches was valid for both samples. This failure rate is consistent with that previously reported (23). No FilmArray runs failed due to invalid quality control. Of the Prodesse assays, ProFlu+ was most frequently affected by sample inhibitors, with 11 (5.7%) samples invalid due to failed internal controls. Inhibition rates in the ProParaflu+, Pro hMPV+, and ProAdeno+ assays were 0, 1% (2 samples), and 3.1% (6 samples), respectively. Inhibitory specimens were not repeated. It is important to note that the sample type tested in our study, NPS, has not been validated by the manufacturers of FilmArray RP or the Prodesse assays.

## DISCUSSION

In this report, we have compared the performance of the premarket version of the FilmArray RP to that of the FDA-cleared Prodesse real-time PCR assays for respiratory viruses. The characteristics examined include the detection of viruses targeted by both assay platforms, the yield of additional pathogens in the larger FilmArray panel, assay throughput, labor, and reagent costs. LDAs were used to resolve discordant FilmArray RP and Prodesse results and to confirm FilmArray RP positive results for pathogens not included in the Prodesse assays.

Among viruses common to both FilmArray RP and the

TABLE 3. Comparison of FilmArray RP and laboratory-developed PCR assays for pathogens not included in Prodesse assays[a]

| Virus | No. of specimens with results: | | | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Kappa (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| | $LDA^-/FA^-$ | $LDA^+/FA^+$ | $LDA^+/FA^-$ | $LDA^-/FA^+$ | | | | | |
| Bocavirus | 179 | 8 | 1 | 4 | 88.9 | 97.8 | 66.7 | 99.4 | 0.75 (0.53–0.97) |
| Coronavirus 229E | 187 | 4 | 1 | 0 | 80.0 | 100 | 100 | 99.5 | 0.89 (0.66–1) |
| Coronavirus NL63 | 190 | 0 | 1 | 1 | 0 | 99.5 | 0 | 99.5 | NA |
| Coronavirus OC43 | 187 | 2 | 2 | 1 | 50.0 | 99.5 | 66.7 | 98.9 | 0.56 (0.07–1) |
| RV/EV | 63 | 118 | 0 | 11 | 100 | 85.1 | 91.5 | 100 | 0.88 (0.80–0.95) |

[a] In addition, all positive FilmArray RP results for coronavirus HKU1 ($n = 1$), *M. pneumoniae* ($n = 1$), and parainfluenza virus 4 ($n = 4$) were confirmed by LDAs. Several specimens were tested in blinded fashion by these LDAs, preventing comparison of LDA and FilmArray RP performances for these pathogens. FA, FilmArray RP; PPV, positive predictive value; NPV, negative predictive value; NA, not applicable.

Prodesse panel of real-time PCR assays, FilmArray RP missed 5 of 11 adenovirus infections and reported 2, 3, and 1 apparent false-positive parainfluenza virus 1, parainfluenza virus 3, and RSV results. Of these six FilmArray RP false positives, three were confirmed by LDAs. The FilmArray RP used a substantially larger patient specimen volume (112 μl of original diluted NPS) than the Prodesse assays (1.25 μl) and the laboratory-developed assays (1.5 μl). This could explain the additional parainfluenza viruses detected by FilmArray and the inability of LDAs to confirm all viruses detected by FilmArray. FilmArray RP missed both adenovirus serotype 2-positive specimens in this study. This serotype is a common cause of upper respiratory tract illness. Two other adenovirus-positive specimens missed by FilmArray RP (collected 2 days apart from the same child) were found to contain serotype 41, species F, which usually causes gastrointestinal symptoms; however, RV/EV was also detected in both of these specimens. According to the manufacturers' package inserts, both FilmArray RP and Prodesse ProAdeno+ will detect species F adenoviruses (FilmArray RP package insert, version 1, May 2011; Prodesse ProAdeno+ package insert, version 16, December 2010). The differences in overall diagnostic performances were not significant. However, the prevalence of some viruses was low, and this may have prevented observed differences for parainfluenza viruses and adenovirus from reaching statistical significance. Influenza viruses were extremely rare in this population of young children with upper respiratory tract symptoms. NPSs were collected over a 28-month period, which included three winter seasons and the entire 2009 H1N1 pandemic. The low subject age and the symptoms likely biased against influenza virus in this study; infants with confirmed influenza may present with more severe symptoms, such as sepsis-like illness, and are more likely to require hospitalization for diagnosis and treatment (24).

TABLE 4. Coinfections detected

| Assay | Viruses[b] | No. of coinfections |
|---|---|---|
| Prodesse | hMPV, PIV3 | 2 |
| | RSV, PIV3 | 1 |
| FilmArray RP[a] | RV/EV, PIV3 | 3 |
| | RV/EV, PIV4 | 2 |
| | RV/EV, ADV | 4 |
| | RV/EV, hMPV | 3 |
| | RV/EV, BoV | 6 |
| | RV/EV, CoV | 6 |
| | PIV3, hMPV | 2 |
| | PIV3, RSV | 1 |
| | BoV, RV/EV, RSV | 1 |

[a] Confirmed by Prodesse or laboratory-developed PCR assay.
[b] PIV3, parainfluenza virus 3; ADV, adenovirus; BoV, bocavirus.

The larger number of targets in the premarket version of FilmArray RP than of the Prodesse panel of PCR assays allowed the detection of more pathogens in our patient specimens. FilmArray RP detected 138 confirmed pathogens not included in the Prodesse assays, 118 of which were RV/EV. Additionally, FilmArray RP detected eight bocaviruses confirmed by an LDA; the clinical significance of detecting bocavirus in respiratory specimens alone remains questionable (18). The FDA-cleared version of FilmArray RP does not include bocavirus. Other respiratory pathogens that were detected only by FilmArray RP in this study (and confirmed by LDAs) were coronavirus ($n = 7$), parainfluenza virus 4 ($n = 4$), and *M. pneumoniae* ($n = 1$). Compared to the LDAs, the sensitivity of FilmArray RP for coronaviruses 229E, NL63, and OC43 was widely variable, although the data should be interpreted with caution due to the low prevalence of these viruses during the study. Excluding RV/EV, 60 specimens were positive for a pathogen by FilmArray RP (and confirmed by LDAs), compared to 45 specimens positive by the Prodesse panel of assays. In this study population of young children (over 80% of specimens were from children less than 6 months of age at the time of illness), RV/EV was the most common cause of upper respiratory tract illness. Previous studies have also shown that picornaviruses are the predominant cause of community-acquired respiratory tract infection in the first year of life (15).

Twenty-eight confirmed coinfections were detected by the FilmArray RP in this study. The majority of coinfections detected by FilmArray RP involved RV/EV. Of eight confirmed bocaviruses detected by FilmArray RP, seven were coinfections. Of six adenoviruses detected by FilmArray RP, four were coinfections (all with RV/EV). Adenovirus, RV, and bocavirus have been shown to have prolonged presence in the upper respiratory tract (13, 14, 18, 25). The two influenza viruses and *M. pneumoniae* were present only as single infections.

While two FilmArray pouches had structural failure (no vacuum), no patient specimens were invalid due to failure of the internal control. The robustness of the FilmArray RP is likely due to the nucleic acid purification step in the pouch and the use of nested PCR. First-stage multiplex PCR products (and any PCR inhibitors) are diluted 100-fold prior to their transfer to individual second-stage PCRs.

We estimated specimen throughput, labor, and instrument

and consumable costs based on the following assumptions: testing performed during one 8-hour shift, one FilmArray instrument, and one easyMAG and two SmartCyclers for Prodesse assays. A FilmArray batch consisted of one patient specimen, for which an assay could be completed in approximately 68 min. Of this time, approximately 3 min was hands on. Therefore, during one 8-hour shift, eight specimens can be tested, with the last run allowed to continue overnight. The total FilmArray hands-on time was 24 min per shift.

A Prodesse batch consisted of 22 patient specimens that were extracted along with one positive extraction control and one negative extraction control using the easyMAG. Each Prodesse amplification batch consisted of the 22 extracted patient specimens and the two extracted controls, plus one amplification control (a total of 25 PCRs). Prodesse assays were performed sequentially; when one assay was complete, another set of PCRs was ready for the next assay. In this manner, three Prodesse assays (including ProAdeno+, which has a shorter amplification program) could be completed during one 8-hour shift, and the forth assay could be started and allowed to run overnight. The total hands-on time was approximately 194 min, or 8.8 min per patient specimen. Prodesse throughput could be increased with additional batch extractions and by adding more Smartcycler blocks. Also, FilmArray RP throughput could be increased by adding more instruments. It is important to note that only one patient sample was positive for influenza A virus in our cohort of young children, so we did not include the time and labor required to perform the proFAST+ influenza A virus subtyping assay. The impact of influenza A virus subtyping on Prodesse throughput would depend on whether subtyping results were generated in real time with each batch of 22 patient specimens or whether influenza A virus-positive specimens were batched and subtyped at a later time. While the FilmArray assay was very simple to perform, with substantially less hands-on time per patient specimen than the complete panel of Prodesse assays, throughput was only 8 patient specimens per shift versus 22 for the Prodesse ProFlu+, ProParaflu+, Pro hMPV+, and ProAdeno+ assays.

Reagent and consumable costs (in U.S. dollars) for the FilmArray RP and the panel of Prodesse assays were calculated to be $129 and $185 per patient, respectively. Again, this does not take into account the cost of ProFAST+ influenza A virus subtyping due to the extremely low prevalence of influenza A virus in our study. The costs were based on reagent list prices provided by the manufacturers. All reagents and consumables necessary for the FilmArray RP are provided in the kit. For the panel of Prodesse assays, we included the cost of easyMAG extraction reagents and supplies (approximately $4.95 per patient), PCR tubes, controls, and miscellaneous supplies, such as pipette tips and test tubes. Considering easyMAG and SmartCycler list prices of $79,500 and $34,399, respectively, the total instrument cost for our Prodesse system was $148,298 (one easyMAG and two SmartCyclers). The FilmArray instrument list price was $49,500. Three FilmArray instruments (a total of $148,500) would be required to achieve a throughput equivalent to that of the Prodesse system described in this study.

In conclusion, the results of the study indicate that the premarket version of the FilmArray RP has diagnostic performance generally comparable to that of the FDA-cleared panel of Prodesse real-time PCR assays for viruses detectable by both platforms. The FilmArray RP may be less sensitive for detection of adenovirus; larger studies are required to further evaluate the performance of the adenovirus-specific reagents in the FilmArray RP. The FilmArray RP detects a large panel of respiratory pathogens in a platform that is simple to use with minimal hands-on time. The low throughput is a significant factor that laboratories must consider.

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