

Validation of a Pre-Market Version of the Idaho Technology Inc. FilmArray™ Respiratory Panel for the Detection of Respiratory Pathogens in Well-Characterized Human Respiratory Specimens

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ABSTRACT (Revised)

Validation of a Pre-Market Version of the Idaho Technology Inc. FilmArray™ Respiratory Panel for the detection of respiratory pathogens in well-characterized human respiratory specimens

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Objectives Respiratory infections are a major cause of illness worldwide. Unbiased molecular detection of respiratory pathogens is complicated by the fact that multiple agents sharing few conserved sequences cause respiratory infections. Massive multiplexed PCR assays for respiratory pathogens exist but are labor intensive and there is a prolonged time to produce a result. Idaho Technology Inc. (ITI) has developed a real-time PCR instrument called the FilmArray and an associated reagent pouch which together are capable of simultaneously detecting multiple organisms in a sample. The FilmArray pouch contains freeze-dried reagents to perform nucleic acid extraction, amplification, reverse transcription, and nested, multiplex real-time PCR. The Respiratory Panel (RP) pouch identifies 20 common and emerging viral and bacterial pathogens and results are available in less than an hour. Our objective was to help validate the RP by testing a set of well-characterized specimens.

Methods 87 specimens positive for adenovirus, influenza A, influenza B, parainfluenza virus, rhinovirus, respiratory syncytial virus, coronavirus, human metapneumovirus, *Bordetella pertussis*, or *Mycoplasma pneumoniae*, as determined by PCR and conventional methods or PCR alone, were tested by the RP assay and the results compared to the earlier testing.

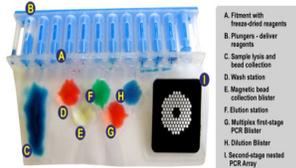
Results 68 specimens positive for a respiratory virus were tested. 9 of 68 (13%) produced invalid FilmArray results due to control failures. 7 of the 9 invalid specimens were available for retesting, resulting in 66 specimens for comparison.

Resolved results were: 66/66 specimens produced valid runs, with 65/66 (98%) of these producing results that agreed with our conventional and/or PCR testing. 19 specimens positive for either *B. pertussis* or *M. pneumoniae* were also evaluated by the FilmArray. 3 of these 19 (16%) specimens produced invalid results due to control failures. After retesting, all 19 specimens produced valid results with 12/19 (63%) valid runs yielding results that agreed with previous testing. Detection of individual agents and data from the ongoing resolution of discrepant and FilmArray failed results will be presented.

Conclusion The ITI FilmArray RP is simple to use with minimal hands-on time and has the ability to quickly detect a wide variety of viral and bacterial agents of respiratory disease. The majority of instances where the FilmArray RP failed to detect the expected agent were due to one or more control failures, an issue that ITI is working to resolve prior to formal clinical studies.

INTRODUCTION & PURPOSE

The laboratory diagnosis of respiratory tract infections is complicated by the number of microbial agents capable of causing these infections. Conventional methods can be slow or ineffective due to the inability to grow the agent in culture or to a lack of detection reagents, while molecular methods are hindered by the lack of sequence conservation between the microbes, necessitating large multiplex reactions for detection. Such multiplex assays exist but can be time consuming and labor intensive. We present here data from a study evaluating a beta version of the Idaho Technology (ITI) FilmArray Respiratory Panel (RP), a self-contained system consisting of a pouch and associated instrument. The pouch contains freeze-dried reagents for nucleic acid extraction, reverse transcription, and nested multiplex PCR capable of detecting 20 viral and bacterial respiratory pathogens. To perform a test, the reagents are rehydrated and the specimen added. The pouch is then placed in the instrument and the results are ready in less than an hour. To evaluate the FilmArray RP we tested a group of specimens that were well characterized by both conventional methods and a panel of specific PCR assays.



FilmArray Reagent Pouch



FilmArray Real-Time Instrument

METHODS

68 specimens positive for adenovirus, influenza A or B, parainfluenza virus, rhinovirus, respiratory syncytial virus (RSV), coronavirus, or human metapneumovirus (hMPV), as determined by conventional methods (culture and/or antigen detection) and PCR, or PCR alone were tested by the RP assay and the results compared to the earlier testing. Additionally, 17 specimens positive for *Bordetella pertussis* and 2 specimens positive for *Mycoplasma pneumoniae*, as determined by previous PCR testing were also included.

RESULTS

In initial testing, 9 of the 68 virus-containing (13%) specimens produced invalid results due to control failures. Because in this pre-market version of the RP assay control parameters were still being defined, after the initial testing, control parameters were adjusted and 7 of 9 specimens with sufficient remaining volume were retested, resulting in 66 specimens available for comparison. Resolved results were: 66/66 specimens produced valid runs, with 65/66 (98%) of these producing results that agreed with our previous testing (Table 1).

Table 1. Results for virus-positive specimens

VIRUS	CONVENTIONAL TESTING*	PCR	FilmArray RP ASSAY
Adenovirus	6/6	6/6	6/6
Coronavirus	N.A.*	11/11	10/11
hMPV	N.A.	5/5	5/5
Influenza A	12/16	16/16	16/16
Influenza B	6/6	6/6	6/6
Parainfluenza	9/9	9/9	9/9
Rhinovirus	6/8	8/8	8/8
RSV	5/5	5/5	5/5

Number positive/Number tested
* Not available

Because the FilmArray RP assay is a multiplex assay it can detect the presence of more than one respiratory agent in a single specimen. 19(29%) specimens were found to contain more than one virus, including 16 with 1 additional virus and 3 with 2 additional viruses. Table 2 lists the additional viruses detected according to the originally detected virus.

Table 2. Additional Viruses Detected by the FilmArray RP

Virus Originally Detected	Additional viruses detected by FilmArray RP (No. of specimens)
Adenovirus	Influenza A (1); hMPV (2); 2 different coronaviruses (1)
Coronavirus	RSV (4); Rhino (1); Inf A & Bocavirus (1)
hMPV	Coronavirus (1); RSV & Bocavirus (1)
Influenza A	Coronavirus (2)
Parainfluenza	Rhino (1); Bocavirus (1)
Rhinovirus	Coronavirus (1)
RSV	Rhino (1); Bocavirus (1)

Specimens containing 19 of the 22 additional viruses detected by the RP assay were available for retesting by the Washington University (WU) PCR assays. The presence of 16 (84%) of the additionally detected viruses was confirmed by the WU PCR assays, with the WU assays failing to detect one each of a coronavirus, RSV and rhinovirus.

17 specimens positive for *Bordetella pertussis* and 2 specimens positive for *Mycoplasma pneumoniae* by PCR testing at Washington University (WU) were also evaluated with the FilmArray RP assay. Initial testing of the 17 pertussis specimens by the RP assay resulted in 3 specimens with control failures. Following adjustment of control parameters, all 17 pertussis specimens produced valid runs, with 10/17 (59%) positive for *B. pertussis*. Interestingly, 7/17 (41%) pertussis specimens were also found to contain rhinovirus RNA, with rhinovirus being the only other respiratory agent detected in the pertussis specimens. The presence of rhinovirus RNA was confirmed by the WU rhinovirus PCR assay in 5 of the 6 pertussis specimens available for retesting. *Mycoplasma pneumoniae* DNA was detected by the RP assay in both specimens positive for *M. pneumoniae* by WU PCR.

An explanation for the lower rate of detection for the RP assay relative to the WU PCR test may have been the targets employed by the two tests. The WU pertussis assay targets the IS481 element, known to be present in the *B. pertussis* genome at 50 to 200 copies, creating an inherently sensitive assay. However, the IS481 element is not specific for *B. pertussis*, having been also detected in some strains of *Bordetella holmselii* and *Bordetella bronchiseptica*. The FilmArray RP assay targets a different *B. pertussis* sequence that may be less sensitive than assays targeting the less specific IS481 sequence.

Of the 8 pertussis specimens that were either negative, or had control failures on initial RP assay testing, retesting at Idaho Technology with a second IS481 PCR assay revealed the presence of IS481 target in all 8 specimens. However, a monoplex nested PCR assay targeting the same pertussis sequence as the RP assay was positive for only 3(38%) of the 8 specimens. As expected, testing of *Bordetella holmselii* DNA with both assays revealed the presence of the IS481 sequence but not the target of the RP assay *B. pertussis* target.

Confirmation of the greater sensitivity of the IS 481 PCR assay was obtained by testing dilutions of *Bordetella pertussis* DNA with both assays. The WU IS481 PCR assay was able to detect a dilution of pertussis DNA corresponding to 0.05 colony-forming units (CFUs) of *B. pertussis* per reaction, while the IT assay had a limit of detection that was 100-fold less sensitive at 5 CFUs (data not shown).

CONCLUSIONS

- We evaluated a beta-test version of the Idaho Technology FilmArray Respiratory Panel assay using a collection of well characterized respiratory specimens that had been previously tested by viral culture, antigen detection, and a panel of PCR assays for respiratory viruses *Bordetella pertussis* and *Mycoplasma pneumoniae*.
- When compared to the previous methods, the FilmArray Respiratory Panel was able to accurately and rapidly detect the presence of adenovirus; bocavirus; coronavirus; human metapneumovirus; influenza A & B viruses; parainfluenza virus; respiratory syncytial virus; rhinovirus and *Mycoplasma pneumoniae*.
- The multi-plex format of the FilmArray Respiratory Panel allowed it to accurately detect the presence of multiple respiratory viruses in nearly one third of the specimens tested.
- The FilmArray Respiratory Panel appeared to be less sensitive than the monoplex WU pertussis PCR assay for the detection of *B. pertussis* DNA. This was likely due to the fact that the WU assay targets the IS481 sequence which is present at 50-200 copies per pertussis genome.
- The FilmArray Respiratory Panel required minimal labor to run and the results were ready in less than an hour. However, the instrument can accommodate only one specimen at a time, potentially making through-put an issue.
- The fact that the assay functions as a closed system and does not require multiple pipetting steps reduces contamination concerns.