Respiratory Virus Detection with FilmArray Respiratory Panel Compared to Conventional Methods in Immunocompromised Patients

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Running Title: Respiratory Virus Detection in Compromised Patients

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Abstract

Respiratory virus infections cause significant morbidity and mortality in immunocompromised patients. Timely diagnosis is needed to provide optimal clinical care. Diagnostic tests routinely available at most institutions are limited by poor sensitivity and slow turnaround time. We collected 90 respiratory samples from 87 immunocompromised patients (56 bronchoalveolar lavage and 34 nasopharyngeal aspirate samples) in order to compare the performance of routine respiratory virus testing available at our institution to the FilmArray® respiratory panel assay, a novel diagnostic tool which utilizes multiplex PCR to test for 21 respiratory pathogens with a one hour turnaround time. Samples with discordant results and 13 samples with concordant results underwent further verification testing by laboratory-developed real-time PCR. The FilmArray assay identified viral pathogens in more samples than clinical testing (30/90 vs. 16/90, McNemar P=0.001). Most of the additional viral pathogens identified by the FilmArray respiratory panel assay that were confirmed by verification testing were pathogens not assessed by routine clinical tests, including rhinovirus/enterovirus, human metapneumovirus, and coronavirus. The FilmArray respiratory panel assay allowed for increased identification of respiratory viral pathogens in this cohort of immunocompromised patients.

Introduction

Patients with hematologic malignancy and recipients of stem-cell and solid organ transplants are at significant risk for severe illness due to viral respiratory tract infection (1, 5). While infection with an upper respiratory tract virus such as rhinovirus or parainfluenza virus typically results in a self-limited illness in a normal host, this type of infection can result in significant morbidity and mortality in an immunocompromised host. The severity of illness in this population is typically attributed to the frequent development of secondary infection with bacteria, fungi or other viruses and also to the spread of the virus to involve the lower respiratory tract (1, 3-6).

In order to provide optimal patient care, rapid and accurate diagnosis of viral respiratory pathogens is needed for immunocompromised patients. Though there are several respiratory viruses that can cause significant illness in this population, the symptoms of different viral respiratory tract infections are similar and do not help distinguish the specific pathogen, thus patients in whom viral respiratory tract infection is suspected need to be tested for a battery of pathogens (1, 5). Rapid and accurate identification of the specific viral pathogen(s) causing illness allows for targeted therapy where treatments exist, timely institution of appropriate infection control measures, appropriate monitoring for secondary infections, and minimization of empiric treatment for possible concerning alternative conditions. Furthermore, while there are no FDA-approved treatments for many respiratory viruses such as parainfluenza or rhinovirus, accurate identification of these viruses will allow for a better understanding of the need for and development of investigational treatment options (2).

Until recently, the primary diagnostic tools for respiratory viruses included direct fluorescent antibody (DFA) assays, enzyme immunoassays, and viral culture. While DFA assays and enzyme immunoassays have a rapid turnaround time, sensitivity is limited. Viral culture is more sensitive but requires several days of incubation before results are available. More recently PCR-based tests and specifically multiplex PCR assays for respiratory viruses have greatly improved respiratory viral diagnostics, particularly in the immunocompromised population (7, 9, 10, 12, 14). However, the technical complexity of PCR-based testing has limited its usefulness. The FilmArray Respiratory Panel (RP) is a multiplexed, fully-automated PCR assay, which is capable of detecting 18 viral respiratory pathogens and three atypical bacterial pathogens with a turn-around time of approximately 1 hour (12). The performance of this assay in the general adult and pediatric populations in comparison to DFA, other multiplex PCR-based assays, and laboratory-based PCR assays has been described (8, 11-13). The goals of the present study are to characterize the performance of the FilmArray RP assay on bronchoalveolar lavage (BAL) and nasopharyngeal aspirate (NPA) samples in the immunocompromised host population in comparison to the standard clinical testing for respiratory viruses.

Materials and Methods

Patients and samples. The study population included 87 adult patients with hematologic malignancy or recipients of hematopoietic stem-cell transplant (HSCT) or solid organ transplant (SOT) who underwent testing for viral respiratory pathogens for any clinical

indication at Dana-Farber Cancer Institute/Brigham and Women's Hospital (DFCI/ BWH) between November 2009 and September 2010. The clinical indications for testing included symptoms of an upper respiratory tract infection (URI), lower respiratory tract infection (LRI), or for surveillance of other infectious or non-infectious conditions.

Study samples were collected consecutively Monday through Friday on those BAL or NPA samples (collected from transplant recipients or patients with hematologic malignancy) from which there was fluid remaining after all aliquots necessary for clinically indicated tests were obtained. Three of the 87 patients each contributed two samples that were collected at least 1 month apart for new clinical indications, for a total of 90 samples. Fluid samples were diluted 3:1 with M4 viral transport media, aliquoted, and stored at -80°C until study testing with a research version of the FilmArray RP assay (Idaho Technology, Inc, Salt Lake City, UT) or individual PCR testing for verification was carried out. Both FilmArray and verification PCR testing were performed retrospectively such that the results had no impact on clinical decision-making.

Electronic medical records were reviewed for clinical details of patients who contributed respiratory samples including gender, age, underlying malignancy, type of transplant, and the reason for the respiratory virus testing. This study was approved by the Office of Human Research Services at DFCI/BWH.

Clinical testing. All study BAL and NPA samples were tested for one or more respiratory viruses based on clinical indications determined by the patient's clinical

providers. During the study period the following DFA respiratory virus tests were available at DFCI/BWH: Influenza A and B (Millipore, Billerica, MA), adenovirus (Millipore, Billerica, MA), respiratory syncytial virus (RSV) (Millipore, Billerica, MA and Trinity Biotech USA Inc, Jamestown, NY), and Parainfluenza 1, 2, and 3 (Diagnostic Hybrids, Athens, OH). Additionally, for BAL samples only, culture for adenovirus and multiplex PCR for Influenza A, Influenza B, and RSV (Prodesse, Gen-Probe Incorporated, San Diego, CA) were also available. Twelve BAL samples were also sent to a reference lab for human metapneumovirus (HMPV) DFA (Focus Diagnostics, Cypress, CA) based on clinical provider orders.

FilmArray testing. Patient samples were retrospectively tested at DFCI/BWH for respiratory pathogens with a pre-market version of the FilmArray RP panel which included testing for the following pathogens: influenza A (H1N1, H1N1 2009, H3N2), influenza B, RSV, parainfluenza 1-4, adenovirus, rhinovirus/enterovirus (the assay does not distinguish between these two pathogens), HMPV, coronavirus (229E, HKU1, OC43, NL63), bocavirus, *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*. The FilmArray instrument and pouch system have been described in detail elsewhere (11-13). The research use only version of the FilmArray RP system reported a cycle threshold for each positive PCR assay.

Verification PCR testing. Study samples for which clinical respiratory virus testing and FilmArray RP assay results were discordant, as well as one sample for which FilmArray and clinical testing were concordant and identified parainfluenza 3, and 12 samples

which were negative by both methods, underwent further verification testing at Idaho Technology using validated real time singleplex PCR assays. The 12 samples which were negative by both methods were randomly selected from all samples which were negative by FilmArray and clinical testing and that had more than one remaining sample aliquot.

Three separate sample preparation methods were used for verification testing including a DNA preparation to assess for Bocavirus, *B. pertussis*, *C. pneumonia*, and *M. pneumonia;* a standard RNA preparation to assess for multiple pathogens including Coronaviruses (229E, HKU1, NL63, OC43), Enterovirus, HMPV, Influenza A (H1, H1N1 2009, H3), PIV4, and Rhinovirus; and a separate standard RNA preparation to assess for RSV. Three samples underwent verification testing using the DNA preparation (including two that were negative for pathogens by both clinical testing and the FilmArray RP assay and one that was positive for Bocavirus only by the FilmArray RP assay). Twenty-four samples underwent verification testing using the standard RNA preparation for multiple pathogens (including seven samples that were negative for pathogens by both clinical testing and the FilmArray RP assay, 14 samples that had discordant results by the two testing methods, and one sample that was positive for parainfluenza 3 by both methods). Six samples underwent verification testing using the standard RNA preparation for RSV (including three samples that were negative for pathogens by both clinical testing and the FilmArray RP assay, two samples that had discordant results by the two testing methods for RSV, and one sample that tested positive for RSV by clinical testing and RSV and coronavirus by the FilmArray RP assay).

The assays used a different chemistry (real-time, singleplex PCR with hydrolysis probes) and targeted different sequences for each virus and bacterium than the assay(s) in the FilmArray (12). The targets for each organism, their primer and probe sequences as well as the Limit of Detection$_{95}$ (LoD_{95} concentrations of organism or nucleic acid at which 95% of the samples are positive) are shown in Supplementary Table 1. Inclusivity and exclusivity testing used essentially the same organisms as were tested on the FilmArray Respiratory panel (FilmArray Respiratory Panel Instruction booklet, available upon request). The comparator assays distinguish between enterovirus and human rhinovirus but this information was not used in the comparison with the FilmArray RP assay results which report only a combined result.

The QIAcube (Qiagen, Valencia, CA) was used to purify nucleic acid for the PCR or reverse transcription PCR reactions. For the DNA purification 500µl of sample was loaded into the instrument and 200µl recovered. For the RNA purifications, 140µl was loaded and 100ul recovered. 10µl of purified nucleic acid was used in each 20µl singleplex PCR reaction. Verification testing at Idaho Technology was performed on coded samples without knowledge of either the clinical testing or the FilmArray RP assay results.

Resolution of concordant and discordant results.

Patients were considered infected with a specific respiratory virus if results from clinical testing were positive and matched the FilmArray RP results. Patients were considered not to be infected if the clinical testing and FilmArray RP testing both yielded negative

results. In cases where clinical test results did not match FilmArray RP results, if verification testing was concordant with the positive clinical test result or FilmArray RP result then the patient was also considered to be infected with the respiratory virus. In cases where the clinical testing result and the FilmArray RP result were discordant and confirmatory testing was negative the patient was considered not to be infected with a respiratory virus.

Because verification testing was primarily used in cases where the clinical testing and FilmArray RP testing results were discordant, because both the FilmArray RP and verification panel tested for more pathogens than clinical testing, and because not all pathogens tested have a "gold standard" test, true positive and negative predictive values could not be estimated. However, patients were designated as having a respiratory viral disease or not (as described above) in order to tabulate a calculated positive predictive value (cPPV) and calculated negative predictive value (cNPV) for the standard clinical testing available at DFCI/BWH and the FilmArray RP assay. The samples that had concordant positive results for clinical testing and FilmArray RP did not have verification assays and may have had false positive results on both assays, so each of the cPPVs may be optimistic. Among the samples that had concordant negative results for clinical testing and FilmArray RP which did not have verification testing there may have been some samples that had false negative results, so each of the cNPVs may be optimistic.

Statistical analysis

Clinical testing and the FilmArray RP assay were compared by the exact two-sided McNemar's test. The cPPV and cNPV (as defined above) and their corresponding exact 95% binomial confidence intervals were calculated separately for clinical testing and FilmArray RP testing. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Ninety samples were obtained from 87 immunocompromised patients who were undergoing respiratory viral testing for clinical indications. Patient characteristics are shown in Table 1. Nearly half (48%) of the patients were HSCT recipients and one third (34%) were SOT recipients; the remainder had hematologic malignancy but had not undergone HSCT. Sample characteristics are shown in Table 2. The majority of samples were obtained for URI or LRI symptoms and a minority for surveillance. The majority of NPA samples were collected for URI or LRI symptoms (only one of 34 was collected for surveillance), while the majority of BAL samples were collected for LRI symptoms but with a significant number collected for surveillance (19 of 56).

The FilmArray RP assay was significantly more likely to detect a respiratory virus than routine clinical testing at DFCI/BWH. Among 90 samples, the FilmArray RP assay identified 30 with viral pathogens (including 2 samples in which 2 pathogens were detected). In contrast, routine testing at DFCI/BWH identified 16 samples with one viral pathogen each among the 90 samples. Detailed results are shown graphically in Figure 1 and in Tables 3 and 4. Among the 19 samples on which the two assays disagreed, the

FilmArray RP assay identified a viral pathogen in 16 when the clinical testing was negative or different and the clinical testing identified a viral pathogen in 2 when the FilmArray RP assay was negative. (McNemar P = 0.001). If only verified positive results were counted, there were 13 samples with discordant results and all had verification of the FilmArray RP result (McNemar P = 0.0002). When FilmArray RP assay results for viruses that could not be detected by clinical testing (coronavirus, rhinovirus/enterovirus, parainfluenza 4, and bocavirus) were excluded from analysis, there was no significant difference in the performance of the FilmArray RP assay in comparison to clinical testing (P = 0.51). No bacterial infections with *B. pertussis*, *C. pneumonia*, or *M. pneumonia* were identified by either routine clinical testing (in cases where specific testing was pursued) or by the FilmArray RP assay in any of the samples.

Three patients had two separate samples collected for different clinical indications (different respiratory tract infection symptoms in each case). Two of these patients each had one NPA and one BAL sample each collected more than a month apart with negative clinical testing and negative FilmArray RP assay results for both samples for both patients. The third patient had two NPA samples collected three months apart for different episodes of illness. The first sample tested positive for parainfluenza 1 by both clinical testing and the FilmArray RP assay and the second sample was negative for respiratory virus infection by clinical testing but was positive by FilmArray RP assay for bocavirus. When the second samples were excluded from analysis, the FilmArray RP assay still identified a viral pathogen significantly more often than clinical testing (McNemar P=0.002)

Verification testing was performed where results were discordant between the FilmArray RP and DFCI/BWH clinical testing (19 samples), on 1 sample with RSV identified by both assays and coronavirus identified only by FilmArrayRP, on one sample identified by both assays as parainfluenza 3, and on 12 concordant negative samples. Altogether 33 samples (37%) underwent verification testing. Results of the verification testing are displayed in Tables 3 and 4. Based on these results, viral disease was considered present in 26 samples (29%) (Table 3) and absent in 64 samples (71%) (Table 4). Verification testing on the 12 samples which were negative on both FilmArray RP and DFCI/BWH clinical testing were all negative.

Among the 26 samples in which viral infection was considered present, 13 (50%) had a positive concordant result by both the FilmArray RP assay and clinically indicated testing (including the sample in which RSV and coronavirus were detected by FilmArray but only the RSV was present by clinical testing). The remaining 13 (50%) had a positive result on the FilmArray RP assay that was concordant with verification testing only (one of them had PIV3 identified by clinical testing and rhinovirus/enterovirus identified by FilmArray RP and verification assays). Other than 2 samples in which RSV was identified, the pathogens detected by FilmArray RP assay and verification testing included pathogens not routinely assessed for by clinically indicated testing including parainfluenza 4, rhinovirus/enterovirus, HMPV, and coronavirus.

Among the 64 samples in which viral infection was not considered present, 4 samples tested positive by the FilmArray RP assay for at least one virus, but had negative clinical testing and verification testing. These four samples included two that tested positive for rhinovirus/enterovirus, one that tested positive for rhinovirus/enterovirus and HMPV, and one that tested positive for bocavirus. The median cycle threshold for the viruses detected by FilmArray RP in these four samples was higher than the median cycle threshold for the other 27 viruses detected by FilmArray RP assay and confirmed by validation testing (26.5 vs. 12.6). Two samples tested positive by clinical testing for parainfluenza 2 and parainfluenza 3 but had negative FilmArray RP assay results. Because the verification testing panels did not include parainfluenza 2 and parainfluenza 3 this discrepancy could not be resolved.

The cPPV and cNPV of the FilmArray RP assay and clinical testing to detect a respiratory viral infection are displayed in Table 5. The two samples that tested positive for parainfluenza by clinical testing and negative by the FilmArray RP assay which did not undergo verification testing for the pathogens detected were excluded from this analysis since the discrepancy could not be resolved. In this context, the overall cPPV of DFCI/BWH clinical testing (1.00) was greater than that of the FilmArray RP (0.87), while the cNPV of the FilmArray (1.00) was greater than that of the DFCI/BWH clinical testing (0.84). Because the indication for collection of BAL samples differed from NPA in that some BAL samples were collected for surveillance while NPA samples were mostly collected for symptoms, the cPPV and cNPV were also calculated for BAL and NPA samples individually. Among both BAL and NPA samples, the cPPV for clinical

testing was greater than for the FilmArray (BAL 1.00 vs. 0.90, NPA 1.00 vs. 0.85) while the cNPV for the FilmArray remained greater than for clinical testing (BAL 1.00 vs. 0.90, NPA 1.00 vs. 0.71). The low cPPV of the FilmArray RP assay can be attributed to the four samples described above that tested positive by the FilmArray RP assay but were not confirmed by validation testing (including two samples that tested positive for rhinovirus/enterovirus, one sample that tested positive for rhinovirus/enterovirus and HMPV, and one sample that tested positive for bocavirus). The cPPV and cNPV of the FilmArray RP assay and clinical testing overall and for NPA and BAL samples specifically did not change much when only the first samples obtained from each patient in the cohort were considered.

Discussion

These data demonstrate that in immunocompromised patients the FilmArray RP assay identified significantly more viral pathogens in BAL and NPA samples than the standard clinical testing available during the study period at our institution. Predictably, the majority of additional pathogens identified by the FilmArray RP assay included those not available by routine testing at DFCI/BWH (rhinovirus/enterovirus, coronavirus, bocavirus) and those that were only available through a reference lab testing (HMPV) which is seldom utilized due to slow turnaround time. The performance of the FilmArray RP assay in this patient population was similar to that reported previously in a general adult and pediatric patient population in which approximately 50% more viral pathogens were identified by FilmArray in comparison to traditional clinical methods, the majority of which were due to viral pathogens not typically detected by traditional

methods (13). Both the wider array of pathogens tested for and the rapid turnaround time of the FilmArray RP assay in comparison to routine testing at DFCI/BWH would fill the need for rapid diagnoses in immunocompromised patients such as those included in this cohort.

This study assessed the performance of the FilmArray RP in both NPA and BAL samples and included the largest number of BAL samples in which the performance of the FilmArray RP has been studied to date (13). The majority of BAL samples in the present study were obtained for symptoms of LRI, though some samples were obtained for surveillance of other conditions such as rejection in lung transplant recipients. In this context in which the overall number of BAL samples with any respiratory viruses detected was relatively low (9/56, 16%), the cNPV of the FilmArray RP panel on BAL samples were higher than those of routinely available clinical testing at DFCI/BWH while the cPPV was lower. In contrast, all NPA samples in the present study except one were collected from a symptomatic immunocompromised host, thus the overall number of samples with respiratory viruses present was relatively high (17/34, 50%). In this context, the cNPV of the FilmArray RP assay was also higher than clinical testing while the cPPV was lower than clinical testing. This low cPPV for the FilmArray RP assay for BAL and NPA samples was likely due to the detection of viral pathogens in one BAL sample and three NPA samples by the FilmArray RP assay that were not confirmed by validation testing.

Clinical testing identified parainfluenza virus in two samples that were not confirmed by FilmArray RP assay, including one sample with parainfluenza 2 and one sample with parainfluenza 3. Both samples were obtained by BAL in patients with symptoms of lower respiratory tract infection. Because only parainfluenza 4 was included in the verification testing panel utilized for the study, it is not clear if these two results reflect false positive clinical testing results or false negative FilmArray RP assay results and thus these samples were excluded from the cPPV and cNPV calculations.

The FilmArray RP assay identified viral pathogens in four samples (three NPA samples and one BAL sample) that were not confirmed either by clinically indicated testing or verification testing, including two samples with rhinovirus/enterovirus, one sample with rhinovirus/enterovirus and HMPV, and one sample with bocavirus. Though these results may be false positive FilmArray RP assay results, it is also possible that these viruses were indeed present in the samples but at a low enough quantity that they were not detected by verification testing. The median cycle threshold of these viruses on the FilmArray RP assay was much higher than that for viruses detected by FilmArray RP assay in other samples and confirmed by validation testing. This difference in median cycle threshold suggests that there may have been very small amounts of virus present in these samples, leading to false negative validation testing. This issue also highlights the difficulty with studying the performance of novel respiratory virus diagnostics where there is no gold standard test for many viral pathogens such as bocavirus.

In addition to the challenge presented by studying viral diagnostics for pathogens in which there is no diagnostic gold standard, this exploratory study was also limited by the relatively small number of samples and the lack of verification testing on all samples. The latter specifically limited our ability to estimate a true positive or negative predictive value, thus the cPPV and cNPV calculated with the available results are optimistic estimates.

In summary, in comparison to the routine clinical testing for respiratory viruses, the FilmArray RP assay detected more viral pathogens among samples obtained from an immunocompromised population. In addition, this assay system performed well on BAL samples. This study provides a practical real-world assessment of the performance of the FilmArray RP assay in a population in whom rapid and accurate diagnosis of viral pathogens is crucial for appropriate clinical management and development of novel therapeutics for respiratory viruses.

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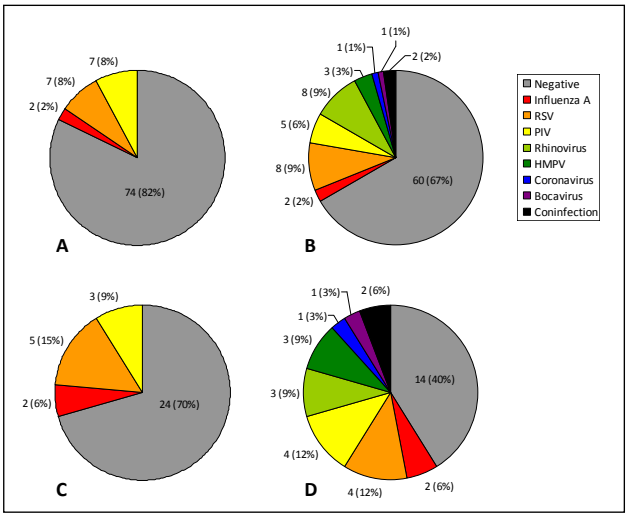

Figure 1: Respiratory virus testing results for tests routinely available at DFCI/BWH and FilmArray RP panel including the number of pathogens and percent of total. (A) Results for routine testing at DFCI/BWH for all samples (90). (B) Results for FilmArray RP assay for all samples (90). Coinfection includes one sample with RSV and Coronavirus and another with HMPV and rhinovirus. (C) Results for routine testing at DFCI/BWH for NPA samples (34). (D) Results for FilmArray RP assay for NPA samples (34). Coinfection includes one sample with RSV and Coronavirus and another with HMPV and rhinovirus.

Table 1: Baseline characteristics of 87 patients from whom respiratory samples were collected

Patient Characteristic	**Number (percent)** N = 87
Median Age, years (range)	55 (19,80)
Male Gender	53 (61)
Underlying condition[a]	
SOT[b]	30 (34)
HSCT	42 (48)
Hematologic malignancy[c]	56 (64)
Type of hematologic malignancy[c]	
Acute leukemia or myelodysplastic syndrome	24 (43)
Chronic leukemia	10 (18)
Lymphoma	18 (32)
Multiple myeloma	4 (7)

[a] SOT, solid organ transplant; HSCT, hematopoietic stem cell transplant.
[b] Includes 28 lung transplant recipients, 1 kidney transplant recipient, and 1 combined heart and kidney transplant recipient.
[c] Includes 15 patients with hematologic malignancy alone, 40 HSCT recipients who underwent transplantation for hematologic malignancy, and one SOT recipient with hematologic malignancy.

Table 2: Clinical characteristics of the 90 respiratory samples collected for clinical indications[a]

Sample Characteristic	Number (percent) N = 90
Type of sample[b]	
NPA	34 (38)
BAL	56 (62)
Clinical indication for test[c]	
URI	28 (31)
LRI	42 (47)
Surveillance	20 (22)

[a] Samples obtained from 87 patients, of whom three had two respiratory samples taken at least 1 month apart for different clinical indications

[b] NPA, nasopharyngeal aspirate; BAL, bronchoalveolar lavage

[c] URI, upper respiratory tract infection; LRI, lower respiratory tract infection

Table 3: Results from clinically indicated testing[a], FilmArray RP testing, and verification testing for samples where a respiratory viral disease was considered present based on concordance between two or more testing methods

Number of samples N = 26	**Clinical results**[b]	**FilmArray RP results**[c]	**Verification results**
2	Influenza A	Influenza A H1-09	---
6	RSV	RSV	---
2	Negative	RSV	RSV
1	RSV	RSV,Coronavirus OC43[d]	RSV[d]
1	PIV 1	PIV 1	---
1	PIV 2	PIV 2	---
2	PIV 3	PIV 3[e]	---
1	Negative	PIV 4	PIV 4
1	PIV 3	Rhinovirus/Enterovirus	Rhinovirus/Enterovirus
5	Negative	Rhinovirus/Enterovirus	Rhinovirus/Enterovirus
3	Negative	HMPV	HMPV
1	Negative	Coronavirus NL63	Coronavirus NL63

[a] Including samples collected for clinical symptoms and for surveillance
[b] RSV, respiratory syncytial virus; PIV, parainfluenza.
[c] HMPV, human metapneumovirus.
[d] Verification testing for Coronavirus OC43 was not performed on this sample, so only RSV infection was confirmed
[e] Verfication testing for other viruses including: coronaviruses (229E, HKU1, NL63, OC43), enterovirus, HMPV, influenza A (H1, H1N1 2009, H3), PIV4, and rhinovirus was performed on one of these two samples and was negative

Table 4: Results from clinically indicated testing[a], FilmArray RP testing, and verification testing for samples where respiratory viral disease was not confirmed

Number of samples N = 64	**Clinical results**[b]	**FilmArray RP results**[c]	**Verification results**
58	Negative	Negative	---[d]
1	PIV 2	Negative	---[e]
1	PIV 3	Negative	---[e]
2	Negative	Rhinovirus/Enterovirus	Negative
1	Negative	HMPV; Rhinovirus/Enterovirus	Negative
1	Negative	Bocavirus	Negative

[a] Including samples collected for clinical symptoms and for surveillance.
[b] PIV, parainfluenza.
[c] HMPV, human metapneumovirus.
[d] 12 samples which were negative for respiratory pathogens by the DFCI/BWH clinical testing and the FilmArray RP assay underwent verification testing. All 12 samples were negative for viral pathogens by verification testing.
[e] Verification testing for PIV2 or PIV3 was not performed but the sample did undergo verification testing for other viruses (coronaviruses (229E, HKU1, NL63, OC43), enterovirus, HMPV, influenza A (H1, H1N1 2009, H3), PIV4, and rhinovirus) which was negative

Table 5: Estimated positive and negative predictive values for FilmArray RP and DFCI/BWH clinically indicated testing

Sample Type[a]	Test[b]	Calculated Positive Predictive Value (CI)[c]	Calculated Negative Predictive Value (CI)[c]
All[d]	DFCI/BWH	1.00 (0.77, 1.00)	0.84 (0.73, 0.91)
	FilmArray RP	0.87 (0.69, 0.96)	1.00 (0.94, 1.00)
BAL[d]	DFCI/BWH	1.00 (0.40, 1.00)	0.90 (0.78, 0.97)
	FilmArray RP	0.90 (0.56, 1.00)	1.00 (0.92, 1.00)
NPA	DFCI/BWH	1.00 (0.74, 1.00)	0.71 (0.49, 0.87)
	FilmArray RP	0.85 (0.62, 0.97)	1.00 (0.81, 1.00)

[a] BAL, bronchoalveolar lavage; NPA, nasopharyngeal aspirate.
[b] DFCI/BWH, Dana-Farber Cancer Institute/Brigham and Women's Hospital; RP, respiratory panel.
[c] The estimated positive and negative predictive values did not change much when the second sample obtained from the three patients who underwent testing twice were excluded
[d] These estimates excluded the two samples in which parainfluenza 2 and parainfluenza 3 were detected by clinical testing but not by FilmArray RP assay since verification testing did not test for either of these pathogens