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Comparison of Viral Isolation and Multiplex Real-Time Reverse Transcription-PCR for Confirmation of Respiratory Syncytial Virus and Influenza Virus Detection by Antigen Immunoassays▽

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We evaluated the Prodesse ProFlu-1 real-time reverse transcription-PCR multiplex assay with the SmartCycler instrument for the detection of human respiratory syncytial virus (RSV) and influenza A and B viruses in comparison to conventional cell culture and antigen immunoassays with the BD Directigen A+B and Binax NOW RSV assays over two successive respiratory virus seasons. Ninety-two percent of the 361 specimens tested were nasopharyngeal aspirates obtained from individual patients, of which 119 were positive for RSV and 59 were positive for influenza virus. The median age of the patients whose specimens were positive for RSV and influenza virus were 6.3 months and 42.4 years, respectively. The specificity of all of the methods tested was ≥99%, and the individual sensitivities of NOW RSV, RSV culture, Directigen A+B, influenza virus culture, and the Proflu-1 PCR for influenza/RSV were 82% (95% confidence interval [CI], 73 to 88), 57% (95% CI, 44 to 69), 59% (95% CI, 44 to 72), 54% (95% CI, 38 to 69), and 98% (95% CI, 93 to 100)/95% (95% CI, 85 to 99), respectively. In a clinical setting where viral isolation is performed to confirm rapid antigen immunoassay results for these common respiratory viruses, one-step real-time reverse transcriptase PCR testing can be a more sensitive and timely confirmatory method.

Human respiratory syncytial virus (RSV) and influenza A and B viruses are respiratory pathogens associated with substantial morbidity and mortality annually (43). Virtually all children become infected with RSV within 2 years after birth, and 1% require hospitalization (15). Although the importance of RSV as a cause of pneumonia and brochiolitis in young children is well recognized (21), the most serious morbidity and highest mortality associated with both RSV and influenza virus circulation occurs disproportionately among elderly persons (43). The first-line tests used to detect these virus infections in many hospitals are antigen-based immunoassays. It has been demonstrated that antigen immunoassays have exceedingly poor sensitivity in detecting RSV and influenza virus infections in the elderly, seriously limiting their utility for detecting and confirming institutional or community outbreaks (7, 13, 38). This study was intended to evaluate the performance of viral isolation in cell culture, one-step real-time multiplex reverse transcription-PCR (RT-PCR), and antigen immunoassays for the detection of influenza virus and RSV in respiratory specimens from adults and children during two respiratory virus seasons.

MATERIALS AND METHODS

Specimens. Upper respiratory tract specimens were collected from 353 individual symptomatic patients during two successive winter respiratory virus seasons encompassing October 2006 to March 2007 and December 2007 to May 2008, when respiratory infection was highly prevalent in our community in southeastern Ontario. The antigen characterization and predominance of the influenza virus strains that were circulating in Canada over these respiratory seasons were 29% A(H1N1), 65% A(H3N2), and 6% B viruses for 2006 to 2007 and 36% A(N1N1), 17% A(H3N2), and 47% B viruses for 2007 to 2008. Patients were tested if they presented with acute respiratory symptoms and were under consideration for admission to the Kingston General Hospital, a 454-bed tertiary-care hospital (410 adult and 44 pediatric beds). Of the 361 specimens tested, 38 were collected by nasopharyngeal swabs, of which 15 (39%) tested positive for RSV or influenza virus. An additional 332 specimens were collected by nasopharyngeal aspiration, of which 178 (54%) tested positive for RSV or influenza virus. Specimens were tested directly within 0.5 h by antigen immunoassay for influenza virus and RSV with the Directigen A+B (BD) and NOW RSV (Binax) assays according to the respective manufacturers' instructions upon receipt at the microbiology laboratory. Specimen aliquots were also frozen at −80°C for subsequent nucleic acid purification and also forwarded at 4°C by courier twice a day at 1200 and 1400 h to a local reference laboratory for virus isolation.

RNA extraction. Eighty-seven percent of the nucleic acid extractions from frozen specimens were performed within 1 week of collection. Specimens with inhibition as determined by failed amplification of the internal control in the PCR assay were reextracted. DNA extraction was performed with the MagNA Pure Compact instrument (Roche Applied Science, Indianapolis, IN) with Nucleic Acid Isolation Kit I. Briefly, a 400-μl respiratory specimen volume was used for extraction without a prior centrifugation step, and an elution volume of 100 μl was selected. The internal control provided with the ProFlu-1 real-time assay kit (Prodesse, Waukesha, WI) was initially diluted 1:10 according to the manufacturer's instructions and diluted a second time 1:2 with water, and 20 μl of it was included and automatically incorporated into the MagNA Pure Compact isolation process.

Real-time RT-PCR. Three real-time reverse transcriptase PCR assays were used. The Prodesse Proflu-1 assay detects highly conserved regions of the RSV polymerase gene, influenza B virus nonstructural genes NS1 and NS2, and the influenza A virus matrix gene. The Cepheid RSV ASR (Cepheid, Sunnyvale, CA) assay detects nucleocapsid protein, and the gene targets for the Cepheid influenza virus (Flu A/B) assay are proprietary. The specificities of individual Cepheid RSV and influenza virus assays were evaluated, and both had 100% agreement with 30 specimens negative by viral culture, antigen testing, and Proflu-1 PCR testing. Similarly, these two assays each had 100% agreement with 30 specimens determined to be positive for their respective virus targets after

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TABLE 1. Accuracy of the Prodesse Proflu-1, Binax NOW RSV, and BD Directigen Flu A+B assays and conventional virus culture[a]

Test method	No. of samples	% Sensitivity	% Specificity	PPV	NPV	TP	FP	TN	FN	Age TP
RSV RT-PCR (Proflu-1)	318	98.2 (93.0–99.7)	100.0 (97.7–100)	100.0 (95.8–100)	99.0 (96.2–99.8)	109	0	207	2	3.6
RSV RT-PCR (Proflu-1) without 2nd PCR	318	98.0 (92.4–99.7)	95.8 (92.0–98.0)	91.7 (84.5–96.0)	99.0 (96.2–99.8)	100	9	207	2	3.8
RSV antigen EIA[b] (NOW RSV)	270	81.7 (73.2–88.1)	98.7 (94.9–99.8)	97.9 (92.0–99.6)	87.9 (81.9–92.2)	94	2	153	21	1.5
RSV culture	332	56.9 (44.1–68.9)	100.0 (98.2–100)	100.0 (88.3–100)	90.5 (86.4–93.5)	37	0	267	28	6.4
Influenza virus RT-PCR (Proflu-1)	286	94.7 (84.5–98.6)	100.0 (97.9–100)	100.0 (91.7–100)	98.7 (96.0–99.7)	54	0	229	3	45.5
Influenza virus RT-PCR (Proflu-1) without 2nd PCR	286	93.3 (80.7–98.3)	95.0 (91.3–97.3)	77.8 (64.1–87.5)	98.7 (96.0–99.7)	42	12	229	3	45.5
Influenza virus antigen EIA (Directigen Flu A+B)	180	58.8 (44.2–72.1)	99.2 (95.1–100)	96.8 (81.5–99.8)	85.9 (79.0–90.9)	30	1	128	21	44.0
Influenza virus culture	329	53.5 (37.8–68.5)	100.0 (98.3–100)	100.0 (82.2–100)	93.5 (89.9–95.9)	23	0	286	20	33.6

[a] PPV and NPV are positive and negative predictive values, TP and FP are true and false positives, and TN and FN are true and false negatives, respectively. The age in years for the true positive results of each testing method is given as a mean. Values in parentheses are 95% CIs. The accuracy of each test method was determined without repeated testing of the same specimen or patient.

[b] EIA, enzyme immunoassay.

testing by viral culture or antigen testing and Proflu-1 PCR. Specimens that produced discrepant results after initial testing with the one-step multiplex Prodesse ProFlu-1 real-time assay kit were tested further with uniplex real-time PCR assays for either influenza virus or RSV with the influenza virus (Flu A/B) (Cepheid) or RSV (Cepheid) primer and probe sets, respectively. The individual uniplex Cepheid ASR PCR assays for RSV and influenza virus that were performed with Proflu-1 PCR-positive specimens that were also negative by viral culture and antigen testing used the same extracted RNA. All three real-time reverse transcriptase PCR assays were performed with SmartCycler II instruments (Cepheid) and the respective reagent manufacturer's recommended cycling parameters. Each reaction mixture of the ProFlu-1 real-time reverse transcriptase PCR was prepared according to the manufacturer's instructions, which included 5 μl of extracted nucleic acid and 20 μl of a mixture containing Platinum *Taq* DNA polymerase (5 U/μl; Invitrogen, Carlsbad, CA), murine leukemia virus reverse transcriptase (50 U/μl; Applied Biosystems, Foster City, CA) diluted 1:10 with RT Enzyme Dilution Buffer (Prodesse), and IA/IB/RSV Mix (Prodesse). Each reaction mixture of the assays developed with the influenza virus (Flu A/B) and RSV assay primer and probe sets was prepared according to the manufacturer's instructions and included 5 μl of extracted nucleic acid and 20 μl of a mixture containing one Flu A/B or RSV ASR lyophilized bead, 50 mM $MgCl_2$ (Invitrogen) for Flu A/B only, RNase inhibitor (20 U/μl; Applied Biosystems), RNase-free water (Qiagen), and OneStep RT-PCR reagents consisting of deoxynucleoside triphosphate mix, enzyme mix, and buffer (5×) (Qiagen, Valencia, CA).

Viral isolation. WI38 (human lung fibroblast) and rhesus monkey kidney cell monolayers in culture tubes were inoculated with 4 drops of antibiotic-treated specimen and incubated at 37°C for 1 h. The cells were then fed with 1.5 ml of cell culture maintenance medium consisting of Eagle's minimum essential medium (Lonaz, Walkersville, MD). The cultures were examined daily for a cytopathic effect. Additionally, cell cultures that were negative for a cytopathic effect at days 5 and 10 had cells scraped off and tested with the D^3 Ultra DFA Respiratory Virus Screening and ID kit (Diagnostic HYBRIDS, Athens, OH) as described by the manufacturer. This culture confirmation immunostaining detected viral antigens for influenza A and B viruses, RSV, parainfluenza virus types 1 to 3, and adenovirus.

Determination of test accuracy. In addition to viral culture, specimens were also defined as true positive for influenza virus or RSV if the Proflu-1 multiplex assay was positive in combination with either a positive antigen immunoassay or a second real-time RT-PCR positive result obtained with a virus-specific real-time RT-PCR assay developed with Cepheid analyte-specific reagents. Data were obtained by testing single specimens from individual patients without repeats.

Statistical analysis. Statistical analysis was performed on all quantitative data which were considered parametric. The results of different comparisons were analyzed by performing the Student *t* test on paired data. All *P* values are two tailed. Calculations were performed with InStat3 (GraphPad Software, La Jolla, CA). Sensitivity, specificity, and positive and negative predictive values were calculated from two-by-two contingency tables for each test. Statistical comparisons were performed on the mean cycle threshold (C_T) values of specimens that were positive and negative by viral culture and antigen testing, respectively. Coefficients of determination (r^2 values) were determined from the linear correlation of C_T values obtained by comparing sequential Proflu-1 and Cepheid singleplex PCR assays by using Microsoft Excel (Microsoft Office 2003; Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

Ninety-two percent of the 361 specimens tested were nasopharyngeal aspirates, and 8% were nasopharyngeal swabs. Of the 38 nasopharyngeal swabs, 5 were positive for RSV and another 10 were positive for influenza virus. The Proflu-1 real-time RT-PCR assay had a specificity of 100% and sensitivities of 94.7% (95% confidence interval [CI], 84.5 to 98.6) and 98.2% (95% CI, 93.0 to 99.7) for the detection of influenza virus and RSV, respectively (Table 1). The accuracy of these results agrees with a previous study of the Proflu-1 assay by LeGoff et al. of a severely diseased pediatric population (28). For our 54 RSV-positive specimens tested by all three methods (119 overall), the RSV positivity rates were 94.7% for PCR, 81.7% for antigen immunoassay, and 56.9% for viral isolation. Similarly, for the 32 influenza virus-positive specimens tested by all three methods (59 overall), the detection rates were 94.7% for PCR, 58.8% for antigen immunoassay, and 53.5% for viral isolation. Other viruses that were isolated in cell culture from individual specimens included 3 isolates of adenovirus, 19 isolates of rhinovirus-like virus, 17 isolates of parainfluenza virus type 3, and 1 isolate each of parainfluenza virus types 1 and 2. One specimen that was positive for RSV by antigen immunoassay and PCR testing was considered a false negative by viral isolation, but a rhinovirus-like virus also grew in cell culture. A second specimen was identified by cell culture as a dual infection with RSV and rhinovirus-like virus. A dual infection with RSV and influenza A virus was detected in only one specimen.

Seventy-one percent of patients who were tested for RSV and 65.6% of those tested for influenza virus were less than 18 years old. Overall, the highest incidence of RSV positivity was found in pediatric patients, of whom 98 (82.4%) were between 1 month and 2 years old and of whom 10 (8.4%) were <1 month old. The mean/median patient ages associated with all of the specimens tested for RSV and the ages of the patients who tested positive for RSV were 19.7 years/1.5 years and 38.1 months/6.3 months, respectively. In contrast, 38 (64.4%) of the

influenza virus-positive samples were from individuals >18 years old, with 20 of those being >65 years old. Overall, with the Directigen A+B assay, the mean/median patient ages associated with the specimens tested and the ages of those patients who tested positive for influenza virus were 22.9 years/1.8 years and 42.0 years/40.5 years, respectively. As a consequence, since viral shedding is generally higher in children, the accuracy of RSV testing in this study should not be generalized to adults and, conversely, the results of influenza virus testing should be generalized to a pediatric population with caution.

An additional 12 influenza virus (22.2% of the total PCR positives)- and 8 RSV (7.3% of total PCR positives)-positive results with the Proflu-1 assay were reclassified from potential false positive to true positive after agreement with either the Cepheid influenza virus A/B or the Cepheid RSV PCR assay. There was highly significant agreement for the correlation of PCR C_T measurements between sequential PCR tests for influenza virus ($r^2 = 0.80$, $P < 0.0001$) and RSV ($r^2 = 0.84$, $P < 0.0001$) with the Proflu-1 and Cepheid analyte-specific reagent-derived assays. These C_T values ranged from 26.47 to 35.98 for RSV and from 20.38 to 35.95 for influenza virus. This correlation of sequential PCR C_T values supports the robustness of the determined PCR specificity since C_T values are inversely proportional to the amount of target nucleic acid detected. This type of supporting evidence for the use of PCR for influenza virus and RSV testing has not been previously demonstrated. For these positive results obtained only by sequential PCR testing, the C_T levels were indicative of an abundant nucleic acid target, defined here as a C_T of <29, in 58% and 38% of the nucleic acid extractions for influenza virus and RSV, respectively.

The sensitivity of the Proflu-1 RT-PCR was substantially higher in comparison to viral culture or the Binax NOW RSV and BD Directigen A+B antigen immunoassays (Table 1). The improved sensitivity of PCR over antigen testing and virus isolation for RSV (14, 16, 44, 45) and influenza virus (17, 20, 40, 41, 48) has been previously demonstrated by uniplex, as well as multiplex, testing (4, 23, 28, 32). Studies have also demonstrated improved sensitivity of PCR in combination with culture as a composite "gold standard" or as the alternate reference test for RSV or influenza virus (1, 19, 33, 36, 38). A very small minority of previous studies evaluating PCR detection of influenza virus and RSV have included controls to monitor inhibition due to inadequate extraction and purification of nucleic acids (16, 23, 28, 36). Estimates of sensitivity are incomplete or compromised without the inclusion of an internal control to monitor these potential false-negative results. The extraction of nucleic acids did not include an initial centrifugation of the respiratory specimens. Less inhibition was seen when respiratory specimens were first centrifuged to remove the inhibitors in cellular debris. However, this cellular material contains respiratory virus and the centrifugation step was found to decrease detection sensitivity by removing it (data not shown). In this study, 13% of the specimens tested by PCR had a failed internal control upon initial testing, which was reduced to 9% on repeat testing of the extracts after a freeze-thaw cycle and reduced further to 4% after repetition of the nucleic acid extraction and purification steps. In this study, 25% of the specimens that were initially negative for RSV or influenza virus by PCR in combination with a failed internal control were subsequently determined to be positive on repeat PCR testing with or without repeated extraction.

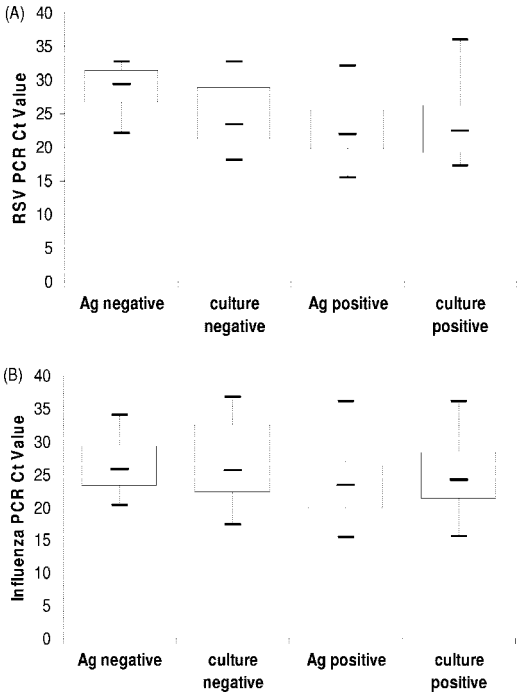

FIG. 1. Comparison of PCR C_T values obtained with the Proflu-1 multiplex assay for specimens that tested positive or negative for RSV (A) or influenza virus (B) by virus isolation or antigen immunoassay. The differences between the mean PCR C_T values of antigen (Ag)-positive and -negative specimens were significant (NOW RSV, $P < 0.0001$; Directigen A+B, $P = 0.0235$).

As might be expected for a test with higher sensitivity, the mean PCR C_T values were lower for the specimens that also tested positive for RSV or influenza virus by antigen testing or cell culture than for those specimens that tested negative (Fig. 1A and B). Significant differences were observed between the mean PCR C_T values of specimens antigen positive and negative by the NOW RSV ($P < 0.0001$) and Directigen A+B ($P = 0.0235$) assays. The simplest explanation for these results is found in the larger number of pediatric patients who tested positive for RSV than for influenza virus and the well-described association of high viral shedding in pediatric patients with RSV infections (13).

The performance of both the NOW RSV and Directigen A+B assays is at least equal to that of other commercially available antigen immunoassays (2, 6, 7, 25, 38, 47). In this study, the NOW RSV assay had a sensitivity of 81.7% (95% CI, 73.2 to 88.1), which agrees well with the results obtained previously by other laboratories when testing nasopharyngeal aspirates from pediatric patients (Table 2). The Directigen A+B assay had an overall sensitivity of 58.8% (95% CI, 44.2 to 72.1) for the detection of influenza virus. Separate sensitivities of 61.4% (95% CI, 45.5 to 75.3) for the detection of influenza A virus and 42.9% (95% CI, 11.8 to 79.8) for the detection of influenza B virus were obtained, although the number of influenza B virus-positive specimens tested was very small (Table 3). While these results compare

TABLE 2. Comparison of reports of the accuracy of the Binax NOW RSV antigen immunoassay

Reference	Yr	% Sensitivity	% Specificity	Age when tested	Total no. of tests	No. antigen positive	Specimen(s)[a]	Reference test(s)
31	2004	87.0	94.0	6.9 mo	306	Not available	NPA	DFA
34	2004	89.0	100.0	6 days to adult[b]	118	31	NPA, NPW, N	Culture
47	2004	94.6	88.5	<17.0 yr[c]	84	35	NPW	Shell vial
2	2004	89.2	100.0	<18.0 yr[d]	310[e]	102	NPW[f]	DFA, PCR, culture[g]
22	2006	87.5	100.0	<5.0 yr	91	14	NPA	DFA, culture
6	2006	73.0	100.0	<18.0 yr	130	33	NPA[f]	DFA, shell vial
10	2007	81.0	93.0	Not available[h]	14,756	794	NW[i]	Culture
This study	2008	84.0	99.0	Median, 1.5 yr[j]	256	92	NPA, NPS[j]	Culture, PCR

[a] NPA is nasopharyngeal aspirate, NW is nasal wash, NPS is nasopharyngeal swab, N is nasal, and NPW is nasopharyngeal wash.
[b] 71% of the specimens were from persons <32 months old.
[c] 81% of the specimens were from persons <3 years old.
[d] 80% of the specimens were from persons <12 months old.
[e] Specimens for antigen immunoassays were initially frozen.
[f] Specimens were in 3 ml of viral transport medium.
[g] Viral culture was performed after shipment to referral laboratory with average 36-h delay before setup.
[h] 6.5% of the specimens were from persons <1 month old.
[i] 96.2% of the specimens were nasal washes.
[j] Data from this study (the median age of RSV-positive patients was 6.2 months) include the testing of 38 nasopharyngeal swab samples, of which 5 were RSV positive.

well with recent studies (7, 25, 35, 39), there nevertheless exists substantial variability in the reported sensitivity of the Directigen A+B assay for the detection of influenza virus (Table 3). Differences due to both the specimen type tested (24) and patient age (8, 26, 38, 41) have been postulated to affect the sensitivity reported for influenza virus antigen immunoassays with the Directigen A+B and other, similar, commercial products. Neither the association nor a definitive explanation for the observed decrease in antigen immunoassay sensitivity for influenza virus detection in adult versus pediatric patients has been conclusively demonstrated to date. Young children have been reported to have higher attack rates and more prolonged viral shedding (19). Similarly, it has been empirically shown that the sensitivity of influenza virus antigen immunoassays is highest in patients ≤5 years old (38, 41). Patterns of virus shedding and differences in the quality of specimens between age groups are plausible explanations (41). This would help explain the low Directigen A+B assay sensitivity reported here, since despite the testing of samples from patients with a wide age range, the mean age of the influenza virus-positive patients was 42 years. Nevertheless, it is important to recognize that greater than 90% of the deaths due to influenza and its complications occur among elderly persons and nursing home residents are at higher risk of serious influenza-related complications than are elderly persons living in the community (18, 46). Oseltamivir postexposure prophylaxis during nursing home outbreaks is used to reduce serious complications and death but must commence within 48 h of the onset of symptoms (18, 46). In this study, PCR was the only method which provided results both rapid and accurate enough for the effective initiation of antiviral therapy.

TABLE 3. Comparison of reports of the accuracy the BD Directigen A+B antigen immunoassay

Reference	Yr	Influenza A virus		Influenza B virus		Age when tested	No. of samples	No. influenza A virus antigen positive	No. influenza B virus antigen positive	Specimen[a]	Reference test(s)
		% Sensitivity	% Specificity	% Sensitivity	% Specificity						
8	2002	96.0	99.6	87.5	96.8	<2 yr to adult[b]	250	24	28	NPA	Culture, PCR[c]
37	2002	82.9	100.0	51.5	100.0	Not available[d]	160	34	17	NPA, TS[d]	Shell vial
11	2003	82.4	100.0	70.0	99.6	Not available	155	14	7	Not available	Shell vial, DFA
38	2003	86.7	97.7	86.3	97.8	1 day to 31 yr[e]	200	13	44	NPA	Culture
25	2004	61.3	100.0	50.0	100.0	Not available[f]	77	19	14	NPA, NPS[g]	Culture, DFA[g]
7	2004	43.0	99.8	44.8	99.9	Mean, 3.2 yr[h]	4,092	49	47	NW[i]	Culture
35	2007	41.0	98.0	50.0	99.0	Median, 44 yr[j]	118	15	3	NPS	Culture
39	2007	53.0	99.7	33.0	100.0	Median, 34 yr	354	38	2	NPW, TS, NW	Culture
This study	2008	61.4	99.2	42.9	100.0	Median, 1.8 yr[k]	180	27	3	NPA, NPS	Culture, PCR

[a] NPA is nasopharyngeal aspirate, TS is throat swab, NPS is nasopharyngeal swab, and NW is nasal wash.
[b] 80% of specimens from persons <6 years old.
[c] PCR used for specimens that were culture negative but positive by antigen immunoassay or direct immunofluorescence antigen microscopy.
[d] Nasopharyngeal aspirates from pediatric patients (62.2% of positives) and throat swabs from adults (37.8% of positives).
[e] 36.5% of the specimens were from pediatric patients.
[f] 56.2% of the specimens were from pediatric patients.
[g] Specimens for antigen testing and culture were initially frozen.
[h] 2% of the patients were >18 years old.
[i] 98% nasal wash, 0.22% nasopharyngeal swab, 1.7% tracheal aspirate, 0.17% bronchoalveolar lavage, 0.1% sinus wash, and 0.06% sputum samples.
[j] Overall estimate with combined specimens for antigen testing and DFA.
[k] Data are from this study (the mean and median ages of influenza virus-positive patients were 42.7 and 42.4 years, respectively), where 33 of 50 samples were influenza A virus positive, 3 of 6 samples were influenza B virus positive, and 10 of 38 nasopharyngeal swab samples were influenza virus positive.

The Binax NOW RSV and Directigen Flu A+B antigen immunoassays had sensitivities that were 25% and 5% higher than that of culture, respectively (Table 1). The thermolability of RSV is well described, and samples must be kept cold during transport without freeze-thawing and be inoculated onto a cell culture as quickly as possible (2, 9). This fact may account for the lower sensitivity of RSV isolation in this study since the inoculation of our specimens onto a cell culture was delayed. The difference between the mean PCR C_T values of culture-positive and -negative specimens was not statistically significant for RSV or influenza virus. When this observation is considered together with the similar wide distribution of the interquartile range of PCR C_T values (Fig. 1), it suggests that viral nucleic acid was present in a range of quantities in both culture-positive and culture-negative specimens. The simplest explanation for this observation is that the loss of virus culturability occurred with minimal RNA and antigen degradation and that this was an important contributing cause of the lower sensitivities observed with culture. A range of different sensitivities for virus isolation have been reported in other studies. Importantly, a small number of studies have described the immediate culture of pediatric patient specimens as having a very high sensitivity for RSV and influenza virus detection when cell culture was performed on site (20, 37). In addition to the importance of immediate virus isolation for accurate cell culture results, the methodology is also heterogeneous and the sensitivity for the detection of these viruses differs when different cell culture methods are used (12, 27, 29, 42).

Isolation of RSV and influenza virus with R-mix cells can provide positive culture results in as little as 1 to 2 days, whereas conventional tube culture methods typically provide a 5- to 6-day turnaround time for positive specimens (12, 42). The average turnaround times in this study for specimens positive by tube cell culture for RSV and influenza virus were 10.6 and 8.8 days, respectively. This prolonged turnaround time for viral isolation reduced its usefulness in patient management. Moreover, in this study, the sensitivity of viral isolation performed off site was shown to be 53.5% (95% CI, 37.8 to 68.5) and 56.9% (95% CI,44.1 to 68.9) for influenza virus and RSV, respectively, greatly diminishing the use of viral culture as a confirmatory method. In contrast, with real-time RT-PCR, eight specimens required an average of 45 min for the extraction and purification of nucleic acids by a semiautomated method, which was followed by 45 min for the preparation of the Proflu-1 assay and 97 min of cycling run time (a total of 3 h).

The definitive diagnosis of RSV and influenza virus infections depends on the microbiology laboratory. A rapid diagnosis of infections with these viruses is required to implement effective infection control measures to limit nosocomial transmission but is also associated with a reduced length of hospitalization and other hospital-related costs (3, 5, 30). In the case of influenza virus testing in particular, the annual impact of this virus is expected to intensify since people aged ≥80 years are the fastest growing segment of the U.S. population (18). PCR testing represents an alternative to the unacceptably low sensitivity of rapid antigen immunoassays for influenza virus in this high-risk group of people. In clinical practice, antigen immunoassays and direct immunofluorescence antigen (DFA) testing with fluorescent antibody have often been relied upon to make a rapid diagnosis of influenza virus and RSV infections. DFA testing with fluorescent antibody remains an excellent screening test, although the technical time required to obtain results and the subjective nature of the results can be serious limitations. Rapid antigen testing by immunoassay methods remains a very attractive option for laboratories because they are convenient and rapid and possess a high positive predictive value. Although rapid results can be obtained in 30 min by this method, the sensitivity can be low and negative results require confirmation by more sensitive testing. Viral isolation by cell culture has traditionally been the method used for this purpose; however, these results can be delayed, thereby negating the potential impact of confirmatory testing on patient care. In a clinical setting where the sensitivity of viral isolation is less than optimal, real-time reverse transcriptase PCR testing is a more accurate and timely confirmatory test for influenza virus and RSV antigen testing.

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