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Evaluation of the Hexaplex Assay for Detection of Respiratory Viruses in Children

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The Hexaplex assay (Prodesse, Inc., Milwaukee, Wis.) is a multiplex reverse transcriptase (RT)-PCR assay for the detection of parainfluenza virus types 1, 2, and 3, respiratory syncytial virus (RSV) types A and B, and influenza virus types A and B. We evaluated the Hexaplex assay in comparison with conventional viral cell cultures and rapid enzyme immunoassays (EIAs) for RSV (Directigen; Becton Dickinson Inc., Cockeysville, Md.) and influenza A virus (Abbott Test Pack; Abbott Laboratories, Abbott Park, Ill.) for the detection of respiratory viruses from pediatric respiratory specimens obtained from children seen at Children's Hospital of Wisconsin from December 1997 through May 1998. A total of 363 respiratory specimens were evaluated. The tissue culture prevalence of parainfluenza virus during this period of time was low (1.1%). The sensitivity, specificity, and positive and negative predictive value of Hexaplex compared to tissue culture for the detection of parainfluenza virus were 100, 95.8, 19.0, and 100%, respectively. Only one specimen was determined to contain influenza B virus by Hexaplex; it was tissue culture negative. A specimen was considered to contain RSV or influenza A virus when it was either culture positive or culture negative but Hexaplex and EIA positive. Prior to the analysis of discrepant results, the sensitivity, specificity, and positive and negative predictive value for the detection of RSV were 91.2, 100, 100, and 98.0%, respectively, for tissue culture; 84.5, 100, 100, and 96.6% for EIA; and 98.5, 91.5, 72.8, and 99.6% for Hexaplex, respectively. The sensitivity, specificity, and positive and negative predictive value for the detection of influenza A virus prior to the analysis of discrepant results were 100, 100, 100, and 100%, respectively, for culture, 78.0, 100, 100, and 89.4% for EIA, respectively, and 95.1, 94.1, 67.2, and 99.3% for Hexaplex, respectively. Culture- and/or EIA-negative, Hexaplex-positive specimens were analyzed by a second RT-PCR assay which used primers specific for a different genomic region than that used in the Hexaplex assay. After analysis of these discrepant results, the sensitivity, specificity, and positive and negative predictive value for the detection of RSV were 74.3, 100, 100, and 93.5%, respectively, for tissue culture; 70.3, 100, 100, and 92.5% for EIA; and 98.6, 97.4, 91.2, and 99.6% for Hexaplex. The sensitivity, specificity, and positive and negative predictive value for the detection of influenza A virus were 83.3, 100, 100, and 97.4%, respectively, for tissue culture; 69.4, 100, 100, and 83.3% for EIA; and 95.8, 98.7, 92.0, and 99.3% for Hexaplex. Hexaplex is a rapid, sensitive, and specific method for the detection of the seven most common respiratory viruses in children.

Respiratory syncytial virus (RSV) types A and B, influenza virus types A (Flu A) and B (Flu B), and parainfluenza virus (PIV) types 1, 2, and 3 are the leading cause of viral lower respiratory tract infections in children (18, 22, 24). Many other viruses, including adenovirus, also play a minor role. Traditional tissue culture methods, shell vial culture methods, and rapid direct specimen antigen tests are currently available. Results of traditional viral respiratory tissue cultures are often not available until after the child has been discharged and thus have little impact on patient care or antiviral therapy. Rapid test results impact positively on patient care by reducing hospital stays, curtailing or preventing antibiotic therapy, and decreasing the need for additional diagnostic procedures (1, 38).

Direct specimen antigen tests include membrane enzyme immunoassays (EIAs) and both direct and indirect immunofluorescent assays for the detection of RSV and, more recently, Flu A and Flu B. Reported sensitivities for EIAs range from 57% (7) to 98% (9, 31) for RSV and 75% (2, 7) to 90% (5, 33, 34) for Flu A. Reported sensitivities for EIAs that detect both Flu A and Flu B are 70 to 96% (29, 30). Monoclonal antibody pools to respiratory viruses used in both direct and indirect immunofluorescent assays demonstrate varying sensitivity depending on the virus detected; 28 to 79% for PIV (4, 36), 65 to 92% for RSV (7, 15, 36), 40 to 65% for Flu A (2, 7, 36), and 58% for adenovirus (21).

Immunofluorescent testing on specimens that have been cytocentrifuged results in improved sensitivities of 98% for RSV and 90% for FluA (3). Due to the low sensitivities of some of these tests, utilization may require that culture be performed on negative specimens. There is a strong need for rapid, sensitive, and specific diagnosis of lower respiratory tract infections in children.

Recent reports suggest the utility of PCR for the rapid detection of respiratory viruses, with reported sensitivities ranging from 94 to 100% for PIV (14, 32), 95 to 98% for RSV (4, 12), and 92 to 95% for Flu A (2, 6).

The Hexaplex assay (Prodesse, Milwaukee, Wis.) employs a multiplex reverse transcriptase (RT)-PCR amplification in an enzyme hybridization probe system for the detection of Flu A, Flu B, RSV A and B, and PIV types 1, 2, and 3. We compared the Hexaplex assay to tissue culture and rapid EIA (for RSV

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and Flu A only) to determine if the Hexaplex assay is an acceptable alternative to EIA and culture.

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MATERIALS AND METHODS

Specimens. Respiratory specimens were collected from patients admitted to Children's Hospital of Wisconsin from December 1997 through May 1998. Acceptable specimens included nasopharyngeal swabs (NP), throat swabs, endotracheal aspirates, and bronchial alveolar lavage specimens (BAL). All specimens except BAL were submitted in Multi-Microbe Medium (M4) (MicroTest, Inc., Lilburn, Ga.). Specimens were stored at 2 to 8°C for up to 24 h. Specimens were vortexed, and the swabs were removed. If particulate matter was present, specimens were centrifuged at 700 × *g* for 20 min at 4°C.

The supernatant was removed and used for testing. Specimens were then aliquoted for culture, rapid EIA, and Hexaplex testing, and the remainder were frozen at −70°C. All specimens were tested by both Hexaplex assay and traditional tube cell culture. From January through May, all respiratory specimens except BAL were tested for RSV prior to freezing using Abbott TestPack RSV (Abbott Laboratories, Abbott Park, Ill.). From January through March, frozen aliquots of specimens received Monday through Friday were tested for Flu A using Directigen Flu A (Becton Dickinson Inc., Cockeysville, Md.). Individuals performing all tests were blinded to the results of the other testing methods.

Culture. An antibacterial-antimycotic solution, Bartels Cul-trol (Bartels, Inc., Issaquah, Wash.) was added to an aliquot of the specimen to yield a final concentration of 50 μg of streptomycin, 10 μg of gentamicin, and 4 μg of amphotericin B per ml. Specimens were allowed to stand at room temperature for 15 min. The medium was removed from one tube each of MRC-5 cells, rhesus monkey kidney cells, and Hep-2 cells (Viromed Laboratories, Minneapolis, Minn.), and 0.2 ml of specimen was inoculated into each tube. The tubes were incubated on a roller drum at 35°C for 1 h, after which time 2 ml of 2% Eagle's minimal essential medium (Viromed Laboratories) was added to the MRC-5 and Hep-2 cells and 2 ml of serum-free Eagle's minimal essential medium (Viromed Laboratories) was added to the rhesus monkey kidney cells. The rhesus monkey kidney tubes had been washed, prior to inoculation, in serum-free Eagle's minimal essential medium. The tubes were incubated at 35°C and examined every 48 to 72 h for cytopathic effect for 10 days. Specimens were also tested for hemagglutination at days 3 and 10, following standard procedures (19, 20). Cultures positive by cytopathic effect or hemagglutination were identified using Bartels viral respiratory screening and identification kit (Bartels, Inc.) according to the manufacturer's recommendations. This kit includes direct immunofluorescence reagents for the detection of Flu A, Flu B, RSV, PIV types 1, 2, and 3, and adenovirus.

Rapid EIA. An aliquot of the specimens was tested for RSV using the Abbott TestPack RSV (Abbott Laboratories). The test was performed according to the manufacturer's directions. The frozen aliquot of selected specimens was thawed and tested for Flu A using the Directigen Flu A Test (Becton Dickinson Inc). The test was performed according to the manufacturer's directions.

Hexaplex assay. An aliquot of the specimen was tested for PIV types 1, 2, and 3, RSV A and B, and Flu A and Flu B using the Hexaplex assay (Prodesse Inc.). The assay was performed according to the manufacturer's directions (11). In brief, viral genomic RNA was extracted from 280 μl of specimen (or plasmid RNA from positive control transcripts) with the QIAamp viral RNA mini kit (Qiagen, Chatsworth, Calif.). cDNA was synthesized with the use of random hexamers, murine leukemia virus reverse transcriptase, and RNA (10). PCR amplification was carried out by adding Super-Mix (Prodesse, Inc.) and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Cetus, Foster City, Calif.) to the previously synthesized cDNA. The Super-Mix contained 6½ pairs of primers to the seven viruses. These primers were designed from highly conserved regions of genetic sequences obtained from Prodesse, Inc., and GenBank (Bethesda, Md.). Specifically, this proprietary mixture contained a pair of primers to the hemagglutinin neuraminidase gene of PIV 1, 2, and 3; a pair of primers to the membrane gene of Flu A; a pair of primers to the nonstructural gene of Flu B; and primers to the 1B and nucleocapsid genes of RSV A and B. Positive controls (RNA transcripts) and negative controls (water, previously tested Hexaplex-negative respiratory samples, and viral transport medium) were added to each assay. The PCR mixture contained 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM each of the four deoxynucleoside triphosphates, 0.5 μM primers, and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Cetus). After denaturation at 95°C for 2 min, aliquots were then amplified by two cycles at 95°C for 1 min, 55°C for 45 s, and 72°C for 45 s and 38 cycles at 94°C for 1 min, 60°C for 45 s, and 72°C for 45 s and were then held at 72°C for 7 min. After amplification, PCR products were purified with the QIAquick PCR purification kit (Qiagen) and added to 96-well neutraviden-coated microtiter plates provided by the manufacturer (Prodesse, Inc.) (11). Peroxidase-labeled probe solutions 1 to 7 (Prodesse, Inc.) were added, each to a single well. These proprietary probes represented highly conserved regions of the previously described genes. A capture and hybridization reaction was then achieved, and substrate solution was added to each well. After 10 min, the reaction was stopped and the optical density of each well was measured at 450 nm on a spectrophotometer. The positive cutoff value was calculated as three times higher than the negative control or ≥0.400, whichever was greater.

Analysis of discrepant specimens. Frozen aliquots of specimens which were EIA or culture negative and Hexaplex positive for RSV or Flu A were thawed and tested using a second RT-PCR assay. Due to the low number of specimens positive for PIV and Flu B, discrepant analysis was not performed on these specimens. Specimens that contained more than one virus were also not included in the discrepant analysis.

Specimens were tested at Prodesse, Inc., using a second RT-PCR assay employing a second set of primers. Viral genomic RNA was extracted and cDNA was synthesized as for the Hexaplex assay. The second RT-PCR employed primers from a highly conserved sequence of the membrane gene from Flu A, with a PCR product size of 236 bp; fusion protein gene of RSV A, with a PCR product size of 182 bp; and G protein gene of RSV B, with a PCR product size of 149 bp. The PCR mixture contained 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM each of the deoxynucleoside triphosphates, 0.5 μM primers, and 2.5 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer Cetus). After denaturation at 95°C for 2 min, aliquots were then amplified by two cycles at 95°C for 1 min, 55°C for 45 s, and 72°C for 45 s and 38 cycles at 94°C for 1 min, 60°C for 45 s, and 72°C for 45 s and were then held at 72°C for 7 min. The PCR products were then analyzed by electrophoresis on a 2% agarose gel in Tris-borate-EDTA buffer at 80 V for 1.25 h, stained with ethidium bromide, and examined using UV fluorescence.

Statistical methods. Sensitivity, specificity, and positive and negative predictive value were calculated using standard methods (28). Calculations were performed prior to discrepant analysis. For these calculations, a specimen was considered to contain a specific virus when it was positive by culture or positive by Hexaplex and rapid EIA. Calculations were also performed after discrepant analysis. For these calculations, a specimen was considered to contain a specific virus when it was positive by culture, positive by Hexaplex and rapid EIA, or positive by Hexaplex and alternate RT-PCR.

RESULTS

A total of 363 respiratory specimens were collected. NP specimens (324) accounted for 89% of the specimens received. Other specimens included 18 BAL (5%), 12 throat (3%), and 9 endotracheal aspirates (2%). RSV was detected in all specimen types. Flu A or B was detected in all specimens except endotracheal aspirates. PIV were not detected in throat or endotracheal aspirates but were detected in NP and BAL specimens.

Fifteen specimens were identified as dual infections by Hexaplex testing (Table 1). One of the two viruses was also detected in tissue culture in 10 of these specimens. None of the dual infections was confirmed by tissue culture.

Hexaplex testing detected 13 PIV in pure culture and 10 PIV in mixed culture from 21 specimens. Excluding the dual infections, two specimens grew PIV in tissue culture, four specimens grew a virus not expected to be detected by the Hexaplex assay, and seven specimens were tissue culture negative. Of the specimens identified as containing PIV in dual infections, two specimens grew PIV in tissue culture, four specimens grew the other virus, and two specimens were tissue culture negative (Table 1). Hexaplex-positive specimens not confirmed by culture were considered false-positives. The sensitivity, specificity, and positive and negative predictive values of the Hexaplex

TABLE 1. Dual viral infections as identified by Hexaplex assay

Positive Hexaplex result	Positive tissue culture result	No. of specimens
PIV 1, PIV 2	PIV 1	1
PIV 1, RSV A	RSV	2
PIV 1, RSV B	RSV	1
PIV 2, RSV B	PIV 2	1
PIV 2, Flu A	Negative	1
PIV 2, PIV 3	Negative	1
PIV 3, RSV A	RSV	1
RSV A, Flu A	RSV	1
RSV A, Flu A	Flu A	1
RSV B, Flu A	RSV	2
RSV B, Flu A	Negative	3

assay for the detection of PIV were 100, 95.8, 19.0, and 100%, respectively (see Table 3).

RSV (Table 2) was identified by Hexaplex testing and either culture or EIA in 67 specimens. One specimen was positive by culture only. No specimens were positive by EIA only. There were 25 specimens that were positive by Hexaplex testing only. The sensitivity, specificity, and positive and negative predictive value prior to discrepant analysis are listed in Table 3. The Hexaplex test can discriminate the A and B subtypes of RSV. The sensitivity, specificity, and positive and negative predictive value for each subtype are listed in Table 3.

Discrepant analysis was performed on 21 of the 25 specimens that were positive by Hexaplex only. Using the second RT-PCR assay, 8 of the 14 RSV A specimens and 6 of the 7 RSV B specimens were confirmed as true positives. Those specimens not confirmed were considered false-positive tests. After resolution of the discrepant results, RSV was confirmed in 82 specimens; 51 specimens were determined to contain RSV A and 31 specimens RSV B. The sensitivity, specificity, and positive and negative predictive value after discrepant analysis are listed in Table 4. The sensitivity, specificity, and positive and negative predictive value for each subtype after discrepant analysis are listed in Table 4.

Flu A (Table 2) was identified by Hexaplex testing and culture in 39 specimens. Two specimens were positive by culture only. No specimens were positive by EIA only. There were 19 specimens that were positive by Hexaplex testing only. The sensitivity, specificity, and positive and negative predictive value prior to discrepant analysis are listed in Table 3. Using the second RT-PCR assay, 8 of the 12 specimens tested were confirmed as true positives. The four specimens that were not confirmed were considered false-positives. After resolution of the discrepant results, 51 specimens were determined to contain Flu A. The sensitivity, specificity, and positive and negative predictive values after discrepant analysis are listed in Table 4. Only one Flu B was detected during the study period.

There were 12 viruses detected in culture that would not have been expected to be detected by Hexaplex testing alone. There were three adenoviruses, three enteroviruses, two herpes simplex viruses, and four rhinoviruses. These included four organisms in mixed infections with PIV.

TABLE 2. Number of specimens containing respiratory viruses by each method

Result			Virus(es) detected (no. of specimens)[a]			
Hexaplex	Culture	Rapid EIA	PIV	RSV A	RSV B	Flu A
+	+	+		31	12	25
+	+	−		5	3	5
+	+	ND[b]	4	5	5	9
+	−	+		1	5	
+	−	−		8* (7)	4* (3)	5* (4)
+	−	ND	17[c]		2* (1)	5* (5)
−	+	−		1		2

[a] *, confirmed as positive by a second RT-PCR assay. Numbers in parentheses are specimens which were not confirmed or not tested by a second RT-PCR assay and are considered false-positive results.
[b] ND, not determined.
[c] Confirmatory testing not performed. For statistical analysis, these are considered false-positive results.

DISCUSSION

RSV, Flu A and B, and PIV types 1, 2, and 3 cause the majority of viral lower respiratory tract infections in children (16, 18). In adults, these viruses cause 8 to 10% of all lower respiratory tract infections and 75% of viral lower respiratory tract infections (8, 25, 26). Traditional respiratory viral tissue cultures for these agents have demonstrated lack of speed and little impact on patient care (1, 38). Although rapid test results impact positively on patient care, the low sensitivity of some of these tests often requires culture to be performed on negative specimens. RT-PCR can provide rapid definitive diagnosis with sensitivities equal to or better than culture (2, 6, 13, 14, 32).

Hexaplex, a multiplex RT-PCR assay for RSV A and B, Flu A and B, and PIV types 1, 2, and 3, has been shown to have an analytical sensitivity of 100 to 140 copies/ml, depending on the virus (11). Cross-reactions with other viruses have not been reported, demonstrating the analytical specificity (11) of the assay. This study was undertaken to determine the clinical sensitivity and specificity of the test in routine use. It has been shown that many assays employing PCR technology demonstrate better sensitivity than the gold standard cultures to which they are compared. In an attempt to determine if the culture-negative, Hexaplex-positive specimens were true positives, a second RT-PCR assay employing primers to a different part of the genome was employed. The use of this second method for discrepant analysis tends to result in improved sensitivity, specificity, and positive predictive value. Analysis without it will tend to lower sensitivity, specificity, and positive predictive value (27; R. Hoffman, personal communication). The true estimate of the sensitivity probably lies somewhere in between. For this reason, we have reported the data both with and without the discrepant analysis.

In this study, only 23 PIV were detected by Hexaplex as pure or mixed infections from 21 specimens, with only 4 being confirmed by culture. The 17 specimens that were not confirmed were considered false-positive tests. This resulted in a specificity of 95.3% and, in our low-prevalence population, a positive predictive value of only 19%. It may be that some of these specimens did contain PIV. Growth of other viruses in eight specimens may have precluded the recovery of PIV. Tissue culture methods optimized for the recovery of PIV or discrepant analysis employing an alternate RT-PCR method may have been able to confirm the presence of PIV in these specimens as

TABLE 3. Sensitivity, specificity, and positive and negative predictive value of Hexaplex, tissue culture, and EIA for PIV, RSV A and B, and Flu A prior to discrepant analysis

Virus	Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PIV	Tissue culture	100	100	100	100
	Hexaplex	100	95.3	19.0	100
Total RSV	Tissue culture	91.2	100	100	98.0
	TestPack RSV	84.5	100	100	96.6
	Hexaplex	98.5	91.5	72.8	99.6
RSV A	Hexaplex	97.7	95.3	73.7	99.7
RSV B	Hexaplex	100	97	71.4	100
Flu A	Tissue culture	100	100	100	100
	Directigen Flu A	78.0	100	100	89.4
	Hexaplex	95.1	94.1	67.2	99.3

well. A previous study on specimens from asymptomatic children reported negative Hexaplex results, suggesting these are true-positive specimens and confirming the clinical specificity of this assay for PIV (11). Other studies have also demonstrated that the Hexaplex is sensitive and specific for the detection of PIV types 1, 2, and 3 compared to tissue culture or immunofluorescence (K. J. Henrickson, unpublished data).

There were three culture-positive, Hexaplex-negative specimens, one containing RSV and two containing Flu A. The presence of an inhibitor in specimens can cause false-negative tests. The Hexaplex assay includes an optional internal inhibitor control. When 5% of the samples were tested for the presence of an inhibitor, none was detected, suggesting that the presence of inhibitors is unlikely. Sampling error, loss of RNA during extraction, or degradation of RNA could account for the false-negative test. Despite the use of primers to a conserved region of the genome, a mutation in the genome or a rare genotype also could account for the virus's not being detected using the Hexaplex primers. Alterations in the genome that result in the virus being undetectable by the assay are a potential problem for all molecular assays.

The overall sensitivity of the Hexaplex assay for RSV and Flu A after discrepant analysis was 98.6 and 95.8%, respectively. This is in sharp contrast to a sensitivity of 74.3 and 83.3% for RSV and Flu A, respectively, from tissue culture and 70.3 and 69.4% for RSV and Flu A, respectively, from EIA. This is somewhat lower than other reports (2). The reported high sensitivity of some rapid tests may be due to the use of an imperfect gold standard. Indeed, when tissue culture alone is employed as the gold standard, the EIA appeared to have a much higher sensitivity of 81% for RSV and 78% for Flu A.

Six specimens were positive for RSV by EIA and negative by culture. RSV was detected in each of these specimens by the Hexaplex assay, suggesting that these were true positives. Low viral titer and delay in culture setup can decrease the recovery of virus from clinical specimens (37), with less effect on the Hexaplex assay (11). Other reports of low specificity of EIA tests compared to culture may be a reflection of this as well.

There were 33 culture- and/or EIA-negative, Hexaplex-positive specimens that were tested by an alternate RT-PCR method. Of those, 16 were not confirmed. These specimens all contained a lower number of viral RNA copies (data not shown), as determined by Hexaplex testing, suggesting a low viral titer. These specimens were also detected in different runs on different days, supporting that these false-positives were not due to contamination. The alternate RT-PCR assays developed have not undergone the rigorous testing that the Hexaplex assay has. Although the analytical lower limit of detection is 10-fold less sensitive than the Hexaplex assay, other performance characteristics of these assays have not been determined. Therefore, these specimens may, in fact, be true positives. Despite these false-positives, the specificity of the Hexaplex was 97.4% for RSV and 98.7% for Flu A. In our

TABLE 4. Sensitivity, specificity, and positive and negative predictive value of Hexaplex, tissue culture, and EIA for RSV A and B and Flu A after discrepant analysis

Virus	Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Total RSV	Tissue culture	74.3	100	100	93.5
	TestPack RSV	70.3	100	100	92.5
	Hexaplex	98.6	97.4	91.2	99.6
RSV A	Hexaplex	98.0	98.0	88.9	99.7
RSV B	Hexaplex	100	99.7	96.1	100
Flu A	Tissue culture	83.3	100	100	97.4
	Directigen Flu A	69.4	100	100	83.3
	Hexaplex	95.8	98.7	92.0	99.3

high-prevalence population, this resulted in a positive predictive value of 91.2% for RSV and 92.0% for Flu A.

One concern when employing a test for a limited number of specific agents is the presence of other agents that could go undetected. A limitation of the Hexaplex test is that it does not detect adenovirus, an important respiratory tract pathogen. It also does not detect PIV type 4. Twelve viruses were detected in tissue culture that would not be detected by the Hexaplex assay; 25% of these were adenovirus and 33% were rhinovirus. The tissue culture assay employed would not have been expected to detect PIV 4. These other viruses accounted for 6% of the viruses detected from respiratory specimens during this time period.

The number of dual infections (11% of the total infections) was higher than that reported from other studies based on tissue culture (14). However, the results are similar to studies utilizing molecular methods (13, 14). In one study, RSV was more commonly associated with mixed infections (13), while both RSV and PIV were involved in dual infections in this study. It is likely that dual viral infections play a greater role in disease than has previously been appreciated.

The rapid EIA tests may provide faster turnaround yet are significantly less sensitive and may require tissue culture testing to confirm negative specimens. These tests are further limited by testing for only RSV, Flu A, or Flu A and B. Immunofluorescent testing on cytocentrifuged specimens also provides faster turnaround and has higher reported sensitivities. Tests are available for adenovirus in addition to the same viruses as the Hexaplex. These same-day rapid-turnaround tests provide cost-effective reporting which could positively impact patient care as well as use of antiviral therapy. The Hexaplex assay is a rapid, sensitive, and specific assay for detection of seven of the most common viral causes of lower respiratory infection in children and adults. The test is sensitive enough to eliminate the need for viral tissue culture backup on negative specimens for patients at risk for infection with these viruses. Certainly, high-risk or immunocompromised patients at risk of infection with other viruses as well would require traditional tissue cell culture. The Hexaplex assay can be performed in 7 h; however, for use in the routine diagnostic laboratory, overnight testing would most likely be utilized. The overnight turnaround time could also positively impact patient care as well as use of antiviral therapy. Improved detection of dual infections could improve our understanding of the epidemiology of these viruses.

Cost-effective implementation of molecular testing in routine diagnostic laboratories is difficult. Cost benefits could result from decreased hospitalization due to rapid diagnosis, decreased nosocomial spread of infection, and decreased antibiotic use (1, 23, 35, 38). Utilization guidelines, testing algorithms, or other innovative strategies may be necessary to best benefit from this test.

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