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Research Article

Rapid RSV and Influenza Testing versus Respiratory Viral Panel PCR: A Retrospective Analysis

Landoll AL, Meyer KM, Isava A*, Rowe-King PA and Hardigan Y

Department of Pediatrics, Broward Health Medical Center, USA

***Corresponding author:** Isava A, Department of Pediatrics, Broward Health Medical Center, USA

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Abstract

Background: Accurate and rapid diagnosis of Respiratory Syncytial Virus (RSV) and Influenza (FLU) is important in the prevention of nosocomial infections. This study was performed to determine what method was more accurate in the diagnosis of these infections.

Methods: This single center, retrospective analysis evaluated the reliability of the Xpect Rapid RSV test (Remel, Inc.) and the Xpect Rapid Influenza test (Remel, Inc.) when compared to the Film Array RP nucleic acid test (Idaho Technology, Inc.). The performance was evaluated if both tests were performed on a single patient within 24 hours. A total of two hundred and twelve patients with respiratory symptoms who were less than, or equal to, 18 years of age were included. Sensitivities, specificities, positive and negative predictive values were performed.

Results: For the RSV rapid test, these values were 16.7%, 99.2%, 90.0%, and 72.2%, respectively; for the influenza rapid test, these values were 27.2%, 100.0%, 100.0%, and 95.7%, respectively. Further, Receiver Operator Characteristic (ROC) curves of each test were performed, revealing Area Under the Curve (AUC) for RSV 62.4% and for influenza 73.8%; these rapid tests show a poor to fair level of discrimination.

Conclusion: This study reveals the advantage of nucleic acid respiratory pathogen testing at our institution.

Introduction

Bronchiolitis is a general term for wheezing associated with a viral respiratory infection, affecting around 50% of children in the first 2 years of life [1]. It involves inflammation in the bronchioles, leading to inadequate expiratory airflow; this process can be potentially life-threatening, especially in infants and small children. Due to this risk, bronchiolitis remains the leading cause of hospitalization of infants. The primary cause is Respiratory Syncytial Virus (RSV), followed in frequency by human metapneumovirus, parainfluenza viruses, influenza viruses, adenoviruses, and rhinoviruses [1].

RSV infections are typically seasonal, with prominence during winter months. The virus is spread easily through respiratory droplets from the secretions of affected individuals. The incubation period is three to seven days. The classical presentation is a progressive respiratory illness, leading to audible wheezing and difficulty breathing. Treatment is supportive.

Influenza, although not the most common cause of bronchiolitis, remains a significant respiratory illness affecting 10-20% of the US population yearly. It is a highly contagious infection characterized by abrupt onset of fever, myalgia, headache, cough, and rhinitis. Like RSV, influenza follows a seasonal pattern with prominence during the winter months. It typically resolves in one to two weeks, but carries some morbidity and mortality. Treatment remains supportive, although antivirals may be given if diagnosis is made promptly.

Because these two viruses can be spread so easily, rapid diagnosis of RSV and influenza is important to prevent the spread of nosocomial infections in infants and children requiring hospitalization [2]. If viral status is known, RSV- or influenza-infected patients may be isolated or cohorted with other children infected with the same virus. These infection control methods have been shown to be cost effective [3]. For this reason, rapid and accurate diagnoses are of utmost importance.

The gold standard for diagnosis remains viral culture, but this requires long periods of time and specialized equipment. Instead, rapid RSV and influenza testing can be performed using immunoassay for qualitative detection. Generally, these tests are reliable, especially in infants and younger children. More recently, molecular diagnostic tests using Polymerase Chain Reaction (PCR) have increased viral detection rates over immunoassay. As PCR has been reported to show advantage over rapid RSV and influenza testing, we decided to evaluate the reliability of each test within our institution with a retrospective analysis.

Materials and Methods

Nasopharyngeal swabs were collected from pediatric patients at Broward Health Medical Center, Fort Lauderdale, Florida over a five year period from January 1, 2009 to December 31, 2014. Following hospital protocol, the patient's nasopharynx was swabbed with a flocked applicator and placed in 3 milliliters of transport medium at room temperature. Specimens were then transported to the laboratory for analysis.

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RSV testing was conducted using the Xpect immunoassay (Remel, Inc.), which utilizes a pair of RSV specific antibodies in an immunochromatographic sandwich assay. If a sample is positive, it reacts with an antibody coupled with a colored particle, which migrates along with membrane. An immobilized capture antibody then forms a colored line at the positive region. If negative, this reaction does not take place and a solid line does not appear. To ensure accuracy, a control line is built in. The manufacturer reports sensitivity and specificity to be 95.6% (95% CI = 89.0-98.8%) and 94.1% (95% CI = 80.3-99.3%), respectively, using a retrospective study of 124 samples collected at three clinical sites.

Influenza testing was conducted using the Xpect immunoassay (Remel, Inc.), which is similar in mechanism to its RSV counterpart. The test incorporates separate membrane strips for influenza A and influenza B. If one of these antigens is present, it will bind anti-influenza A or B conjugated antibodies. A line will form where a complex of antibody-antigen-antibody colored particles is captured. If negative, these complexes do not form and no line will appear. A control region is built in to ensure a properly working test. The manufacturer reports sensitivity and specificity to be 88.9% (95% CI = 70.8-97.7%) and 100% (95% CI = 96.2-100%), respectively for influenza A, and 83.3% (95% CI = 35.9-99.6%) and 100% (95% CI= 96.9-100%) for influenza B.

Finally, the Film Array RP (Idaho Technology, Inc.) was used to perform multiplexed nucleic acid testing. This test can identify both RSV and Influenza A and B, along with other organisms including: Adenovirus, Bocavirus, Coronavirus 229, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Parainfluenza Virus [1-5], Rhinovirus/Enterovirus, Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae. The test consists of four major steps. First, the sample is added to a testing pouch and undergoes nucleic acid purification. The sample is lysed by agitation and the liberated nucleic acid is captured. Second, the sample undergoes a reverse transcription step as many detected pathogens are RNA viruses. The resulting cDNA then begins thermo cycling to begin the PCR process. Third, the products of the PCR are diluted and mixed with fresh PCR reagents, which contain a patented fluorescent DNA dye. This product is then distributed among various wells that test for specific pathogens and undergo a second-stage PCR process. Last, the sample undergoes a DNA melting analysis, in which the temperature is slowly increased and fluorescence is monitored to generate a melting curve. This melting curve is consistent and predictable for each pathogen. The Film Array detects these curves and reports if there is a positive reaction. The process from beginning to end takes about one hour.

For Influenza A, the manufacturer of the Film Array RP reported sensitivity 90% (CI 55.5-99.8%) and specificity 99.8% (CI 99.2-100%). For influenza B, specificity was reported at 100% (CI 99.6-100%). RSV had a reported sensitivity of 100% (CI 93.2-100%) and specificity 89.1% (CI 86.8-91.2%).

Patient data were collected by searching inpatient and emergency room records for diagnoses of: acute bronchiolitis, acute bronchiolitis due to RSV, acute bronchiolitis due to other organism, RSV infection, and influenza with pneumonia, acute bronchitis, or influenza with respiratory manifestations. Patient age was limited to equal to, or less than, 18 years old. From there, data were collected including: patient age, results of rapid RSV or influenza tests (if performed), and results of respiratory viral panel (if performed). Data were included in the analysis only if a rapid RSV or influenza test and respiratory viral panel were both performed. Additionally, tests were required to be performed within 24 hours of each other in order to reduce confounding with potential nosocomial infection. Some samples with initial negative rapid tests had subsequent positive respiratory viral tests after multiple days of hospital admission; these were excluded as the possibility of nosocomial infection could not be excluded.

Table 1: Classification statistics for rapid RSV vs. Resp. Viral panel controlling for age.

		Resp. Viral Panel		
		Positive	Negative	Total
Rapid RSV	Positive	9	1	10
	Negative	45	117	162
	Total	54	118	172
Sensitivity	16.70%			
Specificity	99.20%			
Positive predictive value	90.00%			
Negative predictive value	72.20%			
False positive	0.80%			
False negative	83.30%			

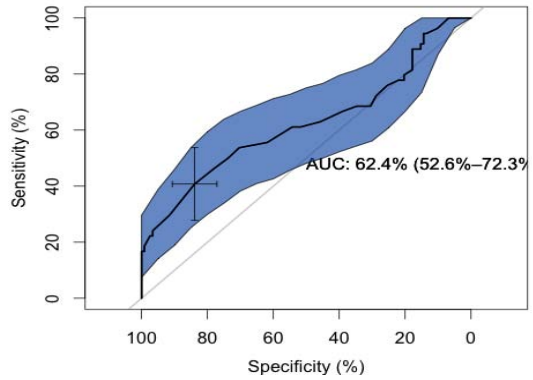

Figure 1: ROC for rapid respiratory syncytial virus test.

Results from the rapid RSV and rapid influenza tests were then compared with results of the respiratory viral panel. Logistic regression, controlling for the subject's age, was used to model the probability of infection with RSV or influenza against the PCR test. The sensitivity and specificity, as well as predictive values and a receiver operator characteristic curve of each of the rapid tests were calculated.

Results

A total of 1,122 patients fell within the seven searched diagnoses. Of these, 212 patients met inclusion criteria of both a rapid RSV or influenza test and the respiratory viral PCR panel within 24 hours. The average age was 24.9 months (± 36.9). The data were further divided into two arms by the type of test being measured: RSV or FLU.

The results from the rapid RSV and rapid influenza tests were compared with results from the respiratory viral panel PCR. Logistic regression, controlling for a subject's age, was used to model the probability of having RSV or FLU against the respiratory viral panel. The sensitivity, specificity, predictive values, and a receiver operator characteristic curve of each of the rapid tests were calculated.

In the RSV arm of the study, 40 subjects were removed as they were not given the rapid RSV test. The average age for the 172 tested patients was 17.9 months (+/- 29.6). Based on the respiratory viral panel, 31% of the subjects tested positive for RSV.

The relationship between the rapid RSV test and the respiratory viral panel are shown in (Table 1). The tests are associated as 73.2% (95% CI: 65.9-79.7%) of the subjects were correctly classified. The calculated sensitivity for the rapid RSV test was 16.7% and the related specificity was 99.2%. The area under the curve is 62.4% (95% CI: 52.6-72.3%), which is considered a marginally acceptable level of discrimination (Figure 1). These results did not differ significantly by the patient's age.

In the FLU arm of the study, 25 subjects were removed as they were not given the rapid flu test. The average age for the tested subjects was 27.3 months (+/- 38.8). Based on the respiratory viral panel, 6.3% of the subjects tested positive for the respiratory virus.

The relationship between the rapid flu test and the respiratory viral panel are shown in (Table 2). The two tests are also highly associated, as 95.7% (95% CI: 91.7%, 98.1%) of the subjects were correctly classified. The sensitivity for the rapid RSV test was 27.3% and the related specificity was 100%. We can attribute the small sensitivity to the few number of children who tested positive via the respiratory viral panel. The area under the curve is 73.8% (95% CI: 59.0-88.6%), which is considered an acceptable level of discrimination (Figure 2). Results did not differ significantly by the patient's age.

For reference we examined the State of Florida, Department of Health, reported number of people who tested positive for the influenza during the flu season of 2014-2015 (October 1, 2014 – May 23, 2015). During this time period 1,197 people were reported tested and 43.9% (526) were positive. In our study 1,122 patients were tested and 6.3% were positive. We note that sensitivity and specificity will change if the population tested is dramatically different, especially if the spectrum of the disease is different.

Discussion

Although detecting antigen through immunoassay remains a widespread procedure, the use of PCR has increased steadily in recent years, especially following US Food and Drug Administration approval of multiplex PCR detection in 2008 [6]. This has not only allowed for the detection of multiple pathogens, but has been shown through multiple studies to have superior accuracy [7]. The higher sensitivity reported in the Film Array RP for RSV versus the Xpect RSV test implies that fewer RSV-infected children will be missed, thereby assisting the clinician to not only guide the course of treatment, but to advise precautions from a public health perspective. Our analysis has shown rapid RSV and rapid influenza testing to be less sensitive at our institution versus the PCR counterpart, consistent with both the data provided by the test package inserts and by other studies [2,7-10]. However, our sensitivity was far less than reported

Table 2: Classification statistics rapid flu vs resp. Viral panel controlling for Age.

		Resp. Viral Panel		
		Positive	Negative	Total
Rapid FLU	Positive	3	0	3
	Negative	8	176	184
	Total	11	176	187
Specificity	100.00%			
Positive predictive value	100.00%			
Negative predictive value	95.70%			
False positive	0.00%			
False negative	72.80%			

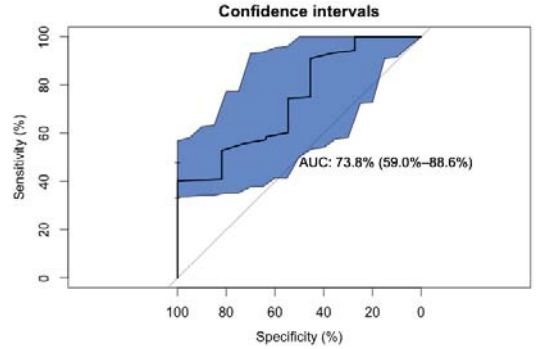

Figure 2: ROC for rapid influenza virus test.

values from the manufacturers; the reliability of screening for the viruses by rapid antigen testing has poor discrimination for the rapid RSV test and marginally acceptable discrimination for the rapid FLU test at our institution.

Our study had some limitations. We accepted Film Array RP positives as true positives, even though there is a potential for false positives, as with all tests. Conversely, the package inserts compared each test to a viral culture. This test is the gold standard, although it has been shown to have low sensitivity as well [2]. For this reason, our sensitivities for both the rapid RSV and rapid FLU tests may be falsely low; a future prospective analysis with viral culture may assist in a better comparison of the two tests.

Additionally, as a retrospective analysis, there were multiple variables that could not be accounted for. First, the method of collection could not be standardized, but both tests used the same nasopharyngeal swab. Second, a limitation to the rapid tests as listed by the manufacturer is a false negative probability for bloody samples, which we were unable to account for. Inclusion of these samples may have led to the increase in negative results with the rapid tests versus the PCR viral panel. Third, we could not control for the number of days the patient had been symptomatic; the quantity of influenza viral particles shed decreases dramatically after 72 hours of symptoms and could affect results [11,12]. Last, we had to exclude a large number of patients who received only the rapid test or the PCR; a prospective study could ensure that we obtain samples consistently and for both assays.

The rapid tests, having high specificities (99.2% for rapid RSV and

100.0% for rapid FLU), are adequate tests for confirming a diagnosis; however, our study illustrated the poor sensitivities (16.7% for rapid RSV, 27.2% for rapid FLU), leading to a high false negative rate. The PCR requires a slightly longer timeframe to process (one hour versus 15 minutes for a rapid test) and carries a higher cost ($1,718 versus $117 for rapid RSV and $234 for rapid influenza), but may be the superior method for screening at our institution.

Conclusion

Based on the data collected at our institution, the respiratory viral panel may be a more accurate method of determining infection with RSV or influenza than the rapid tests. However, as the respiratory viral panel carries a higher cost, a longer processing time, and may not affect clinical decision-making, careful consideration must be applied before conducting this test.

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