

# Comparison of BD Directigen™ EZ RSV and Remel X/pect™ RSV Rapid Chromatographic Immunoassays to Direct Immunofluorescence for Detection of Respiratory Syncytial Virus in Pediatric Patients

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## ABSTRACT

Two rapid lateral flow chromatographic immunoassays (RLFCIs), the BD Directigen™ EZ RSV (BD Diagnostics, Sparks, MD) and X/pect™ RSV (Remel Inc., Lenexa, KS) tests, were compared to a direct immunofluorescence (DFA) assay for detection of respiratory syncytial virus (RSV) in nasopharyngeal aspirates from pediatric patients. Our study of RLFCIs was focused on testing frozen patient specimens. When compared to the DFA, the sensitivity, specificity and positive/negative predictive values of the BD test were 83%, 95%, 88%, and 92%, respectively. For X/pect, the values were 81%, 98%, 97%, and 91%, respectively. The RLFCIs were easy to perform and interpret and the results were available in a short time frame. Although the sensitivity of these assays is lower than DFA, they are a useful alternative method for rapid diagnosis of RSV during the winter respiratory viral season and because results are obtained quickly, can be a benefit to busy laboratories. Because of the lower sensitivity, specimens with negative rapid test results should be further tested by either DFA or viral culture.

## INTRODUCTION

Respiratory syncytial virus (RSV) is an important cause of lower respiratory tract viral disease in infants and young children.<sup>6, 7, 8, 11</sup> RSV is a major cause of bronchiolitis and pneumonia in infants under one year of age.<sup>7</sup> It has worldwide distribution and disease occurs annually beginning in the late fall and lasts through early spring.<sup>7</sup> RSV is a concern due to the potential for hospital-associated transmission. It is a particular hazard for premature infants, infants with congenital heart disease or bronchopulmonary dysplasia, and infants and children who are immunodeficient.<sup>7</sup> Rapid identification of patients with RSV facilitates isolation and cohorting patients, allowing for reduction of hospital-associated infections with RSV.<sup>3, 5, 9, 11, 12</sup>

Laboratory diagnosis of RSV disease is essential because many respiratory illnesses have similar signs and symptoms.<sup>2, 4</sup> RSV can be detected in respiratory secretions by antigen capture enzyme immunoassay (EIA), lateral flow chromatographic immunoassay or direct immunofluorescence (DFA). The sensitivity of DFA ranges from 80 to 90% and the specificity is at least 94%.<sup>7</sup> Advantages of DFA include the ability to perform direct microscopic examination of clinical respiratory specimens for epithelial cells, and the ability to provide relatively rapid results at a low cost.<sup>1, 7</sup> The DFA method, however, requires considerable expertise and is time-consuming.<sup>1, 6, 11</sup> Membrane-EIA and RLFCIs are commonly performed in clinical virology labs. They are ideal platforms for a number of situations including STAT labs, emergency room testing areas, both high and low volume testing laboratories, and high pressure test situations where rapid results are needed. They produce results in less than 30 minutes and are easy to perform.<sup>1, 3</sup> Despite the ease and rapidity of RLFCIs, the patient specimen, once collected, can remain frozen for up to 7 days at -20°C prior to testing. Our premise was that laboratories of all sizes, physician office laboratories, and collection sites will occasionally batch the testing of rapid tests to aid their overall workflow and efficiency. A common storage and maintenance parameter is to freeze the specimens, especially when an extended time delay is anticipated. We decided to test representative aliquots of frozen patient specimens using the RLFCIs and compare the results to DFA obtained immediately after collection from the patient.

The lateral flow immunoassay consists of a test cassette containing a cellulose-like membrane. Following a short incubation time, an antigen-antibody reaction occurs at the control and test lines. The reaction is read visually. Built-in controls on the membrane provide convenient monitoring to insure proper test performance. The availability of sensitive and specific RLFCI kits for the detection of RSV antigen in clinical respiratory specimens provides a rapid, reliable, and relatively inexpensive diagnostic test.<sup>1, 6, 7, 8, 11</sup> Currently available rapid tests for RSV in pediatric specimens have sensitivities and specificities ranging from 80 to 95%.<sup>1</sup>

In this study, we evaluated two RLFCIs, the BD Directigen™ EZ RSV (BD Diagnostics, Sparks, MD) and the X/pect™ RSV (Remel Inc., Lenexa, KS), for the direct detection of RSV antigen from freeze-thawed respiratory specimens. Performance characteristics were compared to results obtained with the DFA technique, which was used as the reference "gold standard."

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## MATERIALS AND METHODS

**Specimens.** Nasopharyngeal (NP) washes were collected from pediatric patients at St. Christopher's Hospital for Children in Philadelphia, Pennsylvania, during the 2004 – 2005 winter season. Specimens were sent to the virology laboratory where DFA testing was performed for RSV. To facilitate workflow and to enable batch testing of samples by the RLFCIs, an aliquot of each NP wash was frozen at -70°C for later testing by lateral flow immunoassay. Storage of specimens prior to testing allowed for assessment of performance characteristics with samples that had been stored frozen for up to 7 days.

**Lateral flow immunoassay testing.** The X/pect™ RSV test was performed according to the package insert. This test utilizes RSV-specific antibodies in a qualitative, immunochromatographic sandwich assay. 150 µL of specimen was added to the test device. The test result was read between 15 – 30 minutes. If a mucoid or

bloody specimen was used, 250 µL of specimen was mixed with an extraction buffer and added to the test device through a filter. The BD test was performed as directed by the manufacturer. This assay is also a qualitative, immunochromatographic sandwich assay. 250 µL of specimen was mixed with an extraction reagent and was filtered while being added to the test device. Results were read between 15 and 60 minutes post sample addition. Both lateral flow manufacturers' devices contained internal controls to monitor their test membranes.

**DFA testing.** Light Diagnostics RSV DFA (Chemicon International, Temecula, CA) was performed according to the manufacturer's instructions. The NP washes were centrifuged and resuspended in PBS. One drop was placed on a slide for testing by DFA. The assay was considered positive if at least one intact epithelial cell was observed to fluoresce.

## RESULTS

A total of 306 samples were tested in this study. 190 specimens were negative by all three assays and 82 samples were positive by all three assays. 31 of the remaining 34 results were discrepant (where only 2 tests were in agreement). The results of the two RLFCIs compared to DFA are presented in Table 1. Sensitivity, specificity and true positive and negative values for the lateral

flow assays were calculated against the DFA results and are presented in Table 2. The BD test produced no invalid results, while 3 specimens gave uninterpretable results with the X/pect™ RSV test. Therefore, the calculations reflect the total number of specimens tested with the BD test at 306 but only 303 for the X/pect™ RSV test.

Table 1. Comparison of lateral flow immunoassay results to DFA

		DFA		
		Positive	Negative	
BD Directigen™ EZ	Positive	86	12	
	Negative	17	191	
	Uninterpretable	0	0	Tot = 306
X/pect™	Positive	83	3	
	Negative	19	198	
	Uninterpretable	3		Tot = 303

Table 2. Sensitivity, specificity, and predictive values of lateral flow compared to the DFA test

	Sensitivity	Specificity	PPV	NPV
BD Directigen™ EZ	86/103	191/203	86/98	191/208
	(83%)	(95%)	(88%)	(92%)
X/pect™	83/102	198/201	83/86	198/217
	(81%)	(98%)	(97%)	(91%)

## DISCUSSION

In this study we compared frozen patient specimens tested with two RLFCIs to the “gold standard” DFA for rapid detection of RSV in nasopharyngeal aspirates from symptomatic pediatric patients; this is the first report directly comparing RLFCIs to the DFA assay. The RLFCIs were rapid and reliable for detection of RSV antigen, with both assays demonstrating acceptable levels of sensitivity, 81% and 83%, and specificity, 98% and 95%, respectively.

The lateral flow assays were easy to perform and interpret. They are single-step methods requiring only the addition of patient sample to a membrane pad containing conjugate. These are “walk away” tests requiring no addition of reagents. The rapid tests have a turn-around-time of 15 – 30 minutes from start to finish.<sup>11</sup> They are suitable for single specimens or batch testing and are inexpensive compared to cell culture.<sup>1,7</sup> Our study was performed on samples that had previously been frozen and stored for testing at a later time. The results of our study indicated that freeze-thawing specimens did not have a significant effect on the sensitivity of the assays: storing samples at freezer temperature did not reduce the sensitivity of the lateral flow assays. This is a feature which may be useful to laboratories that may need to freeze samples for transport to another facility prior to testing or may need to hold specimens for a certain period of time for later testing.

There are some disadvantages to RLFCIs, including: specimen quality cannot be evaluated for appropriate cells; potential false-positives may result when samples with blood or thick mucus are

tested<sup>7</sup>; they are more expensive to run per patient than DFA (not including technologist time); and, according to our findings, have a lower sensitivity than DFA.

The results of this study indicate that both of the lateral flow assays have reduced sensitivity for detection of RSV in clinical specimens when compared to the DFA. This finding is consistent with previously published data.<sup>3, 6, 9, 11</sup> The slightly lower rate of detection may in part be due to the testing of frozen rather than fresh specimens.<sup>7</sup> The specificity of the two assays was also slightly lower compared with DFA, but still adequate for testing specimens when rapid results are desirable to facilitate patient care, shorten hospital stays, and to implement decisions regarding patient isolation and cohorting.

Overall, the lateral-flow assays produce useful results for rapid diagnosis of RSV during the winter respiratory viral season. The majority of laboratories utilizing lateral flow assays will be incorporating these methods into an algorithm with other test methods. The lateral flow assays are an excellent way to streamline workflow and reduce overall costs to the laboratory for RSV testing. Because results are obtained quickly, these tests can be a benefit to busy laboratories and also decrease the cost of hospital visits due to shorter stays, discontinuation of antibiotics, fewer tests ordered with a viral diagnosis, and administration of appropriate anti-viral therapy as required.<sup>1, 2, 4, 10</sup> Our recommendation, as well as that of other studies,<sup>3, 6, 9, 11</sup> is that specimens with negative results be further tested by either DFA or viral culture.

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