

# Comparison of BD Directigen™ EZ RSV and Binax NOW® RSV tests for Rapid Detection of Respiratory Syncytial virus from Nasal Aspirates in a Pediatric Population.

R. Selvarangan, BVSc, PhD, D. Abel, Y. Ballam, BS, K. Nelson, BS, MT(ASCP) and M. Hamilton, MD, PhD.

Department of Pathology and Laboratory Medicine, Children's Mercy Hospitals and Clinics • Kansas City, MO.

## REVISED ABSTRACT

**BACKGROUND:** Immunochromatographic assays (Lateral Flow) are used in the rapid diagnosis of respiratory syncytial virus (RSV) infection in children. BD Directigen EZ RSV (BD) and Binax NOW RSV (BN) assays are two FDA cleared rapid lateral flow kits. A prospective study was undertaken to compare the performance characteristics of these two assays.

**METHODS:** One hundred fresh nasal aspirate samples were tested by the two methods. These samples were transported in Hanks balanced salt solution, which is a compatible transport medium stated in both the product package inserts. All samples were inoculated into tube and shell vials for isolation of RSV. RT-PCR analysis were used for discrepant analysis when results from the two kits disagreed and for the samples that were culture negative but antigen positive. A sample positive by culture or RT-PCR was defined as a true positive.

**RESULTS:** The BD kit identified 51 positive and 49 negative samples, while the BN kit identified 48 positive and 52 negatives. The overall agreement between the two rapid kits was 93% (CI: 86.3 to 96.6%). Neither kit had an uninterpretable result. Using the defined true positive as the gold standard the performance characteristics of both the kits were as follows:

	Sensitivity	Specificity	Positive* predictive value	Negative* predictive value
BD	91%	94%	94%	90%
BN	91%	100%	100%	90%

\*With prevalence at 50%

Eight samples positive by both antigen assays were negative by viral culture, while 3 samples negative by both antigen assays were positive by viral culture.

**CONCLUSION:** The BD and BN assay were comparable in performance characteristics and displayed excellent sensitivity and specificity. The rapid lateral flow assays were easy to perform, easy to interpret and the results were available in less than 15 minutes. Reduced turnaround time offered by these kits is particularly useful in the rapid detection of RSV infections in outpatient settings.

## INTRODUCTION

Respiratory viral infections cause widespread disease during winter months in temperate climate. Respiratory syncytial virus cause infections primarily in the infants and young children. Viral culture, direct fluorescent antibody technique, rapid immunoassays and PCR assays can be used for detection of RSV infection. Viral cultures are laborious, time consuming and results are not available in a timely fashion to impact clinical decisions in an outpatient setting. Although DFA assays have improved the turn-around time they are technically demanding. Recently two new rapid forms of RSV immunoassays have been approved by the FDA for clinical testing of patient samples. The BD Directigen EZ RSV assay and the Binax NOW RSV assays are the two kits in the market with rapid processing times and results are available in less than an hour. When extracted specimens are added to the test device, RSV A and/or B antigens bind to the colloidal gold antibody conjugate in the test strip and produces a visible color reaction on the membrane.

In this study we evaluated both BD and Binax assay using fresh nasal aspirates obtained from pediatric population during the peak RSV viral activity of the 2004-05 season. The aim of the study was to compare the performance characteristics of the two kits in terms of sensitivity, specificity, positive predictive value and negative predictive value. The readability and influence of background on test interpretation was also compared between the kits. The results were compared with RSV culture using traditional tube cultures and R-mix shell vial cultures. RT-PCR analysis was used for discrepant analysis of selected samples that were antigen positive but culture negative.

## MATERIALS AND METHODS

**Specimens:** A total of 100 fresh nasal aspirates were obtained from pediatric patients visiting our hospitals and clinics. The samples were collected in HBSS. Following clinical testing at the individual site excess samples were stored at 4°C and transported within 12 hrs to the main laboratory for the study.

**Rapid Antigen testing:** All of the samples were tested by BD Directigen EZ RSV and Binax NOW RSV kits following manufacturers recommendations. The nasal aspirates were applied to the device either directly (Binax) or after an extraction step (BD). Following incubation for 15 min the test window was viewed for reactivity. Presence of a purple/pink band next to the sample/test window and control window indicates a positive reaction. The assay was performed by two technologists who

also scored the background and readability/intensity of the reactive bands. The readability was scored on a scale of 0.5 to 4 with 0.5 being the weakest and 4 denoting the strongest band.

**Discrepant analysis-Viral Culture and RT-PCR analysis:** The nasal aspirates were cultured by inoculating 0.2 mL in to individual tubes of A549, MRC5 and RMK cells and observed for 10 days. Additionally 3 R-mix shell vials were inoculated with 0.2 mL each and processed at 24, 48 and 96 hrs post-inoculation following manufacturer's recommendation.

Samples that were positive by either kits but negative by culture were tested by RT-PCR by a reference laboratory to detect the presence of RSV nucleic acid sequences.

## RESULTS

Figure 1. Nasal aspirates positive for RSV by testing method (n=100)

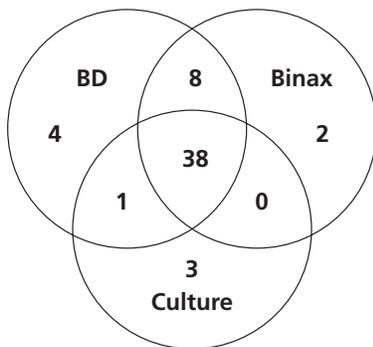


Table 1. Discrepant analysis using RT-PCR assay

Number of samples	BD Directigen EZ RSV result	Binax Now RSV result	Viral Culture result	RT-PCR assay Confirmation (%)
8	pos	pos	neg	7*(100)
4	pos	neg	neg	1 (25)
2	neg	pos	neg	2 (100)

\* 1 sample was not tested by RT-PCR due to insufficient volume.

Table 2. Performance characteristics of BD Directigen EZ RSV assay

	BD positive	BD negative
<b>Mod. Gold std. positive</b>	48	5
<b>Mod. Gold std. negative</b>	3	44

\*Mod. Gold std. = Culture or PCR results  
Analytical Sensitivity 91% Positive predictive value 94%  
Analytical Specificity 94% Negative predictive value 90%

Table 3. Performance characteristics of Binax NOW RSV assay

	Binax NOW positive	Binax NOW negative
<b>Mod. Gold std. positive</b>	48	5
<b>Mod. Gold std. negative</b>	0	47

\*Mod. Gold std. = Culture or PCR results  
Analytical Sensitivity 91% Positive predictive value 100%  
Analytical Specificity 100% Negative predictive value 90%

Table 4. Readability/Intensity of antigen test reactions

Readability of test band	0.5	1	2	3	4
<b>BD</b>	7	7	8	14	15
<b>Binax</b>	4	5	10	10	19

### **CONCLUSION**

- Both BD Directigen EZ RSV assay and Binax NOW RSV assay have comparable performance characteristics; Percent agreement between the two kits with 95% confidence interval is 93% (86-97%) Both kits have similar readability rates and the interference of background was minimal to none with both test kits.
  - Both BD and Binax assays are easy to perform and the results were available in less than an hour. Hence use of these kits in outpatient settings can expedite diagnosis of RSV infections.
  - It is important to note that the predictive value of these rapid antigen tests are affected by the viral disease prevalence rates. Hence during early and late viral respiratory season it is advisable to back up viral antigen test results with culture confirmation.
-

