

# Respiratory Syncytial Virus Detection by Tru RSV, Binax NOW RSV, Direct Immunofluorescent Staining and Tissue Culture

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# Introduction

- Human respiratory syncytial virus (RSV) is a negative-sense, single-stranded RNA virus of the family *Paramyxoviridae*. RSV is a member of the paramyxovirus subfamily *Pneumovirinae*.
- RSV is a common cause of upper and lower respiratory tract infections and the major cause of bronchiolitis and pneumonia in infants and children. Infections and outbreaks due to RSV typically occur yearly in the fall, winter and spring.
- Rapid identification and diagnosis of RSV has become more important due to the availability of effective antimicrobial therapy.
- Rapid identification can lead to reduced hospital stays, reduction in antimicrobial use, and reduction in the cost of hospital care.
- The objective of this study was to determine the performance characteristics of 2 immunochromatographic methods: MeridianTru RSV (Somagen Diagnostics, Edmonton, AB) and Binax NOW RSV (Innovatek Medical Inc., Delta, B.C.) to those of a combined standard of DFA and/or culture with nasopharyngeal specimens (NPS).

# Specimens

- NPS specimens (n=97) collected on flocked swabs (Copan, Murrieta, CA) were submitted from children (age range 2 weeks to 4 years). Specimens were received in viral transport medium with no further dilutions prior to testing.
- A portion of the NPS specimens were inoculated into HEp-2 tube cultures (Viromed). The tubes were incubated at 37° C and examined for CPE every other day for 10 days. RSV growth was confirmed by staining cell scrapings with FITC-conjugated monoclonal antibody (Millipore, Temecula, CA).
- A portion of the NPS specimens were tested for RSV by DFA using Simulfluor Respiratory Screen Kit (Millipore). Specimens were considered adequate if 3 or more cells were present per x200 field.
- The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the immunochromatographic methods were calculated in comparison to a combined standard of DFA and culture results. A sample with a true positive result was defined as any sample that was positive by DFA and/or by culture. A sample with a true negative result was defined as any sample that was negative by DFA and culture.

# Meridian TRU RSV

- Meridian TRU RSV is a single use capture immunoassay to detect RSV antigen in human samples. The test consists of a conjugate tube, a test strip and sample diluent. The conjugate tube contains a lyophilized bead of colloidal gold-linked monoclonal antibodies to RSV fusion and nucleoproteins (detector antibodies). The test strip carries a nitrocellulose membrane with dried capture antibodies placed at a designated test line for RSV.
- The conjugate bead is first rehydrated in the conjugate tube with sample diluent. Patient sample is added, the contents mixed and the test strip added. If RSV antigens are present, they first bind to the monoclonal antibody-colloidal gold conjugate. When the sample migrates up the test strip to the test line, the antigen-conjugate complex is bound to the capture antibody, yielding a pink-red line. When no antigen is present, complexes are not formed and no pink-red line appears at the test line. An internal control line helps determine whether adequate flow has occurred through the test strip during a test run. A visible pink-red line at the control position of the test strip should be present each time a specimen or control is tested. If no pink-red line is seen, the test is considered invalid.

# Binax NOW RSV

- The Binax NOW RSV Test is an immunochromatographic membrane assay used to detect RSV fusion protein antigen in nasopharyngeal swab specimens. Anti-RSV antibody is adsorbed onto a nitrocellulose membrane as the sample line. Control antibody is adsorbed onto the same membrane as a second stripe. Both anti-RSV and control antibodies are conjugated to visualizing particles that are dried onto an inert support. The conjugate pad and the striped membrane are combined to construct the test strip.
- To perform the test, the sample is added to the white pad at the top of the test strip, and the test device is closed. RSV antigen present in the sample reacts to bind anti-RSV conjugated antibody. The resulting antigen-conjugate complexes are captured by immobilized anti-RSV antibody, forming the sample line. Immobilized control line antibody captures a visualizing conjugate, forming a pink control line.
- Test results are interpreted by the presence or absence of visually detectable pink-to-purple coloured lines 15 minutes after sample addition. A positive test result will include the detection of both a sample line and a control line. A negative test result will produce only a control line. Failure of the control line to appear, or the control line remaining blue, indicates an invalid assay.

# Results

## Culture and DFA

- 97 specimens tested
- 41 positive by DFA and/or culture.
- Culture and DFA positive = 16
- DFA only positive = 25
- No specimens were positive by both immunochromatographic tests but negative by the standard tests.

## Meridian TRU RSV

	True Positive	True Negative	Total
Positive	24	0	<b>24</b>
Negative	16	57	<b>73</b>
<b>Total</b>	<b>40</b>	<b>57</b>	<b>97</b>

## Binax NOW RSV

	True Positive	True Negative	Total
Positive	24	0	<b>24</b>
Negative	17	56	<b>73</b>
<b>Total</b>	<b>41</b>	<b>56</b>	<b>97</b>

# Results

	Meridian TRU RSV	Binax NOW RSV
Sensitivity	60%	58.5%
Specificity	100%	100%
PPV	100%	100%
NPV	78%	76.7%

# Conclusions

- The performance characteristics of both the Meridian Tru RSV assay and the Binax NOW RSV assay are comparable. The sensitivities of both assays were approximately 60%.
- Both Meridian and Binax assays are easy to perform and the results were available within 30 minutes. Hence use of these kits in peripheral lab settings could expedite the diagnosis of RSV infections.
- Either assay would be useful for screening RSV in respiratory specimens when large volumes are tested or low levels of staffing occur.
- It is important to note that the predictive value of these rapid antigen tests is affected by the viral disease prevalence rates. If there is low prevalence (e.g. outside of RSV season), then the positive predictive value of rapid tests is low. Therefore during early and late viral respiratory season it is advisable to back up rapid test results with culture and/or DFA confirmation.

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