Respiratory syncytial virus is a member of the Paramyxoviridae family and is the most significant respiratory pathogen for infants and children. Infection usually causes mild to moderate severe upper respiratory illness that may lead to life threatening pneumonia or bronchiolitis. RSV infections are seasonal and are most prominent from December to March in the northern hemisphere. The virus is spherical in shape with a lipoprotein envelope synthesized from the plasma membrane of the infected host cell. The virus is spread rapidly through droplets dispersed in the air of secretions from the respiratory tract of infected individuals. The incubation period is three to seven days. Specimens from patients are obtained by using nasopharyngeal aspiration, washes, and swabs.

Several methods have been developed for the detection of RSV. This includes direct and indirect immunofluorescence on exfoliated cells, enzyme immunoassay (EIA) from nasopharyngeal samples, and isolation of the virus from tissue culture. Tissue culture has remained historically the “gold standard” used for diagnosis, but requires specialized equipment, highly trained personnel, specialized care in specimen collection and transportation, and long periods of time to obtain results. Rapid immunodetection methods have provided a cost effective detection option, which allows for timely patient treatment to prevent possible nosocomial spread.

PRINCIPLE
The Xpect™ RSV test utilizes a pair of Respiratory Syncytial Virus (RSV) specific antibodies in an immunochromatographic sandwich assay. The reaction between a positive sample and the colored particle-conjugated antibody forms a complex that migrates along the membrane. An immobilized capture antibody will form a colored line at the S (specimen) region upon reacting with the colored complex. An internal control line C (control) region is built-in to assure that the test has been carried out correctly.

STORAGE
The product should be stored at room temperature (15-30°C) in the sealed pouches. Do not freeze the test kit or kit reagents.

PRECAUTIONS
- **For In Vitro Diagnostic Use Only.**
- In accordance with the principles of Good Laboratory Practice, it is strongly recommended that all specimens be treated as potentially infectious and handled with all necessary precautions.
- Discard all used test devices into a biohazard container.
- Do not use kits after the stated expiration date and do not mix kit components from different lots.
- Users are cautioned against over reading of membrane immunoassays. Only clearly visible lines in the S region (specimen) should be considered a positive result.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORTATION
Acceptable specimens for evaluation with the Xpect™ RSV test include nasopharyngeal washes, aspirates, and swabs. Specimens should be transported to laboratory immediately after collection. Specimens may be stored at 2-8°C for up to 48 hours or at –20°C for up to one week.

**Transport Media:**
The following transport media have been tested and found to be compatible with Xpect™ RSV test.

- Earle’s Minimum Essential Medium (EMEM)
- EMEM with 1% Lactalbumin Hydrolysate
- M4™
- Phosphate Buffered Saline (PBS)
- PBS with 0.5% Bovine Serum Albumin
- PBS with 0.5% Gelatin
- Saline (normal)
- Todd Hewitt Broth
- Tryptic Soy Broth
- Viral CULTURETTE™

**REAGENTS AND MATERIALS SUPPLIED**
1. **Test devices (30):** Each foil pouch contains one single-use test device.
2. **Extraction Buffer (2 x 10 ml):** Two dropper bottles containing a mucolytic agent and 0.1% sodium azide as a preservative.
3. **Disposable Extraction Tubes (30):** with filtered caps.
4. **Instructions for Use (1).**

**MATERIALS REQUIRED BUT NOT SUPPLIED**
1. **Timer**
2. **Specimen Collection Containers and Transport Media**

**PROCEDURE**
Acceptable specimens include nasopharyngeal washes, aspirates, and swabs.

*Note: Mucoid or bloody specimens may fail to flow properly on the Xpect™ RSV test causing inconclusive test results (see Test Procedure). For excessively mucoid or bloody specimens, it may be helpful to treat the specimen with extraction buffer, followed by brief sonication, prior to addition to the Xpect™ RSV test.

**Sample preparation:**
1. **Nasopharyngeal Washes**
   a. Nasopharyngeal wash volumes of 2 to 4 ml are recommended. Excess wash volume may decrease test performance.
   b. If the specimen is mucoid or bloody, see note above.
2. **Nasopharyngeal Aspirates**
   a. Nasopharyngeal aspirates should be collected in volumes between 0.5 and 1 ml.
   b. Specimens should then be placed in 2 to 4 ml of viral transport medium or physiological saline, depending on the volume of aspirate received.
   c. If the specimen is mucoid or bloody, see note above.
3. **Nasopharyngeal Swabs**
   a. Place swab specimen in 0.75 to 3 ml of transport medium or physiological saline.
   b. Mix the swab and transport media or saline vigorously.
   c. Express excess liquid from the swab.
   d. Dispose of the swab in an appropriate container.

**Test Procedure:**
1. Remove the test device from the foil pouch when ready to perform the test and place it on a flat surface.
2. Label the device with patient or control identification.
3. Pipette 150 µl of the nasopharyngeal specimen into the test device.
4. Read and record the test results visually after 15 minutes. Some positive results may be observed within 30 seconds depending on the concentration of antigen in the specimen. Do not interpret results after 30 minutes.

**Note for mucoid or bloody specimens:** Add 250 µl of the nasopharyngeal wash specimens to the extraction tube. Add 2 drops of RSV Extraction Buffer. Insert filter cap, mix, and dispense 3-4 drops (approximately 150 µl) of extracted specimen from the extraction tube into a fresh test device. Some positive results may be observed within 30 seconds depending on the concentration of the antigen in the specimen. Do not interpret results after 30 minutes.

**INTERPRETATION**

**Positive Test:**
The test is positive if two colored lines appear. One colored line will appear in the S region (specimen) and one in the C region (control). A colored line of any intensity in the specimen region should be considered positive.

**Negative Test:**
The test is negative if a colored line appears only in the C region (control).

**Invalid Test:**
The test is invalid if no colored line appears in the C region (control) even if a colored line appears in the S region (specimen). If this occurs, the test is invalid and should be repeated. Colored lines that appear after 30 minutes are not diagnostic and should be ignored.

**QUALITY CONTROL**

**Internal:** Each test device includes an internal procedural control. The appearance of a control line in the C region (control) of the test device is a positive procedural control. Correct procedural technique, specimen flow, and test device performance is confirmed when a colored line appears in the C region (control) of the membrane. If the colored line fails to appear in the C region (control), the test result is invalid.

**External:** Positive and negative controls for RSV antigen should be tested and the appropriate results obtained with each new test kit lot number. Each laboratory should follow their state and local requirements.

**LIMITATIONS**

1. Both viable and nonviable RSV viruses are detectable with the Xpect™ RSV test. The test is not intended for confirmation of a respiratory infection caused by other etiological agents.
2. The Xpect™ RSV test is dependent on antigen load and may not correlate with other methods used for the detection of RSV such as tissue culture performed on the same specimen.
3. Frozen specimens should be thawed and brought to room temperature prior to testing.
4. False negatives may result from inadequate specimen collection (e.g., over dilution) or improper specimen handling or transport.

5. A negative test result does not rule out the presence of RSV virus. The results from the Xpect™ RSV test should be used in conjunction with other clinical findings to establish a diagnosis.

**PERFORMANCE CHARACTERISTICS**

**Accuracy by Comparison:**

**Laboratory Studies**
Sixty-three (63) frozen patient samples were obtained from several laboratories. An RSV viral culture was performed on each sample. Each sample was thawed and an Xpect™ RSV was performed.

<table>
<thead>
<tr>
<th>Tissue Culture Results</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>57</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>5</td>
<td>63</td>
</tr>
</tbody>
</table>

*Confirmed positive by EIA.

Percent Positive Agreement
98.3% (57/58 x 100); 95% CI = 90.8-99.9%
Percent Negative Agreement
100% (5/5 x100); 95% CI = 47.8-100%
Percent Agreement
98.4% (62/63 x 100); 95% CI = 91.5-99.9%

Ninety-four (94) frozen patient samples were obtained from several laboratories. Each sample was thawed and tested with an Xpect™ RSV and another commercially available test.

<table>
<thead>
<tr>
<th>Other Commercial RSV Test</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>83</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>7</td>
<td>94</td>
</tr>
</tbody>
</table>

*All four were confirmed positive by EIA and tissue culture.

Percent Positive Agreement
95.4% (83/87 x 100); 95% CI = 88.6-98.7%
Percent Negative Agreement
100% (7/7 x100); 95% CI = 59.0-100%
Percent Agreement
95.7% (90/94 x 100); 95% CI = 93.0-99.5%

**Clinical Sensitivity and Specificity:**

**Retrospective Study**
Three clinical sites tested one hundred twenty four (124) clinical samples blindly and retrospectively using the Xpect™ RSV test and compared the results to tissue culture. Samples were stored frozen and thawed prior to testing. The results are shown in the table below.

<table>
<thead>
<tr>
<th>Tissue Culture Results</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>86</td>
<td>2</td>
<td>88</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>34</td>
<td>124</td>
</tr>
</tbody>
</table>

Relative Sensitivity
95.6% (86/90 x 100); 95%CI = 89.0 to 98.8%
Relative Specificity
94.1% (32/34 x 100); 95%CI = 80.3 to 99.3%
Relative Correlation
95.1% (118/124 x 100); 95%CI = 89.8 to 98.2%
Clinical Comparison:
Prospective Studies
Nasal Washes
Two clinical sites tested twenty-six (26) clinical nasopharyngeal wash specimens blindly and prospectively using the Xpect™ RSV test and another commercially available RSV test. The results are shown below.

<table>
<thead>
<tr>
<th>Other Commercial RSV Test</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>22</td>
<td>26</td>
</tr>
</tbody>
</table>

Percent Positive Agreement
100% (4/4 x 100); 95%CI = 39.8% to 100%
Percent Negative Agreement
99.0% (22/22 x 100); 95%CI = 70.1% to 98.9%
Percent Agreement
92.3% (24/26 x 100); 95%CI = 74.9% to 99.1%

Nasopharyngeal Swabs
Two clinical sites tested twenty-eight (28) clinical swab specimens blindly and prospectively using the Xpect™ RSV test and another commercially available RSV test. The results are shown below.

<table>
<thead>
<tr>
<th>Other Commercial RSV Test</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>17</td>
<td>28</td>
</tr>
</tbody>
</table>

Percent Positive Agreement
54.5% (6/11 x 100); 95%CI = 23.4% to 83.3%
Percent Negative Agreement
100% (17/17 x 100); 95%CI = 80.5% to 100%
Percent Agreement
82.1% (23/28 x 100); 95%CI = 63.1% to 93.9%

Combined Study – Nasopharyngeal Washes and Swabs
Four clinical sites tested fifty-four (54) clinical specimens blindly and prospectively using the Xpect™ RSV test and the Other Commercial RSV Test. The Xpect™ RSV test and another commercially available RSV test had an overall agreement of 87%.

<table>
<thead>
<tr>
<th>Other Commercial RSV Test</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>37</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>39</td>
<td>54</td>
</tr>
</tbody>
</table>

Percent Positive Agreement
66% (10/15 x 100); 95%CI = 38.4% to 98.7%
Percent Negative Agreement
94.9% (37/39 x 100); 95%CI = 82.7% to 99.4%
Percent Agreement
87.6% (47/54 x 100); 95%CI = 75.1% to 94.6%

Retrospective Study
Three clinical sites tested one hundred twenty three (123) clinical samples blindly and retrospectively using the Xpect™ RSV test and another commercially available RSV test. Samples were stored frozen and thawed prior to testing.

<table>
<thead>
<tr>
<th>Other Commercial RSV Test</th>
<th>+</th>
<th>-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpect™ RSV Results</td>
<td>84</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>37</td>
<td>123</td>
</tr>
</tbody>
</table>

Percent Positive Agreement
97.7% (84/86 x 100); 95%CI = 91.8% to 99.7%
Percent Negative Agreement
97.3% (36/37 x 100); 95%CI = 85.8% to 99.9%
Percent Agreement
97.6% (120/123 x 100); 95%CI = 93.0 to 99.5%

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)
The limit of detection (LOD) for the Xpect™ RSV test was determined for five (5) RSV Strains. These strains included two (2) RSV A and three (3) RSV B strains.

<table>
<thead>
<tr>
<th>Type</th>
<th>RSV Viral Strain</th>
<th>Limit of Detection (TCID₅₀/0.2 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>RSV (Long)</td>
<td>1.7 x 10⁶</td>
</tr>
<tr>
<td>A</td>
<td>RSV (A-2)</td>
<td>9.9 x 10⁵</td>
</tr>
<tr>
<td>B</td>
<td>RSV (9320)</td>
<td>5.5 x 10⁴</td>
</tr>
<tr>
<td>B</td>
<td>RSV (Washington)</td>
<td>1.1 x 10⁴</td>
</tr>
<tr>
<td>B</td>
<td>RSV (Wild-type)</td>
<td>8.9 x 10³</td>
</tr>
</tbody>
</table>

CROSS REACTIVITY/INTERFERENCE STUDY
To confirm the analytical specificity of the Xpect™ RSV test, bacterial and viral cultures likely to be found in the respiratory tract were tested. Bacterial cultures were tested at 1 x 10⁸ cfu/ml and the viral cultures at 1 x 10³ to 1 x 10⁶ TCID₅₀/0.2 ml. All yielded negative results.

To confirm noninterference of the Xpect™ RSV test, RSV whole virus 9320 at titer 1.11 x 10³ TCID₅₀/0.2 ml was added to bacterial and viral cultures likely to be found in the respiratory tract. Bacterial cultures were tested at 1 x 10⁸ cfu/ml and the viral cultures at 1 x 10³ to 1 x 10⁶ TCID₅₀/0.2 ml. All yielded positive results.

Bacterial Cross Reactivity
- Candida albicans
- Chlamydia trachomatis
- Corynebacterium diphtheriae
- Haemophilus influenzae type A
- Klebsiella pneumoniae
- Mycoplasma pneumoniae
- Neisseria meningitidis
- Pseudomonas aeruginosa

Viral Cross Reactivity Panel
- Adenovirus 5
- Echovirus 3
- Parainfluenza 3
- Adenovirus 7
- Echovirus 6
- Varicella zoster
- Adenovirus 10
- HSV Type 1
- Rhinovirus 1A
- Coxackie A9
- HSV Type 2
- Rhinovirus 2
- Coxackie B5
- Influenza A
- Rhinovirus 13
- Coxackie B6
- Influenza B Hong Kong
- Rhinovirus 15
- Cytomegalovirus
- Parainfluenza 1
- Rhinovirus 37
- Echovirus 11
- Parainfluenza 2
REPRODUCIBILITY
The reproducibility of the Xpect™ RSV test was evaluated at three clinical laboratory sites. The Xpect™ RSV test was tested against a panel of five (5) specimens of which included three levels of positives and two negatives. The low and high positives were from the RSV Long strain, and the medium positive was comprised of RSV A2 strain. Negatives were comprised of either sample diluent or Streptococcus group A. Three (3) different laboratory personnel assayed each specimen at each laboratory facility. The overall reproducibility for the Xpect™ RSV test was 100%.

REFERENCES

PACKAGING
REF 24601, Xpect™ RSV-----------------------------------------------30 Tests/Kit

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<tr>
<td>LOT</td>
<td>Batch Code (Lot Number)</td>
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<tr>
<td>Use By (Expiration Date)</td>
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