READ ALL INSTRUCTIONS BEFORE BEGINNING THE ASSAY

INTENDED USE

CLARITY RSV antigen test kit is a visual and rapid assay for the qualitative detection of Respiratory Syncytial Virus (RSV) antigen directly from nasopharyngeal specimens in neonatal and pediatric patients. The test is for in-vitro diagnostic use only. It is recommended that negative test results be confirmed by cell culture.

BACKGROUND

Respiratory syncytial virus is a member of the Paramyxoviridae family and is the most significant respiratory pathogen for infants and children.1, 7 Infected 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 or secretions from the respiratory tract of infected individuals. The incubation period is 3-7 days. Specimens from patients are obtained by using nasopharyngeal aspiration, washes and swabs.2, 3 Several methods have been developed for the detection of RSV. This includes Direct and Indirect Immunofluorescence on ektolized cells, Enzyme Immunoassay (EIA) from nasopharyngeal samples and isolation of the virus from Cell Culture. Cell 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.1, 3, 4

PRINCIPLE OF THE TEST

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

MATERIALS & REAGENTS PROVIDED

1. Test Device.
2. CLARITY RSV Extraction Buffer (Contains mucolytic agent and 0.1% sodium azide as a preservative).
3. Disposable pipettes (150μl ea).
4. Package insert.

MATERIALS NOT PROVIDED

1. Timer.
2. RSV positive control.
3. RSV negative control.
4. Disposable Transfer Pipettes (1ml ea).
5. Disposable Sterile Swabs

PRECAUTIONS

1. For in-vitro diagnostic use only.
2. In accordance with the principles of Good Laboratory Practice, it is strongly recommended that all specimens be handled potentially infectious and handled with all necessary precautions.
3. Discard all used devices into a biohazard container.
4. Do not use kits after the stated expiration date, and do not mix kit components from different lots.
5. Users are cautioned against over reading of membrane immunasays. Only a clearly visible line in the S area should be considered a positive result.
6. Follow test procedure for each specimen type as written. Extraction tube and dropper tips should only be used with bloody or mucous samples.
7. Do not expose test to extreme temperatures. Test performance may be affected.
8. If the laboratory modifies the test system instructions, then the test is considered high complexity and subject to all applicable CLIA requirements.
9. A Certificate of Waiver is required to perform the test in a waived setting. This waiver may be obtained from your local state agency or by completing Form CMS-116 available at www.cms.hhs.gov/cw.
10. Laboratories with a Certificate of Waiver must follow the manufacturer’s instructions for performing the test. 42 CFR 493.15 (e) (1).

STORAGE CONDITIONS

CLARITY RSV Test devices should be kept at room temperature (15-30°C) in the sealed pouches. Do not freeze the test kit or kit reagents.

TRANSPORT MEDIA

The following transport media have been tested and found to be compatible with CLARITY RSV Test.

- 0.9% Saline
- PBS
- PBS, 0.5% Gelatin
- Triplicate Soy Broth
- Todd Hewitt Broth
- Viral CULTURETTE™
- M4 VTM
- M6 VTM
- EMEEM with Lactobumin hydrolysate

SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION

Acceptable specimens include nasopharyngeal washes, aspirates and swabs.7 Specimens should be transported to laboratory immediately after collection. Samples should be stored at 2-8°C for up to 48 hours or at <25°C for up to one week.

SPECIMEN PREPARATION

Acceptable specimens include nasopharyngeal washes, aspirates, and swabs.

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

Procedure For Use with Nasopharyngeal Washes:

1. Nasopharyngeal wash volumes of 2 to 4 ml are recommended. Excess wash volume may decrease test performance.
2. If specimen is mucoid or bloody see note above.

Procedure For Use with Nasopharyngeal Aspirates:

1. Nasopharyngeal aspirates should be collected in volumes between 0.5 and 1ml.
2. Samples then should be dispersed in 2 or 4 ml of viral transport medium or physiological saline up to 4 ml, depending on volume of aspirate received.
3. If specimen is mucoid or bloody see note above.

Procedure For Use with Nasopharyngeal Swabs:

1. Place swab specimen into 0.75-3 ml of transport medium or saline.
2. Mix the swab and transport media or saline vigorously.
3. Express excess liquid from swab.

5. Dispose of swab into appropriate container.

TEST PROCEDURE FOR SPECIMENS

1. Remove the test from the pouch and lay it on a flat surface.
2. Label test with the specimen type and ID.
3. Squeeze and fill the entire pipette with sample.
4. Squeeze and dispense the entire contents of the pipette into test device.

5. Read results at 15 minutes. Do not read results after 30 minutes.

Note: For Mucoid or Bloody Samples: Add 25μl of the nasopharyngeal wash specimen to extraction tube. Add 2 drops of CLARITY RSV Extraction Buffer. Insert filter cap, mix, and dispense 3-4 drops of extracted specimen from extraction tube into a fresh test device. Some positive results may be seen in as short as 30 seconds depending on the concentration of the antigen. Do not read results after 30 minutes.

PROCEDURE FOR EXTERNAL CONTROLS

1. Remove test device from pouch and lay on flat surface. Label device with specimen type and ID.
2. Pipette 150μl of the external control into test device.
3. Read results at 15 minutes. Some positive results may be observed in as briefly as 30 seconds depending on the concentration of the antigen. Do not interpret results after 30 minutes.

INTERPRETATION OF RESULTS

Negative Result: A pink colored band in the control (C) area without a pink colored band in the specimen (S) area is a negative result.

Positive Result: A pink colored band in the control (C) area without a pink colored band in the specimen (S) area is a negative result.

A Rapid Visual Assay for the Qualitative Detection of Respiratory Syncytial Virus Antigen in Nasopharyngeal Specimens
Each sample was thawed and a CLARITY RSV Test was performed.

Positive Result
Any pink colored band in the specimen (S) area with a pink colored band in the control (C) area is a positive result.

CLARITY RSV Test

Cell Culture

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>67</td>
</tr>
</tbody>
</table>

Percent Positive Agreement: (57/63) = 100% = 99.8% 
(95% CI: 90.8% to 99.9%)

Percent Negative Agreement: (5/63) = 100% = 94.5% 
(95% CI: 85.4% to 100%)

Percent Agreement: (62/63) = 100% = 98.4% 
(95% CI: 91.5% to 99.9%)

Ninety-four (94) frozen patient samples were obtained from several laboratories.

In valid Result
No pink colored band in the control (C) area of the test is an invalid result.

Other Commercial Test

CLARITY RSV Test

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>93</td>
</tr>
</tbody>
</table>

All four were confirmed positive by EIA and Cell Culture.

Percent Positive Agreement: (82/83) = 100% = 95.4% 
(95% CI: 88.6% to 97.8%)

Percent Negative Agreement: (7/83) = 100% = 95.0% 
(95% CI: 90.0% to 100%)

Percent Agreement: (90/83) = 100% = 95.7% 
(95% CI: 90.6% to 98.3%)

CLINICAL SPECIFICITY AND SENSITIVITY

Prospective Study

One hundred thirty-two (131) clinical samples collected over two (2) seasons were tested blindly and retrospectively using the CLARITY RSV Test and compared to Cell Culture. The results are shown in the table below.

Cell Culture

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>131</td>
<td>126</td>
</tr>
</tbody>
</table>

Sensitivity: (5/5) = 100% = 100% 
(95% CI: 56.6 to 100%)

Specificity: (126/126) = 100% = 100% 
(95% CI: 97.1 to 100%)

Correlation: (131/131) = 100% = 100% 
(95% CI: 97.2 to 100%)

Retrospective Study

Three clinical sites tested one hundred twenty-four (124) clinical samples blindly and retrospectively using the CLARITY RSV Test and compared to Cell Culture. Samples were stored frozen and thawed prior to testing. The results are shown in the table below.

Cell Culture

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>88</td>
<td>83</td>
</tr>
</tbody>
</table>

Relative Sensitivity: (86/90) = 100% = 95.6% 
(95% CI: 89.0 to 98.8%)

Relative Specificity: (32/4) = 100% = 94.1% 
(95% CI: 80.3 to 99.3%)

Relative Correlation: (118/124) = 100% = 95% 
(95% CI: 89.8 to 97.8%)

CLINICAL COMPARISON

 Nasopharyngeal Swabs

Two clinical sites tested twenty-eight (28) clinical swab specimens blindly and retrospectively using the CLARITY RSV Test and the Other Commercial RSV Test. The results are shown below.

Other Commercial RSV Test

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>28</td>
</tr>
</tbody>
</table>

Percent Positive Agreement: (6/11) = 100% = 54.5% 
(95% CI: 23.4% to 83.3%)

Percent Negative Agreement: (17/17) = 100% = 100% 
(95% CI: 80.5% to 100%)

Percent Agreement: (23/28) = 100% = 82.1% 
(95% CI: 63.1% to 93.9%)

ANALYTICAL SENSITIVITY (LIMIT OF DETECTION)

The limit of detection (LOD) of the CLARITY RSV Test was determined for five (5) RSV Strains. These strains included three (3) RSV B and two (2) RSV A strains.

CROSS REACTIVITY/INTERFERENCE STUDY

To confirm the analytical specificity of the CLARITY RSV Test, bacterial and viral cultures likely to be found in the respiratory tract were tested. Bacterial cultures were tested at 1.0 x 10^5 CFU/ml and the viral cultures at 1.0 x 10^4 TCID50/ml. All yielded negative results.

To confirm noninterference of the CLARITY RSV Test, RSV whole virus 9320 at titer 1.11 x 10^5 TCID50/ml was added to bacterial and viral cultures likely to be found in the respiratory tract. Bacterial cultures were tested at 1.0 x 10^5 CFU/ml and the viral cultures at 1.0 x 10^4 TCID50/ml. All yielded positive results.

Bacterial Cross Reactivity

<table>
<thead>
<tr>
<th>S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>RSV Viral Strain</th>
<th>Limit of Detection (TCID50/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>RSV (Lang)</td>
<td>1.7 x 10^6</td>
</tr>
<tr>
<td>B</td>
<td>RSV (Washington)</td>
<td>1.1 x 10^8</td>
</tr>
<tr>
<td>C</td>
<td>RSV (H1337)</td>
<td>6.9 x 10^9</td>
</tr>
</tbody>
</table>

Viral Cross Reactivity Panel

Adenovirus 5
Adenovirus 7
Adenovirus 10
Coxsackie A9
Coxsackie B4
Coxsackie B6
Coronavirus 2
Cytopathogenic virus
Echovirus 11
Echovirus 12
Echovirus 3
Echovirus 6
HSV Type-1
HSV Type-2

Reproducibility

Physician Office Lab Study

The reproducibility of the CLARITY RSV Test was evaluated at three physician offices. The CLARITY RSV Test was tested against a panel of five (5) specimens of which included three levels of positives and two negatives. The overall reproducibility for the CLARITY RSV Test was 100%.

Lay Person User Study

Individuals having diverse educational backgrounds evaluated the CLARITY RSV Test at three different sites. Each site tested a coded panel consisting of a negative, low positive and high positive. There was greater than ninety-eight percent (98%) agreement (221/225) of the expected results.

REFERENCES


M4™ is a trademark of REMEL, Inc. ATCC® is a registered trademark of American Type Culture Collection TCC® is a trademark of Beckton Dickson and Company

Revision 12/10

701782/2002