RSV ANTIGEN

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PURPOSE

To provide instructions for performing a rapid immunoassay for Respiratory Syncytial Virus (RSV) antigen

BACKGROUND

RSV is an important cause of pneumonia and bronchiolitis in small children. It is also recognized as a cause of significant respiratory disease in the elderly. RSV can cause a wide range of respiratory symptoms that can be difficult to distinguish clinically from symptoms of other viruses such as influenza. The immunoassay test consists of a tube with a monoclonal antibody-conjugate and when added to the test strip, the antibody-conjugate and RSV antigen complex binds to the capture antibody producing a red line.

RELATED DOCUMENTS

R-W-MB-2209 Nasal Collection

SUPPLIES

Meridian TRU RSV kit (can be stored at 2-8˚C or room temperature)
FLU/RSV external controls (store at 2-8˚C)

SPECIMEN COLLECTION

1. Nasal wash or aspirate
   - Collect specimen in VTM(viral transport media) or M6
   - M4 or M5 media
   - Store sample tubes at 2-8˚C until testing
   - Media can be held for up to 72 hours at refrigeration prior to testing

2. Nasal swabs
   - Cotton, rayon, polyester and flocked swabs with plastic or metal handles are acceptable
   - DO NOT USE Calcium alginate swabs
   - Collection of specimen on flocked swabs and placed in VTM media (VTM supply kit)
   - Since RSV is unstable, the specimen collected on a swab without viral media must be tested within 60 minutes of collection. If testing cannot be tested in this time, add 2-3 mls of VTM media. Store at 2-8˚C until testing can be completed.
   - Specimens that are grossly bloody are unacceptable and can lead to false positive results.

SPECIMEN PREPARATION

1. **For Nasal washes, aspirates or swab specimens in transport media:**
   - Remove 1 Conjugate Tube from its foil pouch and label the tube with a Cerner label

- Remove the cap from the tube and discard.
- Using a transfer pipette from the kit, immediately add 100ul (first mark from the tip of the pipette) of sample diluent to the conjugate tube and dispense into the center of the tube. Vortex or swirl the tube for 10 seconds to mix.
- Mix patient sample well by using a transfer pipette included in the kit and gently squeezing the bulb several times or vortex for 10 seconds.
- Using the same pipette, draw 100ul (first mark from tip of pipette) of the sample and add to the conjugate tube.
- Using the same pipette, mix the sample and conjugate thoroughly squeezing the bulb several times or vortex for 10 seconds. Discard the pipette.

2. **For Nasal or NP swabs without transport media or diluent:**
   - Remove 1 Conjugate Tube from its foil pouch and label the tube with a Cerner label.
   - Remove the cap from the tube and discard.
   - Using a pipette supplied in the kit, immediately add 300ul (third mark from the end of the pipette tip) of sample diluent to the center of the conjugate tube.
   - Vortex or swirl the conjugate tube to mix for 10 seconds.
   - For heavily viscous samples, add an additional 200ul (second mark from the end of the pipette tip) of diluent for a total of 500ul to the conjugate tube, mix.
   - Remove the swab from the culturette and dip into the conjugate tube. Rotate the swab 3 times in the liquid. Press the swab against the side of the tube as it is removed to squeeze out as much liquid as possible. Discard the swab.

NOTE: Dilution errors may affect test performance. Failure to add sufficient respiratory sample to the diluent may result in falsely negative results. Failure to add the full amount of sample diluent may result in falsely positive results. Addition of too much sample may result in an invalid test due to inhibition of proper sample flow.

STEPS

1. Remove the test strip from the foil pouch.

2. Insert the narrow end of the test strip into the prepared conjugate tube and firmly press down on the cap to close the tube.

3. Incubate at room temperature for 15 minutes.

4. **Results must be read at 15 minutes +/- 1 minute. Do not read results beyond this time period**.

5. If results are difficult to read, remove test strip from conjugate tube to read test and control lines.

INTERPRETATION OF RESULTS

RESULT	INTERPRETATION
Negative	A pink-red band appears at Control position (C) and no other bands are present
Positive	A pink-red band appears at Control position (C) and at the Test position (T)
Weakly Positive	A pink-red band appears at the Control position (C) and a faintly visible band appears at the Test position (T). Compare to color illustration on the procedure card in the kit.
Invalid Test	No band at the control position. A pink-red band appears after 16 minutes of incubation Bands of color other than pink-red

If any result is difficult to interpret, the test should be repeated with the same sample to eliminate the potential for error. Obtain a new sample and retest when the original sample repeatedly produces unreadable results.

REPORTING

1. Order test code in Cerner: RSV AG

2. Use Cerner icon MBE, enter:

   - NEG: Negative for RSV antigens
   - POS: Positive for RSV antigens
   - INCON: Result is inconclusive, repeat testing

QUALITY CONTROL

1. Each test strip has its own internal control and is evaluated with each test specimen.

2. A pink-red band at the Control line (C) serves as the positive procedural control. If this is not present the test is invalid.

3. A clean, white background around the control and test lines serves as a negative procedural control. Heavy discoloration around the lines may be an indication of reagent deterioration. Investigate and open a new kit if necessary.

4. External controls are separate from the internal controls and performed with each new lot or shipment of test kits.

   - Bring controls to room temperature
   - Remove two conjugate tubes from the foil pouch. Remove the caps from the tubes and discard. One tube and test strip will be needed for each control. Label tubes, positive and negative.
   - Add exactly 5 drops of the positive control reagent to the tube labeled positive. Hold vial vertical to ensure consistent drop size.
   - Using the pipettes in the kit, add 200ul of sample diluent (second mark from the end of the pipette tip) to the tube labeled negative.
   - Vortex or swirl the tubes for 10 seconds.
   - Remove 2 test strips from their pouches and insert the narrow end of a test strip to the conjugate tube and firmly press down on the caps to close each tube.
   - Incubate tubes for 15 minutes. Read results within 1 minute. Do not read beyond this period.
   - See Interpretation of Results table for correct results.
   - Any results not within expected results should be repeated. Remove the kit from service if control consistently fails and contact manufacturer. Do not report any patient results until expected results are achieved.
   - Document results in Cerner or log.

5. RSV is a seasonal organism and there may not be patient testing during this time. If more than 30 days have elapsed since any testing has been performed the kit and techs performing the test must be assessed for accuracy. To restart testing the following must be met:

   - CAP survey must be performed within 30 days prior to restarting patient testing

   - Method performance verified. Perform external QC.

- Competency of all techs performing Influenza antigens within 12 months of restarting the testing.
- If no CAP surveys have been received prior to restarting the test, an alternate method for assessment of the test must be performed.

LIMITATIONS

1. Overincubation of the test strip, past 16 minutes, may lead to a false positive result.
2. The TRU RSV kit detects viable and non-viable RSV. A positive result depends on antigen viral load present in the sample.
3. Antibodies used in testing may not detect all variants or strains of RSV.
4. A negative test does not exclude infection with RSV nor does it rule-out other microbial caused respiratory infections.
5. A positive result does not rule out co-infection with other microbes.
6. Faintly positive, weak test bands may yield false positive results. A tissue culture may be necessary to obtain correct culture results. The TRU RSV results should be used with other test results and the patient's clinical picture.

REFERENCES

Product insert, TRU RSV, Meridian Bioscience, Inc. 11/2009

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Added perform CAP survey for testing not done in 30 days			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☐ Date: ☐ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Linda D. Blumkhandt, MD 8/19/13