Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1215
VERSION: 3

SUBJECT: TRU RSV Kit for the direct detection of Respiratory Syncytial Virus (RSV)

Principle:

RSV infection is a major cause of lower respiratory tract illness in young children as witnessed by the yearly upsurge of pneumonia and bronchiolitis. RSV has also been implicated as a cause of apnea in infants, otitis media, heart disease and acute exacerbations of chronic bronchitis.

TRU RSV is a lateral flow-based immunoassay for the rapid detection of RSV in human respiratory 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. The test strip carries a nitrocellulose membrane with dried capture antibodies placed at a designated test line for RSV. The test strip holder caps the conjugate tube during testing and subsequent disposal to reduce exposure to potential pathogens.

The conjugate bead is first rehydrated in the conjugate tube with sample diluent. Patient sample is then 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, no complexes are 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.

Specimen:

Specimens should be collected and transported in standard containers and stored at 2-8°C until tested. The specimen should be tested as soon as possible, but may be held up to 72 hours at 2-8°C prior to testing. If testing cannot be performed within this time frame, specimens should be frozen immediately on receipt and stored frozen (<= -20°C) for up to 2 weeks until tested. A single freeze/thaw cycle should not affect test results.

The following transport media are suitable for collection of specimens:
M4, M4-RT, M5, UTM-RT, Stuart's, Hank's Balanced Salt, Amies, Dulbecco's

PBS, 0.85% saline, Meridian Viral Transport Medium. The volume of transport medium should not exceed 3 mL or false-negative results may occur due to sample dilution.

The following swabs are suitable for collection of specimens: Cotton/plastic, rayon/plastic, flocked nylon/plastic, foam/plastic, polyester/metal, polyester/plastic, rayon/metal, cotton/metal.

Do not use calcium alginate swabs. The chemical decreases positive reactions. Elute all approved metal-shafted swabs in Sample Diluent or transport medium within 5 minutes of collecting specimens. Elute all plastic shafted swabs in Sample Diluent or transport medium within 60 minutes of collecting specimens.

Whole blood at concentrations greater than 2.9% may lead to falsely positive test results. Do not use specimens that are obviously contaminated with blood.

Specimen Preparation for nasopharyngeal swabs:

Bring specimens and reagents to room temperature before testing.

1. Remove 1 conjugate tube from its foil pouch and discard the pouch. Label the tube with the patient's name.
2. Remove the cap from the conjugate tube and discard the cap.
3. Using a transfer pipette supplied with the kit, immediately add 300 microliters (fourth mark from the end of the pipette tip) of sample diluent to the conjugate tube. Dispense directly into the center of the tube. Vortex or swirl the contents of the conjugate tube for 10 seconds. For heavily viscous samples, up to 500 microliters of the sample diluent can be added. (use 4th mark on pipette -300ul plus 3rd mark –200ul)
4. Dip the swab into the conjugate tube and rotate it 3 times in the liquid. Press the swab against the side of the tube as it is removed to squeeze out as much fluid as possible. Discard the swab.
5. For the positive external control, add exactly 5 drops of the positive control reagent to a prelabeled conjugate tube marked as a positive control. Vortex or swirl for 10 seconds.
6. For the negative control, using 1 of the transfer pipettes supplied with the kit, add 200 microliters (3rd mark from the end of the pipette tip) of sample diluent/negative control to the conjugate tube. Vortex or swirl for 10 seconds.

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

Procedure:

1. Remove the test strip from its foil pouch and discard the pouch.

2. Insert the narrow end of the test strip into the conjugate tube and firmly press down on the cap to close the tube.
3. Incubate at room temperature for 15 minutes.
4. Read the results on the test strip within 1 minute. Do not read results beyond this period. The test strip can be removed from the conjugate tube if the test or control lines are difficult to read. Recap the test strip holder and discard when testing is complete.

Reagents and Materials:

TRU RSV kit containing:

1. Test Strip: A test strip attached to a plastic holder enclosed in a foil pouch with desiccant.
2. Conjugate Tube: A capped plastic tube containing a conjugate bead. The tube is enclosed in a foil pouch.
3. Sample Diluent/Negative Control: A buffered protein solution provided in a plastic vial. Sodium azide (0.094%) added as a preservative.
4. Plastic transfer pipettes with 50, 100, 200 and 300 microliter volume marks.

Other materials required, but not included in kit:

1. Disposable gloves
2. Vortex
3. Interval timer
4. Meridian Bioscience FLU/RSV Positive Control (Product Code 751110).

Reagent Storage:

Kit is to be stored at Room Temperature. Manufacturer's guidelines are to store at 2-25°C.

Precautions:

1. Do not use reagents beyond their expiration dates.

2. Inspect each foil pouch before use. Do not use if they have holes, or if the desiccant has changed from blue to pink, as this would indicate exposure to moisture. This could lead to false negative results.

3. Do not use if sample diluent buffer is discolored or turbid.

4. All patient samples and controls should be handled and disposed of as if they are biologically hazardous.

Quality Control:

At the time of each use, kit components should be visually examined for obvious signs of microbial contamination, freezing or leakage. Do not use contaminated or suspect reagents.

Internal procedural controls are contained within the test strip and therefore are evaluated with each test. A pink-red band appearing at the control line serves as a procedural control and indicates the test has been performed correctly, that the proper flow occurred and that the test reagents were active at the time of use. A clean background around the control or test lines also serves as a procedural control. Control or test lines that are obscured by heavy background color may invalidate the test and may be an indication of reagent deterioration, use of an inappropriate sample, or improper test performance.

The reactivity of each new lot and each new shipment of TRU RSV should be verified using external positive and negative control reagents. (See preparation and procedure sections for performing the QC testing.) If the positive and negative controls fail, do not report test results to the clinician. Repeat the control tests as the first step in determining the root cause of the failure. Contact Meridian Bioscience Technical Support Services at 513-271-3700 for assistance in troubleshooting when control failures are repeated.

Interpretation of Results:

Positive Test:

A pink-red band at the control and RSV test line positions. The color of the test line can be lighter than that of the control line. Test lines may appear strongly visible or may appear less strongly visible.

Weakly Positive Test:

A pink-red band at the control position and the appearance of a very faintly visible RSV test line. TRU RSV weak positive test lines should be interpreted with caution since weakly positive test results may represent false-positive tests. Weakly positive test results should be considered presumptive positives and should be confirmed by tissue culture or DFA testing.

Negative Test:

A pink-red band at the control line position. No other bands are present.

Invalid Test:

No band at the designated position for the control line. The test is invalid since the absence of a control band indicates the test procedure was performed improperly or that deterioration of the reagents has occurred. A pink-red band appearing at the test line position of the device AFTER 16 minutes of incubation, or a band of any color other than pink-red is also an invalid test. Falsely positive results may occur if tests are incubated too long. Bands with other colors may indicate reagent deterioration.

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 Results:

Negative - report as Negative for RSV antigen, or equivalent computer result and a comment confirming that the internal QC performed as expected, using the Microbiology Coded Response Sheet. Using “LotQC” as the entry code, fill in the kit information.

Positive - report as Positive for RSV antigen, or equivalent computer result and a comment confirming that the internal QC performed as expected, using the Microbiology Coded Response Sheet. Using “LotQC” as the entry code, fill in the kit information.

Limitations

1. Test is qualitative and no quantitative interpretation should be made with respect to the intensity of the positive line when reporting the result.
2. The performance of TRU RSV has not been established in patients greater than 5 years of age.
3. Overincubation of tests may lead to false-positive test results.
4. Anti-microbials, anti-virals and interferon were not evaluated for potentially interfering properties.
5. TRU RSV detects both viable and non-viable RSV. The appearance of positive tests depend on RSV antigen load in the specimen; therefore a TRU RSV positive test may not correlate with the results of tissue culture performed on the same specimen.
6. The antibodies used in the test may not detect all antigenic variants or new strains of RSV.
7. A negative test result does not exclude infection with RSV nor does it rule out other microbial-caused respiratory infections. A positive test result does not rule out coinfection with other microbes.
8. In all immunochromatographic assays, faintly visible or weak test lines are more likely to be falsely positive than are strongly positive test lines. As with any diagnostic procedure, the result of a TRU RSV test should be used in conjunction with other tests and the patient’s clinical picture.

References:

TRU RSV kit, Meridian Bioscience, Inc., product insert SN11167, revised 10/07.

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