Respiratory Syncytial Virus Kit

**INTENDED USE**

PathoDx™ RSV kit is a direct immunofluorescence test for the detection of Respiratory Syncytial Virus (RSV) in prepared direct patient specimens and following growth in cell culture.

**2. SUMMARY AND EXPLANATION OF THE TEST**

Respiratory syncytial virus (RSV) is the major cause of acute viral respiratory disease in infants and young children, with known symptomatic infection occurring in about 10 to 40% of those children infected for the first time.

It is responsible for 50% of all bronchiolitis cases and 25% of all cases of lower respiratory disease in infants and children and adults usually results in symptoms of a common cold. Due to the lack of adequate anti-retroviral treatment in adults, especially the elderly.

RSV is prevalent worldwide, causing annual winter outbreaks of respiratory disease, particularly in temperate climates. The virus is very contagious, with infected children shedding virus for 1 to 2 weeks and 1 to 3 weeks of shedding for adults. Nasopharyngeal washs or swabs, or swabs are the most frequently used specimens for RSV. A minimum of five cell culture specimens should be collected to 3 days after the onset of symptoms.

For the diagnosis of RSV infection, a sample must be collected within the first 5 days of symptoms for detection of RSV virus by isolation in cell culture. Samples collected on or after the 5th day of illness are less likely to yield a positive result. However, collection of respiratory specimens beyond the 7th day is necessary in patients with very severe respiratory disease, such as Long or Rhesus monkey RSV, which may exhibit delayed viral shedding. Continuous human epithelial cell lines, such as HEp-2 or Vero, are recommended for isolation of RSV, since they support primary isolation of RSV. HEp-2 cells are the most sensitive cell line for viral isolation. RSV inoculation in tissue culture should be examined microscopically for cell quality and sub-confluence (50 to 75% confluency) to assure minimum sensitivity.

**3. PRINCIPLE OF THE PROCEDURE**

The PathoDx RSV kit uses two fluorescein-labeled monoclonal antibodies for RSV to detect and identify RSV in direct patient specimens and inoculated cell culture. A single reagent contains all necessary reagents specific for RSV: influenza A and B monoclonal antibody specific for the Fv antigen, Human immunodeficiency virus (HIV) antigen from patient cells, and as well as Acetone (reagent grade or better) stored in glass. Microscope slides with wells 7 to 10 mm in diameter, acetone-resistant, hydrophobic coating. Wells must be coated with a protein-stabilized buffer solution (approximately 5 mm) to allow the slides to air dry completely.

**4. MATERIALS**

- **Specimen Collection:**
  - Respiratory secretions
  - Nasopharyngeal washes
  - Nasopharyngeal swabs
  - Bronchoalveolar lavage
  - Nasal aspirates
  - Respiratory cell suspension
  - Specimen collection and transport that best meet their needs.

- **Ancillary materials:**
  - Sterile Pasteur pipettes
  - Centrifuge with holder capable of holding one-dram vials
  - Phosphate-buffered saline (PBS: 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2–7.4, free of calcium and carbonate, and antibiotics (100–250 U/ml Penicillin G and 100–250 U/ml Streptomycin, or 5–10 μl Gentamicin, or equivalent).
  - Sterile swabs
  - Microscope slides with 7 to 10-mm wells
  - Acetone (not provided) is a flammable organic solvent. Use closed vials.

- **Complementary Tests:**
  - PCR
  - Serology
  - Viral culture

**5. PROCEDURE**

**Step 1** Store at 2°C to 8°C, protected from light. Use on or before the expiration date marked on the label. Allow product to come to room temperature (15 to 25°C) before use.

**RSV Reagent**

One plastic dropper bottle containing 5.0 ml of RSV mounting fluid, consisting of buffered glycerol with 0.01% sodium azide as preservative.

**Mounting Fluid**

One plastic dropper bottle containing 5.0 ml of RSV mounting fluid, consisting of buffered glycerol with 0.01% sodium azide as preservative.

**RSV Control Slides**

Five slides packaged individually in sealed foil with a desiccant. Each control slide contains one positive and one negative well with infected RSV Long (ATCC® VR26) cells in lead and copper in older children and adults usually results in symptoms of a common cold.

**6. SPECIFICATION, STORAGE, AND TRANSPORT**

**Specimen Collection:**

RSV can be used for the detection of antigens in a direct patient specimen, as well as in cell culture.

**RSV Kit Description:**

- **Antigen:**
  - After treatment with a single wash of Acetone, the cell monolayer is fixed with alcohol or acetone and covered with a protein-stabilized buffer solution containing 0.088% sodium azide as preservative.

**Shelf Vial Cell Culture Procedure**

- **Supplemental materials:**
  - Sterile Pasteur pipettes

**Notes on technique**

- **Acetone:**
  - Acetone is not a food-grade or drug-grade alcohol.

- **RSV Kit is a direct immunofluorescence test for RSV in specimens from RSV infected patients and following growth in cell culture.**
negative result. Cells show only dark red counterstaining and invalidate the test for specificity.

10. LIMITATIONS

1. Detection of RSV in direct and cultured specimens is dependent upon proper specimen collection, transport, tissue culture technique, and slide preparation.

2. Prolonged exposure of the stained slide to light can result in a decrease in fluorescence intensity.

3. The working reagent (RSV Reagents) comes ready-to-use and is optimized to detect RSV antigens. Dilution or other alteration of the working reagent may result in loss of sensitivity or diminished fluorescence.

4. The intensity of the observed fluorescence is dependent upon the properties of the fluorescence microspheres being used. The type of microscope and lens, the age and kind of light source, the filter assembly and thickness, etc., may all affect test performance. The use of a proper control helps to monitor the proper functioning of the fluorescence microscope.

5. The presence of a specific fluorescence with a direct specimen test for RSV with a negative result can occur when the test detects the presence of nonspecific RSV antigens in the direct specimen.

6. A result showing only dark red counterstaining cells on the shell vial cover does not exclude the possibility of an infection with RSV. For this reason, these tubes should be utilized as a prop of the CPE. If these cultures are negative at the end of ten days, the cells and supernatant should be passed again into another set of cultures. If at the end of ten days this second set does not show any CPE, the specimen may be reported as having no RSV detected.

7. Interpretation of the results must include critical evaluation of the patient and other diagnostic procedures. Another specimen should be submitted if RSV is strongly suspected.

8. The effects of antiviral therapy on the results from this test kit are unknown.

9. Non-specific staining may be observed as an artifact due to binding between antibody Fc regions and proteins A and G found in the cell wall of some strains of Staphylococcus aureus. This reagent contains an inhibitor of this reaction.

10. EXPECTED VALUES

During fall, winter and spring months it is unusual to find an incidence of positive RSV in 25 to 50% of infants and young children tested for lower respiratory infections. Results may vary with season and the population; at times 100% of adults and children older than 8 years have mild or asymptomatic RSV infections. Infants and young children often have lower respiratory involvement and resulting complications. RSV can also be an unusual cause of significant lower respiratory tract infection in adults, especially the elderly.

11. PERFORMANCE CHARACTERISTICS

To demonstrate the specificity of the PathoDx RSV test, the following viruses and cell lines were stained with the RSV reagent.

- Two bovine strains of RSV ATCC® VR794 and ATCC® VR1339 were found to give positive staining with the PathoDx RSV test and with Kit A, a total of 67 tested positive by both methods and 520 tested negative by both methods; however, 8/520 that were negative by direct smear were confirmed positive by the tube culture reference methods. Two direct smear samples tested positive by PathoDx and negative by Kit A, but these were confirmed positive by the shell vial culture reference methods (Table 1).

- Table 1: PathoDx RSV Direct Smears vs. Kit A Direct Smears (n = 189)

- Table 2: PathoDx RSV Direct Smears vs. Kit A Shell Vial Culture (n = 189)

- Table 3: PathoDx RSV Direct Smears vs. Kit A Tube Culture (n = 189)

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- Table 2: PathoDx RSV Direct Smears vs. Kit A Shell Vial Culture (n = 189)

- Table 3: PathoDx RSV Direct Smears vs. Kit A Tube Culture (n = 189)

- Table 4: PathoDx RSV Shell Vial Culture vs. Kit A Shell Vial Culture (n = 594)

- Table 5: PathoDx RSV Tube Culture vs. Kit A Tube Culture (n = 100)

- Table 6: PathoDx RSV Shell Vial Culture vs. Kit A Shell Vial Culture (n = 100)

- Table 7: PathoDx RSV Direct Smears vs. PathoDx RSV Tube Culture (n = 100)

13. CLINICAL STUDIES

In a study conducted at two sites, one in the midwest and the other for 40 to 48 hours. The shell vials were then stained. In comparing direct-smear stained with PathoDx RSV and with Kit A, a total of 67 tested positive by both methods and 520 tested negative by both methods; however, 8/520 that were negative by direct smear were confirmed positive by the tube culture reference methods. Two direct smear samples tested positive by PathoDx and negative by Kit A, but these were confirmed positive by the shell vial and tube culture reference methods (Table 1).

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The results from both culture methods were then compared.

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14. BIBLIOGRAPHY

- Raven Press, New York, NY.
- Springer-Verlag, New York, NY.
- Remel Europe Ltd. Clipper Boulevard West, Crossways Dartford, Kent, UK 01622 879770
- 95% Confidence Limits for Relative Sensitivity and Specificity, respectively: 65.4% – 86.1% and 96.3% – 99.0%.
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- Table 8: PathoDx RSV vs. Kit A

For technical assistance, please contact your local distributor.