Critical in order to initiate therapy and to control nosocomial infection has made RSV the most frequent cause of nosocomial infection.20,21 Parainfluenza virus serotypes have been identified (types 1, 2, 3, and 4) and are responsible for 50% of all bronchiolitis cases and 25% of all pneumonia cases in infants and children. The clinical diseases caused by parainfluenza viruses, the parainfluenza viruses also possess hemagglutinin, and neuraminidase, which are antigenically more variable in older children and adults usually results in symptoms of a common cold, but it can also cause significant lower respiratory tract infections in adults, especially the elderly.22 RSV is distributed worldwide, causing annual winter outbreaks of respiratory tract infections. Parainfluenza virus infections are caused by viruses. With the availability of anti-viral therapy for some viruses, determination of a viral etiology for respiratory infections is important. Early and appropriate use of effective antiviral therapy can decrease morbidity and mortality associated with lower respiratory infections.

The laboratory diagnosis of viral infections is usually accomplished by serology and cell culture isolation/confirmation. The drawback to using cell cultures is the delay involved in obtaining acute and convalescent phase sera. Culture isolation/confirmation is the standard method for most viral and Rickettsial infections. Although it is not as rapid as detection from direct rapid diagnostic tests, the isolation/confirmation of the most sensitive method for detecting viral respiratory pathogens. Respiratory specimens (nasopharyngeal aspirates or swabs) are tested for the presence of the virus using a test detection using viral enrichment-enhancement and cultured tube culture isolation is the gold standard diagnosis of common viral etiologies of respiratory tract infections.

Parainfluenza Viral Infection (RVP): RSV is the major cause of acute viral respiratory disease in infants and young children. Illness occurs in all age groups, but those over 60 years of age are at particular risk. It is responsible for 50% of all bronchiolitis cases and 25% of all pneumonia cases during the first months of life. RSV infection is much less severe in older children and adults usually results in symptoms of a common cold, but it can also cause significant lower respiratory tract infections in adults, especially the elderly.22 RSV is distributed worldwide, causing annual winter outbreaks of respiratory tract infections. Parainfluenza virus infections are caused by viruses. With the availability of anti-viral therapy for some viruses, determination of a viral etiology for respiratory infections is important. Early and appropriate use of effective antiviral therapy can decrease morbidity and mortality associated with lower respiratory infections.

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1. Acetone will absorb moisture if it is not properly stored. This moisture will cause a non-specific staining during the fixation process. Should this occur, use a fresh batch of acetone to replace the reagent.

2. Do not freeze specimens before inoculation into cell culture unless absolutely necessary. Some viruses may not survive freezing.

3. Do not allow the fluorescence-labeled monoclonal antibody staining magnets to dry on the slide. Drying will cause non-specific staining to occur around the periphery of the wells. Ensure that there is sufficient stain to overlay the well.

4. Background fluorescence resulting from the fluorescent reagent and non-specific surface staining may be the result of insufficient washing leading to the loss of the desired signals on the fluorescent conjugate. Should this occur, remove the coverslip, wash thoroughly with PBS, and repeat the slide again.

5. A dull yellow-green fluorescence can result from conjugate being trapped by aggregated cells that made fixation and washing difficult. Avoid over-dissection of this area of the well.

6. Murine sera should be used as much as possible from a known origin to eliminate the potential for false positive and false negative reactions with the fluorescent antibodies. Extreme care should be exercised in interpreting direct immunofluorescence results from the sera.

7. Stained slides must be carefully washed to avoid reagent carryover to adjacent wells. If dual infection occurs or is suspected, it is recommended that separate, fixed slides be used for each of the suspected virus-specific reagents to minimize this possibility.

8. The detection of respiratory viruses in cultured specimens is dependent upon proper specimen collection, transport, tissue culture technique, and slide preparation.

9. Non-specific staining may occur due to binding between the Fab antibody regions and proteins (Ab) found in some strains of bacteria or fungi or other extraneous agents. The non-specific binding could yield results with a decreased positive predictive value.

10. A negative PathoDx Respiratory Virus Panel test result does not preclude the possibility of respiratory virus infection in vitro. The interpretation should be based on other clinical information obtained on the patient and other diagnostic procedures. A negative test result must be confirmed by a viral respiratory panel strongly supported.

11. Frozen vials of maintenance medium are recommended for optimal storage. Do not store or freeze the maintenance medium at temperatures below 0°C or above 37°C.

12. Mount the microscope slide with a 4% formalin in 50% ethanol before mounting the coverslip with the mounting fluid. Remove any air bubbles and excess mounting fluid with absorbent paper.

13. Various types of epifluorescence microscopes using either a 40X, 60X, or 100X oil-immersion objective lens are recommended for the viewing of the fluorescent reagents. The use of the 40X objective lens with an epi-illumination system is recommended for overall fluorescence examination.

14. The reagents (all fluorescein-labeled reagents) come ready-to-use and are optimized to detect their respective viral antigens. Dilution or other alteration of the working reagent to-use and are optimized to detect their respective viral antigens. Dilution or other alteration of the working reagent should not be made.

15. All virus-specific monoclonal antibodies showed no fluorescence when tested on the following: Bacterial, fungal, yeast, and mycoplasma; and negative for the respiratory viruses included in the staining panel.

16. A positive test result on the PathoDx Respiratory Virus Panel will be reported as “no respiratory virus detected”.

17. The PathoDx Respiratory Virus Panel was evaluated in two clinical sites in the mid-western United States to determine the clinical performance of the panel and its individual constituents in detecting the presence of the seven respiratory viruses. The final clinical site conducted two studies. The first study included all fresh specimens and 50 randomly selected retrospective samples. The second study included all fresh specimens and 50 randomly selected retrospective samples. The first study included all fresh specimens and 50 randomly selected retrospective samples.

18. To demonstrate the specificity of the PathoDx Respiratory Virus Panel, viral and bacterial detection reagents were used with each virus-specific monoclonal antibody reagent. The following are the results obtained when tissue specimens were studied. All organs are from American Type Culture Collection (ATCC®), unless indicated otherwise.

19. PathoDx Respiratory Virus Panel is a sensitive, specific, and reproducible test. The PathoDx Respiratory Virus Panel is intended for use by qualified laboratory personnel who are familiar with standard laboratory practices and with the precautions necessary in the handling of live microorganisms.

20. The results for the second study included 168 respiratory, from cell culture isolates consisting of 30 specimens negative for all seven respiratory virus infections.

21. All 185 specimens were tested with the shell vial culture procedure using both PathoDx and Kit B reporters reassess.

22. The PathoDx Respiratory Virus Panel is intended for use by qualified laboratory personnel who are familiar with standard laboratory practices and with the precautions necessary in the handling of live microorganisms.

23. The use of serum-free tissue culture maintenance medium is recommended for optimal storage. Do not store or freeze the maintenance medium at temperatures below 0°C or above 37°C.

24. The virus-infected cells may be observed in the shell vials by the shell vial culture procedure using both PathoDx and Kit B reporters reassess.

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