antibodies directed against adenovirus and RSV will detect all identified serotypes of the respective virus. The Bartels antibodies directed against influenza and parainfluenza viruses are type specific, while monoclonal antibodies directed against adenovirus and RSV will detect all identified serotypes of the respective virus. The Bartels Viral Respiratory Screening and Identification Kit uses indirect fluorescent antibody technology.

**SUMMARY**

Respiratory viral diagnostics brings many benefits to the clinical laboratory, such as allowing the definition of current trends of infection, eliminating unnecessary antibacterial therapy, and helping to define the disease process and prognosis of each viral agent. Identifying potential epidemic and endemic trends in viral infections can reveal valuable information to be used in disease control and in limiting the spread of nosocomial infections. While each respiratory virus shares common upper and lower respiratory tract syndromes, the probability of viral involvement in these syndromes and their severity is often linked closely with a particular virus. Bartels Viral Respiratory Screening and Identification Kit allows the above benefits to the clinical laboratory through the use of high quality monoclonal reagents in the detection of the most common viral respiratory agents.

**Adenovirus**

Adenovirus infections are endemic in nature, showing little tendency to occur seasonally or in outbreaks. Forty-one serotypes with strict specificity for humans show a predominant association with upper and lower respiratory tract syndromes, the probability of viral involvement in these syndromes and their severity is often linked closely with a particular virus. Barrels Viral Respiratory Screening and Identification Kit utilizes an indirect fluorescent antibody staining technique for identifying virus in infected tissue culture and prepared patient specimens. The test consists of two immunological reagents. An anti-viral mouse monoclonal antibody which is unconjugated is applied to fixed cells and binds the viral antigen in question, if present in the cell substrate. A wash with buffered saline (pH 7.0-7.6) removes all unbound anti-viral antibody. Next, an anti-mouse immunoglobulin which is conjugated to fluorescein isothiocyanate (FITC) is added to the specimen. Again a wash is used to remove any unattached reagent, and the specimen is observed under a fluorescence microscope with the correct filter combination for FITC (See Figures 1a and 1b). Since several conjugated antiglobulins can attach to a single antiviral antibody, an augmentation of fluorescence is achieved resulting in a highly sensitive staining technique. The use of monoclonal antibodies maximizes specificity. A positive reaction is one in which bright apple-green fluorescence is observed. Other cells available as alternatives include “Bristol” HeLa, A549 lung cell line, and in vitro substrate. The use of monoclonal antibodies maximizes specificity. A positive reaction is observed in vitro. (See Figures 1a and 1b).

**Para outras línguas**

**Pour d'autres langues**

**Para outras línguas**

**Parainfluenza types 1, 2, and 3**

Parainfluenza virus types 1, 2, and 3 are members of the paramyxovirus family, and along with RSV, constitute the most important viral respiratory pathogens for infants and children. Type 1 and 2 infections are noted to occur in conjunction with RSV and are of limited clinical significance, with a unique pattern of every other year and type 2 infections occurring unpredictably. Type 3 Parainfluenza virus type 3, however, has an endemic nature with occasional small rises in infection lasting for 2 to 3 months. Types 1 and 2 infections involve the respiratory tract, whereas type 3 infection may involve the respiratory and ocular tract. Biologically, when infection occurs includes colds, lyrngitis, cough (type 1 is the most important cause of this syndrome), bronchitis/bronchotracheitis and broncholitis (type 3 is second only to RSV in cause of these syndromes), and pneumonia (type 3 only). As with RSV, a problem arises with the differentiation of these respiratory symptoms and are generally widespread by the age of 2 for type 3 and age 5 for type 1 and 2 infections. Though parainfluenza viruses produce their most serious disease states in children from 2 to 4 years of age, recurrent infections, particularly due to parainfluenza type 3, have been noted in adults. Type 2 infection of respiratory epithelial cells allows a rapid, clinically useful diagnosis in which virus lymphocytosis is not encountered as a limitation. Immunosuppression has thus far been shown to be the best and most widely utilized technique for direct detection, as with RSV or influenza infections. Monoclonal antibodies are useful in the diagnosis of current trends of infection, eliminating unnecessary antibacterial therapy, and helping to define the disease process and prognosis of each viral agent. Identifying potential epidemic and endemic trends in viral infections can reveal valuable information to be used in disease control and in limiting the spread of nosocomial infections. While each respiratory virus shares common upper and lower respiratory tract syndromes, the probability of viral involvement in these syndromes and their severity is often linked closely with a particular virus. Barrels Viral Respiratory Screening and Identification Kit utilizes an indirect fluorescent antibody staining technique for identifying virus in infected tissue culture and prepared patient specimens. The test consists of two immunological reagents. An anti-viral mouse monoclonal antibody which is unconjugated is applied to fixed cells and binds the viral antigen in question, if present in the cell substrate. A wash with buffered saline (pH 7.0-7.6) removes all unbound anti-viral antibody. Next, an anti-mouse immunoglobulin which is conjugated to fluorescein isothiocyanate (FITC) is added to the specimen. Again a wash is used to remove any unattached reagent, and the specimen is observed under a fluorescence microscope with the correct filter combination for FITC (See Figures 1a and 1b). Since several conjugated antiglobulins can attach to a single antiviral antibody, an augmentation of fluorescence is achieved resulting in a highly sensitive staining technique. The use of monoclonal antibodies maximizes specificity. A positive reaction is observed in vitro. (See Figures 1a and 1b).
1. 2, and 3; and RSV have been pooled as a specimen screening reagent. Antibodies are diluted in phosphate buffered saline (0.15 M sodium chloride and 0.01 M sodium phosphate) with 0.75% protein stabilizer. Sodium azide and ProClin® 950 are added at 0.1% as preservatives. Store at 2-8°C. Replacement catalogue number for 10 mL is B1029-86A.

3. Anti-Viral Antibody Identification Reagents – 2 mL of each. Affinity purified mouse monoclonal antibodies directed against adenovirus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, parainfluenza type 3 or RSV are supplied separately for identification of specimens determined to be positive through screening with the anti-viral screening reagent. Antibodies are diluted in phosphate buffered saline (0.15 M sodium chloride and 0.01 M sodium phosphate) with 0.75% protein stabilizer. Sodium azide and ProClin® 950 are added at 0.1% as preservatives. Store at 2-8°C. Replacement catalogue number for 2 mL is: adenovirus—B1029-87A, influenza A—B1029-87B, influenza B—B1029-87C, parainfluenza type 1—B1029-87D, parainfluenza type 2—B1029-87E, parainfluenza type 3—B1029-87F, and RSV—B1029-87G.

4. Anti-Mouse IgG F(ab')2, FITC Conjugate– 2 vials, 10 mL each. Affinity purified goat or sheep anti-mouse IgG F(ab’)2 antibodies conjugated to fluorescein isothiocyanate (FITC) are diluted in phosphate buffered saline (0.15M sodium chloride and 0.01 M sodium phosphate) with 0.75% protein stabilizer. Sodium azide and ProClin® 950 are added at 0.1% as preservatives. Store at 2-8°C. Replacement catalogue number for 10 mL is B1029-68B.

5. Non-Immune Mouse Antibodies – 1 vial, 10 mL each. Affinity purified non-immune mouse antibodies are diluted in phosphate buffered saline (0.15 M sodium chloride and 0.01 M sodium phosphate) with 0.75% protein stabilizer. Sodium azide and ProClin® 950 are added at 0.1% as preservatives. Store at 2-8°C. Replacement catalogue number for 10 mL is B1029-66C.

6. Buffered Glycerol Mounting Medium – 3 vials, 5 mL each. Buffered glycerol mounting medium is to be used to prepare antigen slides and patient samples for microscopic observation. Store at 2-30°C. Replacement catalogue number for 5 mL is B1029-45B.

7. Phosphate Buffered Saline – 2 vials. Phosphate buffered saline is stable for sixty days after reconstitution. Discard if solution becomes cloudy. Replacement catalogue number for one vial is B1029-45F.

8. Buffered glycerol mounting medium is to be used to prepare antigen slides and patient samples for microscopic observation. Store at 2-30°C. Replacement catalogue number for 5 mL is B1029-45B.

9. Phosphate Buffered Saline – 2 vials. Each vial of powdered buffer, when reconstituted in 1 litre of distilled water, is a physiological saline solution buffered with 0.01 M sodium phosphate to a pH range of 7.0-7.6. Prior to reconstitution, store powder at 2-30°C. Phosphate buffered saline is stable for sixty days after reconstitution. Discard if solution becomes cloudy. Replacement catalogue number for one vial is B1029-45F.

10. Antigen Control Slides - 5 slides. Eight well antigen control slides with one well each of adenovirus (Ad type 5 CDC V-002), Vero and Hep-2 cells mixed control, influenza A (Victoria/Port Chalmers CDC V-002), influenza B (Hong Kong, CDC V-004), parainfluenza type 1 (P1 CDC V-004), parainfluenza type 2 (P2 CDC V-003), parainfluenza type 3 (P3 CDC V-005) and RSV (RSV Clinical isolate) have been provided as reagent staining controls. Viruses have been spotted from infected culture cells on each slide in the above order and then fixed with acetone for maximum exposure to antige nic sites. Slides should be stored at 2-8°C to preserve antigen integrity.

11. 2 mL of each Anti-Viral antibody identification reagent containing mouse monoclonal antibodies directed against: a) Adenovirus, (Adeno), b) Influenza A, (Infl A), c) Influenza B, (Infl B), d) Parainfluenza type 1, (Para 1), e) Parainfluenza type 2, (Para 2), f) Parainfluenza type 3, (Para 3). g) RSV.

12. 20 mL FITC labelled goat or sheep anti-mouse IgG F(ab’)2 antibodies with Evans’s blue counterstain. (FITC).

13. 10 mL non-immune mouse antibodies for use as a negative antibody control. (NMA).

14. 15 mL buffered glycerol mounting medium.

15. 2 vials phosphate buffered saline; each for reconstitution to one litre (to be used only in fixation of cell culture and rinses during staining). (PBS).

Materials Required, but not Supplied

1. Cell culture for isolation of respiratory viruses – Cells most often used include MOCX, A549, HEK, HEp-2, Hela, LLC-MK2, and diploid fibroblasts (W38, HMF, MRC5). Different strains of tissue culture cells as well as different passages of the same strain may vary greatly in their sensitivity to different respiratory viruses. Consequently, it is recommended that each laboratory establish its own quality control procedures for each acceptable culture. Known positive cultures for preparing positive control tubes are available from American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852.

2. Viral transport medium which is noninhibitory to adenovirus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, parainfluenza type 3, RSV, and the tissue culture cells used (Bartels® Catalogue Number B1029-35C, B1029-35D, or a suitable equivalent such as Hank’s balanced salt solution plus antibiotics and a protein stabilizer).
3. Tissue culture maintenance medium such as Eagles Minimal Essential Medium with 2% precolloital fetal bovine serum, 15 mL HEPES, 0.8 gram/litre sodium bicarbonate, and antibiotics (Bartels® Catalogue Number B1029-37).

4. Acetone, reagent grade. Note: Acetone fixative contaminated with water or saline may cause a hazy appearance on the substrate in fluorescence assays. Ensure acetone container is tightly closed to avoid hygroscopic absorption of water.

5. Sterile graduated pipettes: 10 mL, 5 mL, 1 mL.


7. Acetone rinsed and dried glass slides – Bartels® Catalogue Number B1029-86E (100, two-well screening slides) and Catalogue Number B1029-86F (100, eightwell identification slides) allow easy spotting of cells and staining with reagents. Acetone rinsing for 5 to 10 minutes and thoroughly drying may help to prevent hazy nonspecific background fluorescence caused by interfering substances found on glass microscope slides.

8. Sodium hypochlorite solution (0.5%): 1:10 dilution of household bleach.

9. Humid chamber.

10. Coverslips (22 x 50 mm) for patient slides and control slides.

11. Fluorescence microscope with the correct filter combination for FITC (exciitation peak = 490 nm, emission peak = 520 nm) (See Figures 1a and 1b).

12. Incubator, 35-37°C (CO2 or non-CO2).

13. Sterile phosphate buffered saline (0.15 M sodium chloride and 0.01 M sodium phosphate, pH 7.0 – 7.6) for specimen collection and processing.


15. Distilled water.

16. Sterile swabs (DACRON® or cotton) noninhibitory to respiratory viruses and tissue culture cells.

17. Sterile 1 mm glass beads.

18. Sonicator capable of 10 kc/sec (optional).

19. Low speed centrifuge capable of 2000 x g.

20. Acetone-resistant marker.

### TRANSMITTED LIGHT SOURCE

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<td>Suppression filter</td>
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**Figure 1A.** Filter combinations for FITC fluorescence assay observation with a transmitted light source.

### INCIDENT LIGHT SOURCE

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**Figure 1B.** Filter combinations for FITC fluorescence assay observation with an incident light source.

### Quality Control of Reagents

A control slide from the kit should be removed and stained each time the test is performed to insure reagent activity. Control slides must demonstrate appropriate staining reaction for results to have validity.

### Handling of Transport Systems Utilizing Swabs

Upon arrival in the laboratory, transport systems containing swabs should be vigorously shaken while the swab is immersed in the transport medium to dislodge cells trapped within the swab fibres. Swabs should then be discarded into sodium hypochlorite solution.

### Processing Specimens for Direct Examination

**If specimens are to be used for both direct detection and culture isolation/confirmation, one-half of the cells should be removed by centrifugation at 300 to 500 x g and used with the procedure for processing specimens for direct examination. Supernatant and remaining cells from the specimen should then be used with the following procedure:**

1. To enhance the release of cell-associated viral antigen, vortex specimen with sterile glass beads for 30-60 seconds or sonicate at 10 kc/sec for the same length of time.

2. Centrifuge specimens at 2000 x g for 10 minutes to remove bacterial contaminants and cellular debris. Supernatant is then used as the inoculum.

### Cell Culture Isolation and Fixation

1. Prior to inoculation, cells should be examined microscopically to ensure cell quality. Use of suboptimal cells for inoculation should be avoided since false negative results may be obtained due to decreased sensitivity. It is further recommended that personnel have experience with tissue culture before attempting this procedure.

2. Using a sterile pipette, remove all old medium from cell culture container and replace with fresh prewarmed (25-37°C) cell culture refeeding medium. Glass 16 x 125 mm culture tubes should have at least 2 mL of fresh medium added while dram vials should have 1 mL of fresh medium added. Use aseptic technique and sterile equipment and materials for all tissue culture procedures.

3. Using a sterile pipette, add 0.2 to 0.5 mL of the clinical specimen into each cell culture tube or vial. It is recommended that the specimen be inoculated in duplicate. Viral adsorption of the specimen (inoculum is added to the cell monolayer prior to fresh culture medium being added, the culture incubated from 30 to 60 minutes, and then the culture is refed with fresh medium) may enhance viral isolation.1-3,10,11,26 Additionally, low speed centrifugation of specimens (500-700 x g for 30 minutes) has also been reported to enhance isolation of some viruses.12,13,12,26 For centrifugation, culture medium is drawn off, inoculum is added to the dram vial, the vial is centrifuged, and then fresh culture medium is added. Note: Since cell lines differ in their ability to withstand centrifugation, it is recommended that both inoculated and un inoculated controls be centrifuged during each specimen run.

4. Incubate all cell culture at 35-37°C in a stationary position with media bathing the cells. Cell culture tubes may be rotated on a roller drum at a rate of one revolution per minute to enhance virus isolation.

5. Cells should have their culture medium renewed every 3 to 4 days to enhance CPE formation.

6. Observe cells for CPE daily. Rapid detection of some respiratory viruses may be evidenced by staining cell cultures before CPE is observed. “Blind staining” of inoculated tissue culture, particularly from centrifuged shell vials, may detect viruses such as influenza A or B, parainfluenza viruses, and occasionally adenoviruses and RSV as early as one to three days post-inoculation.14,15,16 Viral adsorption or centrifuge-enhanced cultures may show CPE within 2 to 5 days. Review summary section for CPE involved with each viral agent. When CPE is observed, cells should be fixed as follows in preparation for staining confirmation.
Note: Hemadsorption with fresh guinea pig erythrocytes may be used in place of observation for CPE, but erythrocytes should be washed off cultures before staining. 30, 31, 34

7. Remove culture medium from the culture tube or vial using a sterile pipette and save in a sterile tube until staining has been completed. If the culture is destroyed during fixation or staining, subculturing from the culture medium can be conducted. As a secondary alternative, the original frozen specimen may be thawed in an attempt to reisolate the virus.

8. Gently rinse cells 2 to 3 times with 1 mL volumes of PBS. Discard rinses into sodium hypochlorite solution.

9. For preparation of slides using cells scraped from tubes or vials, add 0.5 to 1.0 mL of PBS to the tube or vial.

10. Using a sterile glass rod, scrape cells from the tube or vial surface. A sterile Pasteur pipette may then be used to gently resuspend cells.

11. Using a sterile Pasteur pipette, spot cells on at least one screening and one identification acetone-cleaned glass slide. To clean slides, rinse in acetone for 5 to 10 minutes and allow to air-dry. Slides are available from Trinity Biotech for screening (100, two-well slides, Catalogue Number B1029-86E) and for identification (100, eight-well slides, Catalogue Number B1029-86F).

12. Mark one screening slide and one identification slide for each patient with the patient identification number and date, using an acetone-resistant marker. Note: Several sets of slides may be made from a single tube or vial if additional staining is desired.

13. Rapidly air-dry specimens completely at room temperature.

14. Fix slides in chilled acetone (2-8°C) for 10 minutes and air-dry completely.

15. After fixation, slides may be held for several days at 2-8°C before staining. Slides held at 20°C are good for up to a year, while those held at -40°C may be good for up to 4 or 5 years. Slides should always be stored in air-tight containers to prevent moisture penetration. 35

Quality Control of Cell Culture

To ensure viral sensitivity, cell culture samples may be inoculated with representative strains of adenovirus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, parainfluenza type 3, and RSV. Positive control cultures are available from ATCC. An un inoculated tube and/or viral from the same lot should be kept and retested every 3 to 4 days to observe normal growth, and to be used as a negative control when observing for cytopathic effect (CPE). A negative control also allows the user to observe any effects of adverse storage conditions on cell cultures.

Staining of Specimen and Control Slides

1. Remove specimen screening slides (with two wells) and one antigen control slide from storage and allow the slides to equilibrate to room temperature.

2. Add enough anti-viral antibody screening reagent to cover each well of each specimen screening slide and each virus control well of the antigen control slide. The antibody screening reagent is used to screen cell culture samples for the presence of adenovirus, influenza A virus, influenza B virus, parainfluenza type 1 virus, parainfluenza type 2 virus, parainfluenza type 3 virus or respiratory syncytial virus.

3. Add enough non-immune mouse antibody reagent to cover a second well of each specimen screening slide and the cell control well of the antigen control slide. This negative control allows for the screening of any non-specific fluorescence.

4. Incubate specimen screening and antigen control slides in a humid chamber at 35-37°C for 30 minutes.

5. Wash slides in PBS by first rinsing antibodies from slide with a squirt bottle. Use a steady, gentle stream to insure that cells are not washed from the slide. Next, immerse slides in PBS in a coplin-type jar to complete the rinse procedure for 5-10 minutes.

6. Apply enough anti-mouse FITC conjugate to cover all wells on specimen screening and antigen control slides.

7. Incubate specimen screening and antigen control slides in a humid chamber at 35-37°C for 30 minutes.

8. Wash slides in PBS for 5 minutes as done in step 5.

9. Using buffered glycercol mounting medium, mount slides with coverslips. When mounting, avoid trapping air bubbles as a hazy fluorescence may occur.

10. Observe slides with a fluorescence microscope at 250X to 400X magnification. Slides should be observed immediately after staining.

11. If screening slides are negative, the results may be reported. If screening slides are found to be positive, continue with step 12.

12. Remove the matching identification slide (with eight wells) for all specimen screening slides found to be positive in step 10 and an antigen control slide from storage and allow slides to equilibrate to room temperature.

13. Add enough of each individual antibody identification reagent to cover a separate well on each specimen identification slide. Note: Ensure each reagent remains in a separate well to avoid cross-contamination of reagents and possible erroneous results.

14. Add enough of each individual anti-viral antibody identification reagent to cover the corresponding virus control on the antigen control slide.

15. Add enough of the non-immune mouse antibody reagent to cover a separate well on each specimen identification slide and to the cell control well of the antigen control slide.

16. Incubate specimen identification and antigen control slides in a humid chamber at 35-37°C for 30 minutes.

17. Wash slides in PBS as done in step 5.

18. Apply enough anti-mouse FITC conjugate to cover all wells on specimen identification and antigen control slides.

19. Incubate specimen identification and antigen control slides in a humid chamber at 35-37°C for 30 minutes.

20. Wash slides in PBS for 5 minutes as done in step 5.

21. Using buffered glycercol mounting medium, mount slides with coverslips. When mounting, avoid trapping air bubbles as a hazy fluorescence may occur.

22. Observe slides with a fluorescence microscope at 250X to 400X magnification. Slides may be stored in the dark in an air-tight container at 2-8°C for 24 hours without significant loss of fluorescence; however, immediate observation after staining is recommended. For longer storage, slides may be held in the dark in an air-tight container at 20°C or lower. Note: Slides which have been held under refrigeration or frozen must be brought to room temperature before reading to allow condensed moisture, which will obscure reading, to evaporate from the slide surface.

RESULTS

Quality Control of Reagents

A control slide from the kit may be stained each time the test is performed to ensure reagent activity. Positive antigen control wells will show multiple infected cells with bright apple-green fluorescence. The cell control well should show an absence of fluorescence with cells staining a dull red due to the included counterstain. Control slides must demonstrate appropriate staining reaction for results to have validity.

Interpretation of Patient Slides

Scraped cell culture

1. Adenovirus: Apple-green fluorescence is cytoplastic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while the cytoplastic staining is often punctate. Extracellular virus may also be noted, but is insufficient without corresponding intracellular fluorescence to determine a positive specimen.

2. Influenza A and B: Apple-green fluorescence is cytoplastic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while cytoplastic staining is often punctate with large inclusions.

3. Parainfluenza types 1, 2, and 3: Cytoplastic, apple-green fluorescence is punctate with irregular inclusions.

4. RSV: Cytoplastic staining is punctate with small inclusions. Apple-green fluorescence is also found in association with syncytium formation not generally noted in vivo.

5. Negative cells: Cells will stain a dull red due to the included Evan’s blue counterstain. For ease of interpretation, the negative antibody control well (nonimmune mouse antibody) should be observed first so that any background fluorescence will be noted prior to observation of the specimen test well(s). Slides must show three or more cells per field of view at 400X magnification to be considered adequate for detection. Specimens exhibiting two or more intact culture cells with staining as described above, are considered positive for the particular viral agent. For reporting specimens which exhibit no positively fluorescing cells, see “Limitations of the Procedure, Section 6.” Cell fragments should be evaluated with caution as conjugate trapping is possible. If the screening slide shows a positive reaction and the identification slide shows no positive reaction, the test should be repeated to confirm results. In clinical testing, 100% correlation was observed between screening and identification results.

Direct examination slides

1. Influenza A: Apple-green fluorescence is cytoplastic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while the cytoplastic staining is often punctate with large inclusions. Infection is usually found in columnar epithelial cells.

2. Influenza B: Apple-green fluorescence is cytoplastic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while the cytoplastic staining is often punctate with large inclusions. Infection is usually found in columnar epithelial cells.

3. Parainfluenza types 1 and 3: Cytoplastic, apple-green fluorescence is punctate with irregular inclusions. Columnar epithelial cells may be infected.

4. RSV: Cytoplastic staining is punctate with small inclusions. Staining is often more evident toward the periphery and is found in columnar epithelial cells.

5. Negative cells: Cells will stain a dull red due to the included Evan’s blue counterstain. For ease of interpretation, the negative antibody control well (nonimmune mouse antibody) should be observed first so that any background fluorescence will be noted prior to observation of the specimen test well(s). Slides must show three or more cells per field of view at 400X magnification to be considered adequate for detection. Specimens exhibiting two or more intact cells with staining, as described above, are considered positive for the particular viral agent. For reporting specimens which exhibit no positively fluorescing cells, see “Limitations of the Procedure, Section 6.” Cell fragments should be evaluated with caution as conjugate trapping is possible.
Clinical Comparison of Screening and Identification Slide Results

In clinical evaluation of direct specimen detection versus confirmation of culture isolation, three laboratories evaluated variation in staining of screening and identification reagents. Laboratory #1 evaluated 189 total specimens with 86 identified as positive by either method (86/189, 46% detection rate). Two specimens (2/189, 1.1% of total specimens or 2/86, 2.3% of positive specimens) exhibited positive staining on both identification slide and control. Seven specimens (7/189, 3.7% of total specimens or 7/86, 8.1% of positive specimens) exhibited negative screening results with positive staining results on the identification slide. During the course of the testing, 11 direct specimens were determined to have an inadequate number of cells to confirm as negative. Laboratory #2, with an overall detection rate of 44% (234/527 by either method, obtained 15 specimens (15/527, 2.8% of total specimens or 15/234, 6.4% of positive specimens) with positive staining on the screening slide and negative identification slide staining. Laboratory #3 evaluated a total of 277 specimens with 175 exhibiting positive staining by either method (176/277, 49% detection rate). Two specimens (2377, 0.5% of total specimens or 2176, 1.1% of positive specimens) demonstrated positive staining on the screening slide and negative staining on the identification slide. Additionally, 11 specimens (11/277, 3.9% of total specimens or 11/1716, 0.6% of positive specimens) stained negative during screening and positive on identification. During the course of the testing, 11 direct specimens were determined to have an inadequate number of cells to confirm as negative. In consideration of clinical performance characteristics, if sufficient specimen material can be collected, optimal results are obtained when both screening and identification slides are stained for evaluation.

Dual Infections

It is possible to detect dual infections, especially with hospitalized infants where one of the agents identified is RSV. When a dual infection is suspected, it is suggested to confirm the results by utilizing separate slides for each of the suspected anti-viral reagents. This will eliminate the possibility that a cross-reaction, caused by reagent carryover, has occurred.

Reasons for Nonspecific Immunofluorescence in Patient Specimens

1. Hazy glow throughout with a yellow-green color. Staining is not identified with specific morphology. Possible sources include immersion oil, dirty lens, or unclean slide. Use glycerol in place of immersion oil being used, clean lens, and reline both sides of slide.

2. Dull yellow-green fluorescence associated with grouping of cells. Source is cell piling. Avoid observation of this area of the specimen since specimen is too thick and conjugate has become trapped.

3. Apple-green fluorescence only at the very edge of specimens. Staining may appear specific but is due to drying of antibodies or conjugate during staining. When staining, the volume of reagents used should be increased and humid chamber checked to assure that proper humidity is maintained during staining. Note: Most nonspecific fluorescence should easily be identified by comparing the non-immune mouse control well on the specimen slide.

4. Uninoculated LCMV cultures may exhibit dim, green staining characteristics. Compare weakly-stained inoculated cultures with controls to verify specific staining.

LIMITATIONS OF THE PROCEDURE

1. Virus culture isolation and direct detection is greatly dependent upon the specimen quality and its subsequent handling; therefore, a negative result does not rule out the presence of viral infection. Results of the test should be interpreted in conjunction with information available from epidemiological studies, clinical evaluation of the patient, and other diagnostic procedures.

2. All findings of adenovirus infection must be interpreted cautiously since the virus is capable of latency and recrudescence. Asymptomatic shedding may occur in some cases up to 18 months after acute infection.

3. Dull yellow-green fluorescent staining may occur with specimens contaminated with Staphylococcus aureus strains containing large amounts of protein A. Protein A will nonspecifically bind Fc fragments of antibodies.

4. Due to variability of monoclonal antibody reagent staining response, nonspecific perinuclear and/or diffuse cytoplasmic staining may occur with CMV-infected cell cultures. When CMV infection is suspected, results should be interpreted with caution. As with all herpes viruses, nonspecific binding of antibodies due to Fc receptor sites within infected cells is possible.

5. Performance of this kit can only be assured when components used in the assay are supplied by a Trinity Biotech Company.

6. Negative specimens should be reported as: No virus isolated or no virus detected. However a negative test does not exclude the possibility of a viral infection with adenovirus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, parainfluenza type 3 or RSV. Failure to detect the virus in question may be a result of such factors as collection of specimen at an improper time during the course of the disease, improper collection of specimen, handling, or state of cell culture used in the isolation procedure.

7. This test detects type specific influenza A and B, and parainfluenza 1, 2, and 3 antigens and group specific adenovirus and RSV antigens. It cannot be used for the differentiation of influenza or parainfluenza strains or adenovirus types.

8. Performance characteristics of this kit have only been established for use on direct patient specimens with influenza A, influenza B, parainfluenza type 1, parainfluenza type 3, and RSV agents. Results with other reagents should be interpreted with caution and confirmed by culture.

9. The interpretation of direct specimens requires personnel who have gained experience by comparing direct results with culture confirmation results.

EXPECTED VALUES

Isolation/detection rates using specimens collected from persons suffering from respiratory symptoms may vary depending on geographical location, time of year, testing population, age of patient, quality and handling of the submitted specimen, and cell culture or direct test system used. In addition, some viruses such as parainfluenza type 1, parainfluenza type 2, and influenza B may not be isolated at all during some respiratory seasons.

Cell culture isolation

During clinical evaluation of respiratory specimens from September 1986 through February of 1987, in a children's hospital, located in the eastern region of the U.S., RSV was detected in 7.0% (27385) of the specimens, adenovirus was detected in 1.6% (6385) of the specimens, and parainfluenza virus was detected in 0.3% (1385) of the specimens using the Bartels® Viral Respiratory Screening and Identification Kit. The primary method of specimen collection used was a nasal wash. In a university hospital setting with a pediatric clinical population, located in the southeastern region of the U.S., clinical evaluation of respiratory specimens during the 1986/1987 respiratory season yielded a detection rate of 7.0% (11/155) for RSV, 1.9% (3/155) for adenovirus, and 0.6% (1/155) for parainfluenza virus using the Bartels® Viral Respiratory Screening and Identification Kit. The choice method for specimen collection during the season was a nasal wash.

A reference laboratory in the western region of the U.S., with a varied submission of specimens types, reported a detection rate of 2.8% (8276) for influenza virus, 1.0% (3276) for RSV, and 1.0% (3276) for adenovirus while using the Bartels® Viral Respiratory Screening and Identification Kit. Specimen for evaluation were collected between October 1986 and January 1987.

Direct Specimen Detection

Five clinical evaluations of direct antigen detection versus confirmation of culture isolation on respiratory specimens took place during the respiratory season (September through March) of 1987/1988 and 1988/1989. During the 1987/1988 season, a children's hospital, located in the eastern region of the U.S., obtained a prevalence rate of 3.5% (183/527) for RSV and 0.6% (3/527) for parainfluenza type 1. Two university hospitals located in the southeastern and southwestern regions of the U.S., both with predominantly pediatric populations, observed prevalence rates of 3.3% (125377) and 2.7% (81189) for RSV, 1.4% (4377) and 2.1% (4189) for parainfluenza type 1, and 4.8% (19377) and 4.2% (81189) for influenza A. The southwestern hospital also obtained a number of parainfluenza type 3 specimens, noting a prevalence rate of 2.1% (4189).

Specimens collected by two hospitals, located in the midwestern region of the U.S., during the 1988/1989 respiratory season showed a prevalence rate of 3.6% (41104 and 19950) for influenza B.

SPECIFIC PERFORMANCE CHARACTERISTICS

Viral and bacterial detection studies performed with each of the anti-viral monoclonal antibodies yielded the expected results when prepared antigen slides were stained.

Note: Cultures obtained from American Type Culture Collection (ATCC), Rockville, MD, are identified with designated ATCC strain numbers. Chlamydia psittaci and Chlamydia trachomatis strains were obtained as a prepared control slide from the Institute of Ophthalmology, London, England. All other cultures are isolates.
Clinical Accuracy for Cell Culture Confirmation

Testing of respiratory specimens from sixteen clinical laboratory sites throughout the U.S. was conducted comparing Bartels® Viral Respiratory Screening and Identification Kit to standard clinical isolation and confirmation by fluorescent antibody reagents or virus neutralization with type specific antisera. Respiratory specimens evaluated from persons with symptoms of respiratory disease included 49 adenovirus isolates, 117 influenza A isolates, 57 influenza B isolates, 51 parainfluenza type 1 isolates, 35 parainfluenza type 2 isolates, 29 parainfluenza type 3 isolates, and 58 RSV isolates. A total of 864 specimens showed a negative staining reaction with the kit including 30 CMV isolates, 15 HSV isolates, 12 enterovirus isolates, 1 picornavirus isolate, 1 cosacke B4 isolate, 1 echovirus 6 isolate, and 1 echovirus 5 isolate. Adenovirus comparison yielded a sensitivity of 100% (48/48), a specificity of 99.9% (1202/1202), a predictive value positive of 99.9% (1202/1202), and an overall test efficiency of 100% (1202/1202). Parainfluenza type 3 isolates, dating back from the 1984 respiratory season through a sensitivity of 100% (35/35), specificity of 99.9% (1215/1215), a predictive value positive of 99.9% (1215/1215), and an overall test efficiency of 100% (1215/1215). Influenza A yielded a sensitivity of 100% (116/116), specificity of 99.9% (1214/1214), a predictive value positive of 99.9% (1214/1214), and an overall test efficiency of 100% (1214/1214). Influenza B comparison using isolates obtained during testing seasons from 1986 to 1989 showed a sensitivity of 100% (58/58), specificity of 100% (1202/1202), predictive value positive of 100% (58/58), and an overall test efficiency of 100% (1202/1202). Influenza B comparison using isolates obtained during testing seasons from 1986 to 1989 showed a sensitivity of 100% (58/58), specificity of 100% (1202/1202), predictive value positive of 100% (58/58), and an overall test efficiency of 100% (1202/1202). Parainfluenza type 1 isolates collected from 1986 to 1987 were evaluated and showed a sensitivity of 100% (81/81), a specificity of 100% (1209/1209), a predictive value positive of 100% (81/81), and an overall test efficiency of 100% (1209/1209). Influenza A yielded a sensitivity of 100% (116/116), specificity of 99.9% (1214/1214), a predictive value positive of 99.9% (1214/1214), and an overall test efficiency of 100% (1214/1214). Influenza B comparison using isolates obtained during testing seasons from 1986 to 1989 showed a sensitivity of 100% (58/58), specificity of 100% (1202/1202), predictive value positive of 100% (58/58), and an overall test efficiency of 100% (1202/1202). Influenza B comparison using isolates obtained during testing seasons from 1986 to 1989 showed a sensitivity of 100% (58/58), specificity of 100% (1202/1202), predictive value positive of 100% (58/58), and an overall test efficiency of 100% (1202/1202).
Clinical Accuracy for Direct Specimen Detection

During the 1987/1988 respiratory season (September through March), one reference laboratory, two children’s hospitals, one community hospital, two university hospitals (predominantly pediatric specimens), and one state health department, located all across the U.S., evaluated direct detection of antigen in respiratory specimens with confirmation of cell culture isolation using fluorescent antibody reagents. All specimens were collected from persons exhibiting symptoms of respiratory disease. A total of 438 RSV, 29 parainfluenza type 1, 16 parainfluenza type 3, and 30 influenza A specimens positive by either method were evaluated. RSV detection showed a sensitivity of 95% (367/387), specificity of 96% (786/807), predictive value positive of 9% (357/378), and predictive value negative of 93% (788/846). Parainfluenza type 1 yielded a sensitivity of 52% (15/29), specificity of 100% (123/123), predictive value positive of 100% (15/15), and predictive value negative of 99% (1213/1214), predictive value positive of 79% (1114/1228). Direct detection of influenza A specimens resulted in a sensitivity of 86% (67/78), specificity of 99% (1162/1164), predictive value positive of 79% (1213/1572), and predictive value negative of 99% (1213/1227). Parainfluenza type 3 direct detection showed a sensitivity of 86% (357/417), specificity of 97% (786/807), predictive value positive of 94% (357/378), and predictive value negative of 99% (1542/1543).

The American Academy of Pediatrics recommends the use of rapid diagnostic tests for the detection of respiratory viruses in children and young adults. These tests include polymerase chain reaction (PCR) assays, enzyme immunoassays (EIAs), and cell culture methods. PCR assays are highly sensitive and specific, but require specialized equipment and personnel. EIAs are less expensive and easier to perform, but may require additional tests to confirm the results.

**REFERENCES**

GUIDE TO SYMBOLS

Consult Instructions for Use

Catalogue number

For in vitro Diagnostic Use

Contents

Manufacturer

Batch code

Anti-viral antibody screening reagent

Adenovirus reagent

Influenza A reagent

Influenza B reagent

Parainfluenza type 1 reagent

Parainfluenza type 2 reagent

Parainfluenza type 3 reagent

Respiratory syncytial virus reagent

Non-immune mouse antibodies

Phosphate buffered saline

Store at 2-8°C

Store at 2-30°C

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