Trinity Biotech

Bartels® VRK (Viral Respiratory Kit)

REF B1029-86

Pour d'autres langues	Para outras línguas
Für andere Sprachen	Για τις άλλες λώσσες
Para otras lenguas	För andra språk
Per le altre lingue	For andre språk
Dla innych języków	For andre sprog

www.trinitybiotech.com

INTENDED USE

Bartels® Viral Respiratory Screening and Identification Kit is intended for use in cell culture confirmation and direct specimen detection. The screening reagents and the individual antibodies directed against adenovirus, influenza A, influenza B, parainfluenza type 1,2, and 3, and respiratory syncytial virus (RSV) can be used for cell culture confirmation. The influenza A, influenza B, parainfluenza type 1, parainfluenza type 3, and respiratory syncytial virus (RSV) individual antibodies can be used for direct specimen detection. Specimens found to be negative on direct specimen examination should be confirmed by cell culture confirmation. Monoclonal 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 probable diagnosis 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.

Historically, three types of laboratory methods (direct detection, culture isolation/confirmation, serology) have been utilized in the diagnosis of viral infections. The Bartels® Viral Respiratory Screening and Identification Kit utilizes the culture isolation/confirmation method for respiratory virus identification through the use of high quality immunofluorescent group and type specific monoclonal antibody reagents. In addition, performance characteristics for influenza A, influenza B, parainfluenza types 1 and 3, and RSV have been established for prepared direct patient specimens.

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 respiratory and ocular disease symptoms. While adenovirus infections show little significance in adults, with the exception of occasional outbreaks observed in military recruits (types 4, 7, 21), they play a major role with children under 6 in febrile respiratory illnesses. Adenovirus may be involved in most major types of respiratory disease syndromes. It is the most common cause of pharyngitis in children under 6[29] and is also a cause of laryngitis, croup, bronchitis/tracheobronchitis (especially type 7), and bronchiolitis.[22] Adenovirus types 1, 2, 3, 5,12, and 19 have been closely linked to pertussis-like syndromes [10,26] while types 3, 7, and 21 may cause pneumonia.[4,20] In ocular involvement, types 8, 19, and 37 are involved with epidemic keratoconjunctivitis and types 3 and 7 are associated with "swimming pool conjunctivitis."[13] Types 40 and 41, which are for the most part noncultivable in cell culture, have been reported to cause significant diarrheal disease in hospitalized pediatric patients.[17]Worldwide, types 1, 2, 3, 5, and 7 are isolated more frequently, types 4, 6, and 8 less often, and all other types much less frequently.[40] Direct detection using IF techniques is simple to use and tends to be negative in patients carrying the virus incidentally.[16, 20, 28, 35] Thus, identification by IF from respiratory sites during illness is more likely to be of etiological importance than adenovirus recovered in culture isolation.[16, 20, 28, 35] Isolation of the virus has traditionally been carried out in primary human embryonic kidney (HEK) cells, but these may be very expensive and difficult to obtain. Other appropriate cell lines include HeLa, HEp-2, human newborn foreskin fibroblast (HNF), and A549 lung carcinoma.[20,41] Confirmation of infection has been most often accomplished by IF or ELISA.[25] Serological techniques such as neutralization, HI, and CF test systems[39] may be of little value for initial screening since not all infections result in a response in infants and young children.[9] No matter what detection method is utilized, 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.[20] At present, direct detection by IF proves to be the best method for adenovirus infections. However, cell culture isolation/confirmation, if carefully linked by collection of appropriate specimens and scrutiny of disease syndromes involved, is probably more sensitive.

Influenza A & B

Influenza viruses A and B, members of the orthomyxovirus family, occur during cold weather seasons and are associated with large outbreaks of one circulating type. Influenza viruses cause abrupt acute febrile respiratory illnesses and are associated with such respiratory syndromes as pharyngitis, laryngitis, croup, bronchitis/tracheobronchitis, bronchiolitis, influenza, and pneumonia.[20,31,35] For the young and old, influenza infections can be extremely hazardous since complications of compromised pulmonary or cardiac function may result. Additionally, Reyes syndrome, characterized by noninflammatory encephalopathy and fatty infiltration of the liver, has been closely associated with influenza virus infections. Influenza A epidemics generally occur 2 out of every 3 years, while influenza B epidemics are encountered less often; on the average every 3 to 5 years.

Isolation in primary monkey kidney (PMK), A549 lung carcinoma, $LLCMK_2$, BGM or Madin-Darby canine kidney (MDCK)[14,42,43] cell lines as well as embryonated hens eggs, constitutes the classical method for diagnosis of influenza viruses. While direct detection of influenza has shown promise in shortening the time required for diagnosis, isolation/confirmation remains the diagnostic method of choice at the present time.[3] Direct antigen detection followed by isolation and confirmation would thus be the ideal method for diagnosis of influenza infections.

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 respiratory viral pathogens for infants and children.[5,7,8,32] Type 1 and 2 infections are noted most frequently during the autumn months with type 1 occurring in a unique pattern of every other year and type 2 infections occurring unpredictably.[20,34] Parainfluenza virus type 3, however, has an endemic nature with occasional small rises in infection lasting for 2 to 3 months.[20,34] Syndromes involved with parainfluenza infections include colds, laryngitis, croup (type 1 is the most important cause of this syndrome), bronchitis/tracheobronchitis and bronchiolitis (type 3 is second only to RSV in cause of these syndromes), and pneumonia (type 3 most often).[20] As with RSV, parainfluenza viruses reinfect with great frequency and are generally wide spread by the age of 2 for type 3 and age 5 for type 1 and 2 infections.[34] 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.[21,23] Direct detection of parainfluenza viruses in respiratory epithelial cells allow a rapid, clinically useful diagnosis in which virus lability is not encountered as a limitation. Immunofluorescence has thus far been shown to be the best and most widely utilized technique for direct detection.[20,34,37] In experienced hands, IF may approach or exceed the sensitivity observed in cell culture isolation.[15] Culture isolation is most often accomplished with PMK, $LLCMK_2$, and HEK cell lines.[5,6,14] Additionally, parainfluenza viruses are easily recovered on alternate cell lines such as Vero, A549 lung carcinoma, and human diploid fibroblasts. Cytopathic effect, when observed, is evident as an increase in small rounded cells for type 1, syncytial cell formation (similar to SV40 or foamy virus) for type 2, and a distinct stretching of cells at the edge of the cell sheet producing "stringy edges" for type 3.[34] Maximum CPE occurs 4 to 7 days postinoculation. Presumptive confirmation of infection has historically been accomplished by the use of hemadsorption of guinea pig erythrocytes.[6] Immunofluorescence with monoclonal antibodies now provides a rapid, inexpensive detection system which is capable of type specificity. Direct detection, as with RSV specimens, proves to be the most practical method of detection for rapid diagnosis of infection. Cell culture isolation/confirmation can be carried out alone, or in conjunction with direct detection testing for an increase in overall test sensitivity.

Respiratory Syncytial Virus

Respiratory syncytial virus, a member of the paramyxovirus family, is characteristic in its distinct seasonal outbreaks observed each year starting in November to December and continuing for approximately three months. Respiratory syncytial virus is the major cause of bronchitis/tracheobronchitis and bronchiolitis, and plays a contributing role in other syndromes such as croup, colds, and pneumonia, especially in infants and young children.[20,34,35] In fact, the virus is extremely widespread in children by the age of two, having its most serious effects in infants age one to nine months.[35] Though RSV is possibly the most important viral respiratory pathogen for infants and young children, it has also been shown to occur in adult infections as recurrences of the virus are common.[21,23] Enzyme-linked immunosorbent assay and IF techniques are most often utilized for direct detection.[20,37] Culture isolation/confirmation can be utilized alone or to supplement findings of direct detection. For isolation, HEp-2 cells have traditionally been the cell line of choice.[23] Other cells available as alternatives include "Bristol" HeLa, A549 lung carcinoma, PMK, and some human diploid cell lines.[1] Based on availability of reagents, cost, and ease of use, IF is the best choice for confirmation using an immunoreagent.[2,38] Currently, direct detection of RSV has been shown to be the best method for laboratory diagnosis since the need to prevent the spread of nosocomial infections often outweighs the increase in sensitivity obtained by "bedside inoculation" of cell culture. In addition, RSV is extremely unstable and sensitive to freezing and thawing[24], resulting in poor isolation rates for most laboratories.

PRINCIPLE

Bartels® 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 phosphate 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. Uninfected cells are counterstained with Evan's blue which is included in the conjugate, and appear dull red. As with all fluorescence assays, the quality of diagnosis depends on the sensitivity and specificity of reagents, capacity and condition of the fluorescence microscope, meticulous specimen collection and storage, and the skill of the laboratory worker in preparing patient specimens and interpreting the findings.

REAGENTS

For *in vitro* diagnostic use only.

1. **Antigen Control Slides – 5 slides.** Eight well antigen control slides with one well each of Adenovirus (Ad type 5 CDC V5-002), Vero and Hep-2 cells mixed control, influenza A (Victoria/Port Chalmers CDC V7-002), influenza B (Hong Kong, CDC V4-004), parainfluenza type 1 (P1 CDC V6-004), parainfluenza type 2 (P2 CDC V7-003), parainfluenza type 3 (P3 CDC V5-003) 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 antigenic sites. Slides should be stored at 2-8ºC to preserve antigen integrity. *Replacement catalogue number for ten slides is B1029-86D.*

2. **Anti-Viral Antibody Screening Reagent – 1 vial, 10 mL each.** Affinity purified mouse monoclonal antibodies directed against adenovirus; influenza A and B; parainfluenza types

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 2-8ºC. *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 2-8ºC. *Replacement catalogue numbers for 2 mL are: 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. Evan's blue is the included counterstain at 0.008%. Store at 2-8ºC in the dark. *Replacement catalogue number for 10 mL is B1029-86B.*

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 2-8ºC. *Replacement catalogue number for 10 mL is B1029-86C.*

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 2-30ºC. *Replacement catalogue number for 5 mL is B1029-45B.*

7. **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 2-30ºC. Phosphate buffered saline is stable for sixty days after reconstitution. Discard if solution becomes cloudy. *Replacement catalogue number for one vial is B1029-45F.*

WARNINGS AND PRECAUTIONS

1. **For *in vitro* diagnostic use only.**

2. Any reagents containing sodium azide or ProClin® 950 should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with a large volume of water to prevent azide build-up. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as a hazardous waste. MSDS for sodium azide and ProClin® 950 are available by phoning a Technical Service Representative in the U.S. at 1-800-325-3424, or outside the U.S. at (353) 1 276 9800.

3. Reagents are supplied at working strength. Dilution of reagents will decrease sensitivity.

4. Reagents should not be used beyond their expiration date.

5. Microbial contamination of reagents may cause a decrease in sensitivity.

6. All specimens and materials should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel. Decontamination is most effectively accomplished with a 0.5% solution of sodium hypochlorite (1:10 dilution of household bleach).

7. Although control slides have been inactivated, they should be handled and disposed of like other potentially infectious materials.

8. Never pipette reagents or clinical samples by mouth.

9. Avoid splashing, generation of aerosols, and contact with broken skin when processing clinical specimens or virus stocks.

10. Do not substitute reagents from other manufacturers.

11. Incubation times or temperatures other than specified in the test instructions may give erroneous results.

12. Cross-contamination of reagents could cause false results. If staining multiple patients on the same slide, keep each specimen within the perimeter of its well to insure that reagents will not be drawn out of the well when stained.

13. Do not allow wells to dry once assay has begun.

14. Reusable glassware must be washed and thoroughly rinsed free of all detergents.

15. Evan's blue dye is a potential carcinogen. If skin contact occurs with the conjugate, flush with water immediately.

16. Due to the affinity of adenovirus for the occular area, hand to eye contact should be avoided during processing of respiratory specimens and when performing diagnostic tests.[29]

17. Use aseptic technique and sterile equipment and materials for all tissue culture procedures.

STABILITY AND STORAGE

Reagent components will retain their full potency until the expiration date shown on the label of each vial when stored at recommended temperatures. Slides stained with fluorescent conjugate will demonstrate quenching or fading if exposed to light. Slides should be protected as much as possible during the assay. The fluorescent antibody conjugate should not be left out in the light for prolonged periods.

SPECIMEN COLLECTION AND STORAGE

Proper collection and handling of the patient specimen are the most important factors in a successful laboratory diagnosis. The possibility of virus isolation is increased when specimens are collected as soon as possible (3 to 7 days) after disease onset. Depending on the type of respiratory syndrome experienced by the patient, several different specimens may be collected as outlined below.[11,20,27,29,31,33,34,35] The specimen of choice for direct specimen testing is nasopharyngeal aspirates. Use of this type of specimen will provide the best cellular yield for evaluation. Other specimen types are - nasal washes and aspirates, nasopharyngeal swabs, throat washings, and throat swabs.

Methods of Collection

Correct collection of each of the above listed specimens will help to assure a timely diagnosis. Specimen collection, processing, and culture isolation of respiratory viruses should be attempted only by experienced personnel. Swabs should be taken with a DACRON® or cotton swab only.

Specimen Transport and Storage

All collected specimens should be transported on wet ice to the laboratory immediately after collection according to Title 42 and 49 CFR (Code of Federal Regulations) for interstate transport of etiologic agents. Viral transport medium with at least 1% protein allows stabilization of virus for inoculation. Also, antibiotics incorporated into the transport medium are valuable in controlling bacterial contaminants which may interfere with culture isolation. Specimens should be inoculated into tissue culture as soon as possible. Most respiratory viruses are unstable and sensitive to repeated freezing and thawing. Therefore, specimens should be held at 2-8ºC prior to inoculation. If specimen processing cannot be accomplished within 72 hours, freezing at –70ºC or below is recommended. A quick freeze in an acetone/dry ice bath will help in maintaining viral infectivity. However, it should be noted that once specimens are frozen, the chance of virus isolation is greatly reduced.[34]
Additionally, frozen specimens are much more difficult to interpret with direct detection since most cells are broken apart during the freeze-thaw process.

PROCEDURE

Materials Supplied

1. Antigen Control Slides - 5 slides. Eight well antigen control slides with one well each of Adenovirus (Ad type 5 CDC V5-002), Vero and Hep-2 cells mixed control, influenza A (Victoria/Port Chalmers CDC V7-002), influenza B (Hong Kong, CDC V4-004), parainfluenza type 1 (P1 CDC V6-004), parainfluenza type 2 (P2 CDC V7-003), parainfluenza type 3 (P3 CDC V5-003) 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 antigenic sites. Slides should be stored at 2-8ºC to preserve antigen integrity.

2. 10 mL anti-viral antibody screening reagent containing mouse monoclonal antibodies directed against adenovirus; influenza A and B; parainfluenza types 1, 2, and 3; and RSV. (AVSR).

3. 2 mL of each Anti-viral antibody identification reagent containing mouse monoclonal antibodies directed against:

   a) Adenovirus, (Adeno).
   b) Influenza A, (Influ A).
   c) Influenza B, (Influ B).
   d) Parainfluenza type 1, (Para 1).
   e) Parainfluenza type 2, (Para 2).
   f) Parainfluenza type 3, (Para 3).
   g) RSV.

4. 20 mL FITC labelled goat or sheep anti-mouse IgG F(ab')$_2$ antibodies with Evan's blue counterstain. (FITC).

5. 10 mL non-immune mouse antibodies for use as a negative antibody control. (NIMA).

6. 15 mL buffered glycerol mounting medium.

7. 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 MDCK, A549, HEK, HEp-2, HeLa, LLC-MK$_2$, and diploid fibroblasts (WI38, HNF, MRC5).[1,11,14,20,31,34,41,42,43,44] 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.[34] Consequently, it is recommended that each laboratory establish its own quality control procedures for ensuring acceptability of the tissue culture cells employed for isolation. 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 Hanks balanced salt solution plus antibiotics and a protein stabilizer).

3. Tissue culture maintenance medium such as Eagles Minimal Essential Medium with 2% precolostral fetal bovine serum, 15 mM 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.

6. Sterile Pasteur pipettes.

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 (excitation peak = 490 nm, emission peak = 520 nm) **(See Figures 1a and 1b)**.

12. Incubator, 35-37ºC (CO_2 or non-CO_2).

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.

14. Fine-tipped forceps.

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		
LIGHT SOURCE: Filter type	**MERCURY VAPOR LAMP HBO 200W, HBO 50W**	**TUNGSTEN-HALOGEN LAMP100W**
Excitation filter:	KP 490, BG 12	KP 490
Barrier filter:	LP 510, LP 530	LP 510, LP 530
Red Suppression filter:	BG 38	BG 38

Figure 1A. Filter combinations for FITC fluorescence assay observation with a transmitted light source.

INCIDENT LIGHT SOURCE		
LIGHT SOURCE: Filter type	**MERCURY VAPOR LAMP HBO 200W, HBO 100W, HBO 50W**	**TUNGSTEN-HALOGEN LAMP 100W**
Excitation filter:	KP 500	KP 500
Dichromatic splitting filter	K 510	K 510
Barrier filter	LP 510, LP 530	LP 510, LP 530

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, half of the cells should be removed by centrifugation at 300 to 500 x g and used with the following procedure. Supernatant and remaining cells from the specimen should then be used under the procedure for processing specimens for cell culture isolation.

1. Add 2 mL of phosphate buffered saline (PBS: 0.15 M sodium chloride and 0.01 M sodium phosphate, pH 7.0-7.6) to the specimen. Resuspend cells and add an additional 6 mL of PBS.
2. Centrifuge specimen at 300 to 500 x g for 10 minutes to pellet epithelial cells.
3. If the specimen contains mucous, it will be observed as a hazy layer immediately above the cell pellet. Using a sterile Pasteur pipette, remove all of the supernatant including any mucous layer and discard into sodium hypochlorite solution.
4. Add 2 mL of PBS to the specimen and resuspend cell pellet. Add an additional 6 mL of PBS.
5. Centrifuge specimen at 300 to 500 x g for 10 minutes to pellet epithelial cells.
6. Remove all of the supernatant including any mucous layer and discard into sodium hypochlorite solution.
7. Add 2 mL of PBS to the specimen and resuspend cell pellet. Add an additional 6 mL of PBS.
8. Centrifuge specimen at 300 to 500 x g for 10 minutes to pellet epithelial cells.
9. Remove all of the supernatant including any mucous layer and discard into sodium hypochlorite solution.
10. Add 2 to 8 drops of PBS to make cell suspension slightly cloudy. An acceptable specimen should show at least three epithelial cells per 400X field. Less cells per field of view makes the slide difficult to read if positive, and inadequate if negative.
11. Using a sterile Pasteur pipette, spot cells on acetone-cleaned glass slides. To clean slides, dip in acetone for 5 to 10 minutes and allow to air-dry. Mark one identification slide for each patient with the patient identification number and date using an acetone-resistant marker. Teflon-coated slides with wells of 4 to 6 mm are convenient for spotting cells and staining with reagents. **Note:** It may be best to make multiple slides per patient in case restaining is necessary.
12. Rapidly air-dry specimens **completely** at room temperature.
13. Fix slides in chilled acetone (2-8ºC) for 10 minutes.
14. After fixation, slides may be held for several days at 2-8ºC before staining. Slides held at -20ºC are good for up to one year, while those held at -40ºC may be good for up to 4 or 5 years. Slides should always be stored in air-tight containers to prevent moisture penetration.[35] Proceed to **Step 13** under section entitled "Staining of Specimen and Control Slides."

Processing Specimens for Culture Isolation/Confirmation

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 virus, 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 refeed with fresh prewarmed (25-37ºC) 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 1 mL calibrated 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.[11,20,31,34] Additionally, low speed centrifugation of specimens (500-700 x g for 30 minutes) has also been reported to enhance isolation of some viruses.[12,18,23,36] 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 uninoculated controls be centrifuged during each specimen run.
4. Incubate all cell culture at 35-37ºC 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 adenovirus and RSV as early as one to three days post-inoculation.[45,46] 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.[20,31,34]

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. **Note:** When using frozen specimen for source of virus in repeat isolation attempts, thaw the inoculum just until the last ice crystals are no longer visible to avoid overheating the suspension and inactivating 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 (2-8ºC) for 10 minutes and air-dry completely.

15. After fixation, slides may be held for several days at 2-8ºC before staining. Slides held at -20ºC are good for up to a year, while those held at -40ºC 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 uninoculated tube and/or vial from each lot should be kept and refed 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 one 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 35-37ºC 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 35-37ºC for 30 minutes.

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

9. Using buffered glycerol 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 35-37ºC 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 35-37ºC for 30 minutes.

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

21. Using buffered glycerol 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 2-8ºC 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 -20ºC 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 cytoplasmic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while the cytoplasmic 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 cytoplasmic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while cytoplasmic staining is often punctate with large inclusions.

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

4. **RSV:** Cytoplasmic 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 cytoplasmic, nuclear, or both. Nuclear staining is uniformly bright with little definition, while the cytoplasmic staining is often punctate with large inclusions. Infection is usually found in columnar epithelial cells.

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

3. **Parainfluenza types 1 and 3:** Cytoplasmic, apple-green fluorescence is punctate with irregular inclusions. Ciliated and columnar epithelial cells may be infected.

4. **RSV:** Cytoplasmic 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 2 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 screening results and negative staining on identification slides. 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, 17 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 377 specimens with 176 exhibiting positive staining by either method (176/377, 49% detection rate). Two specimens (2/377, 0.5% of total specimens or 2/176, 1.1% of positive specimens) demonstrated positive staining on the screening slide and negative staining with the identification slide. Additionally, 11 specimens (11/377, 2.9% of total specimens and 11/176, 6.3% of positive specimens) stained negative during screening and positive on identification. During the course of the testing, 11 direct specimens were determined to have 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 colour.** 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 rerinse 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 specimen.** 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 $LLCMK_2$ 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.[20]

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.[30]

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 site of collection, 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% (27/385) of the specimens, adenovirus was detected in 1.6% (6/385) of the specimens, and parainfluenza virus was detected in 0.3% (1/385) 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 specimen types, reported a detection rate of 2.8% (8/276) for influenza virus, 1.0% (3/276) for RSV, and 1.0% (3/276) for adenovirus while using the Bartels® Viral Respiratory Screening and Identification Kit. Specimens 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[2] and 1988/1989.[1] During the 1987/1988 season, a children's hospital, located in the eastern region of the U.S., obtained a prevalence rate of 35% (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 33% (125/377) and 27% (51/189) for RSV, 1% (4/377) and 2.1% (4/189) for parainfluenza type 1, and 4.8% (18/377) and 4.2% (8/189) for influenza A. The southwestern hospital also obtained a number of parainfluenza type 3 specimens, noting a prevalence rate of 2.1% (4/189).

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 3.8% (4/104 and 19/500) 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. C*hlamydia psittaci* and C*hlamydia trachomatis* serovars were obtained as a prepared control slide from the Institute of Opthamology, London, England. All other cultures are clinical isolates.

ANTI-VIRAL MONOCLONAL ANTIBODIES Agent Tested	Adeno.	Inf.A	Inf.B	Para. 1	Para. 2	Para. 3	RSV
adenovirus type 1 ATCC VR-1 strain Adenoid 71	+	-	-	-	-	-	-
adenovirus type 2 ATCC VR-2 strain Adenoid 6	+	-	-	-	-	-	-
adenovirus type 3 ATCC VR-3 strain G.B.	+	-	-	-	-	-	-
adenovirus type 5 ATCC VR-5 strain Adenoid 75	+	-	-	-	-	-	-
adenovirus type 6 ATCC VR-6 strain Tonsil 99	+	-	-	-	-	-	-
adenovirus type 7 ATCC VR-7 strain Gomen	+	-	-	-	-	-	-
adenovirus type 8 ATCC VR-9 strain Trim	+	-	-	-	-	-	-
adenovirus type 14 ATCC VR-15 strain de Wit	+	-	-	-	-	-	-
adenovirus type 18 ATCC VR-19 strain D.C.	+	-	-	-	-	-	-
adenovirus type 31 ATCC VR-357 strain 1315/63	+	-	-	-	-	-	-
adenovirus type 40 ATCC VR-931 strain Dugan (79-18025)	+	-	-	-	-	-	-
adenovirus type 41 ATCC VR-930 strain Tak (73-3544)	+	-	-	-	-	-	-
influenza A(H1N1) ATCC VR-897 strain A/New Jersey/8/76	-	+	-	-	-	-	-
influenza A1(H1N1) ATCC VR-97 strain A1/FM/1/47	-	+	-	-	-	-	-
influenza A(H3N2) ATCC VR-822 strain A/Victoria/3/75	-	+	-	-	-	-	-

ANTI-VIRAL MONOCLONAL ANTIBODIES Agent Tested	Adeno.	Inf.A	Inf.B	Para. 1	Para. 2	Para. 3	RSV
influenza A (H0N1) ATCC VR-95 strain A/PR/8/34	-	+	-	-	-	-	-
influenza A(H1N1) ATCC VR-95 strain A/PR/8/34, Puerto Rico	-	+	-	-	-	-	-
influenza A(H3N2) ATCC VR-810 strain A/Pt Chalmers/1/73	-	+	-	-	-	-	-
influenza A2 ATCC VR-544 strain A2/Hong Kong/8/68	-	+	-	-	-	-	-
influenza A(H1N1) ATCC VR-825 strain A/WS/33	-	+	-	-	-	-	-
influenza A ATCC VR-99 strain A/swine/1976/31	-	+	-	-	-	-	-
influenza A ATCC VR-545 strain A1/FLW/1/52	-	+	-	-	-	-	-
influenza A1 ATCC VR-479 strain A1/Ann Arbor/1/57	-	+	-	-	-	-	-
influenza B ATCC VR-790 strain B/Russia/69	-	-	+	-	-	-	-
influenza B ATCC VR-791 strain B/Hong Kong/5/72	-	-	+	-	-	-	-
influenza B ATCC VR-296 strain B/Maryland/1/59	-	-	+	-	-	-	-
influenza B ATCC VR-101 strain B/Lee/40	-	-	+	-	-	-	-
influenza B ATCC VR-523 strain B/Mass/3/66	-	-	+	-	-	-	-
influenza B ATCC VR-786 strain Brigit	-	-	+	-	-	-	-
influenza B ATCC VR-295 strain B/Taiwan/2/62	-	-	+	-	-	-	-
influenza B ATCC VR-103 strain B/GL/1739/54	-	-	+	-	-	-	-
influenza B ATCC VR-788 strain R_{22} "Barbara"	-	-	+	-	-	-	-
influenza B ATCC VR-102 strain B/Allen/45	-	-	+	-	-	-	-
parainfluenza 1 ATCC VR-94 strain (HA-2) C-35	-	-	-	+	-	-	-
parainfluenza 1 ATCC VR-105 strain Sendai/52	-	-	-	+	-	-	-
parainfluenza 1 ATCC VR-907 strain Sendai/Cantell	-	-	-	+	-	-	-
parainfluenza 2 ATCC VR-92 strain (CA virus) Greer	-	-	-	-	+	-	-
parainfluenza 3 ATCC VR-93 strain (HA-1) C243	-	-	-	-	-	+	-
parainfluenza 4 ATCC VR-279 strain M-25	-	-	-	-	-	-	-
parainfluenza 4b ATCC VR-579 strain CH 19503	-	-	-	-	-	-	-
parainfluenza 5 ATCC VR-263 strain DA	-	-	-	-	-	-	-
RSV ATCC VR-26 strain Long	-	-	-	-	-	-	+
RSV ATCC VR-955 strain 9320	-	-	-	-	-	-	+
HSV type 1 ATCC VR-539 strain MacIntyre	-	-	-	-	-	-	-
HSV type 2 ATCC VR-540 strain MS	-	-	-	-	-	-	-
CMV ATCC VR-807 strain Davis	-	-	-	-	-	-	-
varicella ATCC VR-916 strain Webster	-	-	-	-	-	-	-
rubeola ATCC VR-24 strain Edmondston	-	-	-	-	-	-	-
echovirus 2 ATCC VR-32 strain Cornelis	-	-	-	-	-	-	-
echovirus 4 ATCC VR-34 strain Pesascek	-	-	-	-	-	-	-

ANTI-VIRAL MONOCLONAL ANTIBODIES Agent Tested	Adeno.	Inf.A	Inf.B	Para. 1	Para. 2	Para. 3	RSV
echovirus 5	-	-	-	-	-	-	-
echovirus 6	-	-	-	-	-	-	-
echovirus 20 ATCC VR-50 strain JV-1	-	-	-	-	-	-	-
echovirus 22 ATCC VR-52 strain Harris	-	-	-	-	-	-	-
Mycoplasma Pneumonia	-	-	-	-	-	-	-
Mycoplasma Hominis	-	-	-	-	-	-	-
Mycoplasma arginini ATCC 23838	-	-	-	-	-	-	-
Mycoplasma orale ATCC 23714	-	-	-	-	-	-	-
Mycoplasma hyorhinis ATCC 17981	-	-	-	-	-	-	-
Acholeplasmi laidlawii ATCC 23206	-	-	-	-	-	-	-
Legionella pneumophila group 3	-	-	-	-	-	-	-
Pneumocystis Carinii	-	-	-	-	-	-	-
Bordetella Bronchiseptica	-	-	-	-	-	-	-
Mycobacteria avium	-	-	-	-	-	-	-
Mycobacteria Intercellulare	-	-	-	-	-	-	-
Mycobacteria Tuberculosis	-	-	-	-	-	-	-
Ureaplasma Urealyticum	-	-	-	-	-	-	-
B-95-8 Marmoset leukocyte, EBV transformed cells ATCC CRL-1612	-	-	-	-	-	-	-
Chlamydia trachomatis LGV 1,2,3, A-1, K	-	-	-	-	-	-	-
Chlamydia pneumoniae C.IOL-207	-	-	-	-	-	-	-

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 coxsackie 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% (1211/1212), a predictive value positive of 97.9% (48/49), a predictive value negative of 100% (1211/1211), and an overall test efficiency of 99.9% (1259/1260). Influenza A yielded a sensitivity of 100% (116/116), specificity of 99.9% (1143/1144), predictive value positive of 99.1% (116/117), predictive value negative of 100% (1143/1143), and a test efficiency of 99.9% (1259/1260) when isolates collected from 1981 to 1987 were evaluated. Influenza B comparison using isolates obtained during testing seasons from 1980 to 1987 showed a sensitivity of 100% (57/57), a specificity of 100% (1203/1203), a predictive value positive of 100% (57/57), and a test efficiency of 100% (1260/1260). Parainfluenza type 1 isolates collected from 1968 to 1987 were evaluated and showed a sensitivity of 100% (51/51), a specificity of 100% (1209/1209), a predictive value positive of 100% (51/51), a predictive value negative of 100% (1209/1209), and a test efficiency of 100% (1260/1260). Parainfluenza type 2 isolates obtained during respiratory seasons from 1969 to 1987 also yielded a sensitivity of 100% (35/35), specificity of 100% (1225/1225), predictive value positive of 100% (35/35), predictive value negative of 100% (1225/1225), and a test efficiency of 100% (1260/1260). Parainfluenza type 3 isolates, dating back from the 1984 respiratory season through 1987, were evaluated and yielded a sensitivity of 100% (28/28), specificity of 99.9% (1231/1232), predictive value positive of 96.5% (28/29), predictive value negative of 100% (1231/1231), and test efficiency of 99.9% (1259/1260). Respiratory syncytial virus isolates evaluated resulted in a sensitivity of 100% (58/58), a specificity of 100% (1202/1202), a predictive value positive of 100% (58/58), a predictive value negative of 100% (1202/1202), and an overall test efficiency of 100% (1260/1260). Overall testing results with the viral respiratory panel showed a combined sensitivity of 100% (393/393), specificity of 99.6% (864/867), predictive value positive of 99.2% (393/396), predictive value negative of 100% (864/864), and a test efficiency of 99.8% (1257/1260). During the 1987/1988 viral respiratory season, an eastern regional community hospital serving all ages of male and female patients compared centrifugation of respiratory specimens onto cell culture monolayers cultivated in 1 dram vials with conventional culture tube cell cultures. A total of 205 specimens were evaluated using the Bartels® Viral Respiratory Screening and Identification Kit for confirmation of isolates showing an increase in both isolation and rate of detection. Compiled results are outlined below:

ISOLATE TYPE	# DRAM VIAL ISOLATES / AVG # DAYS TO ISOLATE	# CULTURE TUBE ISOLATES / AVG # DAYS TO ISOLATE
adenovirus	9 isolates/2 days	6 isolates/4 days
para type 2	7 isolates/2.5 days	5 isolates/8 days
para type 3	1 isolate/2.5 days	1 isolate/8 days
RSV	22 isolates/2.5 days	16 isolates/6 days
influenza A	10 isolates/2.5 days	9 isolates/10 days
Influenza B	2 isolates/1 day	2 isolates/10 days

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 80 influenza A specimens positive by either method were evaluated. RSV 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 93% (786/846). Parainfluenza type 1 yielded a sensitivity of 52% (15/29), specificity of 100% (1213/1213), predictive value positive of 100% (15/15), and predictive value negative of 99% (1213/1227). Parainfluenza type 3 direct detection gave a sensitivity of 85% (11/13), specificity of 99% (1226/1229), predictive value positive of 79% (11/14), and predictive value negative of 99% (1226/1228). Direct detection of influenza A specimens resulted in a sensitivity of 86% (67/78), specificity of 99% (1162/1164), predictive value positive of 97% (67/69), and predictive value negative of 99% (1162/1173). Specimens negative with Bartels® Viral Respiratory Screening and Identification Kit included five rhinovirus isolates, one enterovirus isolate, five poliovirus isolates, six cytomegalovirus (CMV) isolates, seven herpes simplex virus (HSV) isolates, one coxsackie B2 isolate, and one coxsackie B3 isolate.

During the 1988/1989 respiratory season (September through March), two university hospitals in the midwestern and southeastern regions of the U.S. and one regional community hospital in the midwestern region of the U.S. evaluated direct detection of influenza B 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. Influenza B detection showed a sensitivity of 65% (35/54), specificity of 98% (159/163), predictive value positive of 90% (35/39), and predictive value negative of 89% (159/178). Test efficiency was increased when only nasopharyngeal aspirates were evaluated, giving a sensitivity of 77% (23/30), specificity of 100% (130/130), predictive value positive of 100% (23/23), and predictive value negative of 95% (130/137).

TECHNICAL INFORMATION

For further information or assistance, contact: Technical Services in the U.S. at 1-800- 331-2291, or outside the U.S. at (353) 1 276 9800.

REFERENCES

1. Anderson, J.M., and Beem M.O. 1966. Use of human diploid cell cultures for primary isolation of respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. 121:205-209.
2. Bell, D.M., Walsh E.E., Hruska J.F., Schnabel K.C., and Hall C.B. 1983. Rapid detection of respiratory syncytial virus with a monoclonal antibody. J. Clin. Microbiol. 17:1099-1101.
3. Benjamin, D.R., and Ray C.G. 1974. Use of immunoperoxidase for the rapid identification of human myxoviruses and paramyxoviruses in tissue culture. Appl. Microbiol. 28:47-51.
4. Brandt, C.D., Kim H. W., Vargosko A.J., Jeffries B.C., Arrobio J.O., Rindge B., Parrott R.H., and Chanock R.M. 1969. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. Am. J Epidemiol.. 90:484-500.
5. Chanock, R.M., Parrott R.H., Johnson K.M., Kapikian A.Z., and Bell J.A. 1963. Myxoviruses: parainfluenza. Am. Rev. Respir. Dis. 88:152-166.
6. Chanock, R.M., K.M. Johnson, Cook M.,Wong D.C., and Vargosko A. 1961. The hemadsorption technique, with special reference to the problem of naturally occurring simian parainfluenza virus. Am. Rev. Respir. Dis. 83:125-129.
7. Chanock, R.M., Kim H.W., Vargosko A.J., Deleva A., Johnson K.M., Cumming C., and Parrott R.H.. 1961. Respiratory syncytial virus. I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and other minor respiratory diseases in children. J. Am. Med. Assoc. 176:647-653.
8. Chanock, R.M., and Finberg L. 1957. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II Epidemiological aspects of infection in infants and young children. Am. J. Hyg. 66:291-300.
9. Chernesky, M.A., Ray C.G., and Smith T.F. 1982. Cumitech 15, Laboratory diagnosis of viral infections. Coordinating ed.,W.L. Drew. American Society for Microbiology,Washington, D.C.
10. Connor, J.D. 1970 Evidence for an etiologic role of adenoviral infection in the pertussis syndrome. N. Eng. J. Med. 283:390-394.
11. Cooney,M.K. 1985.Adenoviruses, p. 701-704. In Lennette E.H., Balows A., Hausler Jr.W.J.,, and Shadomy H.J. (eds.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology,Washington, D.C.
12. Espy, M.J., Smith T.F., Harmon M.W., and Kendal A.P. 1986. Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. J. Clin. Microbiol. 24:677-679.
13. Foy, H.M., Cooney M.K., and Hatlen J.B.. 1968. Adenovirus type 3 associated with intermittent chlorination of a swimming pool. Arch. Environ. Health 17:795-802.
14. Frank, A.L., Couch R.B., Griffis C.A., and Baxter B.D. 1979. Comparison of different tissuecultures for isolation and quantitation of influenza and parainfluenza viruses. J. Clin.Microbiol. 10:32-36.
15. Gardner, P.S. and McQuillin J. 1974. Rapid Virus Diagnosis Application of Immunofluorescence. Butterworth and Co., Ltd., London.
16. Gardner, P.S., McGuckin R., and McQuillin J. 1972. Br. Med. J. 3:175.
17. Gary, G.W., Jr., Hierholzer J.C., and Black R.E. 1979. Characteristics of noncultivable adenoviruses associated with diarrhea in infants: a new subgroup of human adenoviruses. J. Clin. Microbiol. 10:96-103.
18. Gleaves, C.A.,Wilson D.J.,Wold A.D., and Smith T.F. 1985. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h postinoculation. J. Clin. Microbiol. 21:29-32.
19. Gleaves, C.A., Smith T.F, Shuster E.A., and Pearson G.R. 1984. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J. Clin. Microbiol. 19:917-919.
20. Greenberg, S.B., and Krilov L.R. 1986. Cumitech 21, Laboratory Diagnosis of Viral Respiratory Disease. Coordinating ed., Drew W.L. and Rubin S. J. . American Society for Microbiology, Washington, D.C.
21. Gross, P.A., Green R.H., and McCrea Curnen M.G. 1973. Persistent infection with parainfluenza type 3 virus in man. Am Rev. Respir. Dis. 108:894-898.
22. Hall, C.B., and Hall W.J. 1985. Bronchiolitis, p. 390-394. In Mandell G., Douglas R.C., and Bennett J.E. (eds.), Principles and Practice of Infectious Diseases. John Wiley and Sons, Inc. New York.
23. Hall,W.J., Hall C.B., and Spears D. M. 1978. Respiratory syncytial virus infection in adults. Ann. Intern. Med. 88:203-205.
24. Hambling, M.H. 1964. Survival of the respiratory syncytial virus during storage under various conditions. Br. J. Exp. Pathol. 45:647-655.
25. Harmon, M.W., and Pawlik K.M. 1982. Enzyme immunoassay for direct detection of influenza type A and adenovirus antigens in clinical specimens. J. Clin. Microbiol. 15:5-11.
26. Harrison, H.R., Howe P., Minnich L., and Ray C. G. 1979. A cluster of adenovirus 19 infection with multiple clinical manifestations. J. Pediatr. 94:917-919.
27. Jalowayski, A.A., England B.L., Temm C.J., Nunemacher T.J., Bastian J.F., MacPherson G.A., Dankner W.M., Stroube R.C., and Connor J.D. 1987. Peroxidase-antiperoxidase assay for rapid detection of respiratory syncytial virus in nasal epithelial specimens from infants and children. J. Clin. Microbiol. 25:722-725.
28. Kalter, S.S., Armour V., and Reinarz J.A. 1969. Arch. Gesamte Virusforsch. 28:34-40.
29. Kasel, J.A. 1979 Adenoviruses, p. 229-255. In Lennette E.H. and Schmidt N.J. (eds.), Diagnostic procedures for viral, rickettsial and chlamydial infections, 5th ed. American Public Health Association,Washington, D.C.
30. Keller, R., Peitchel R., Goldman J.N., and Goldman M. 1976. An IgG-Fc receptor induced in cytomegalovirus-infected human fibroblasts. J. of Immunol. 116:772-777.
31. Kendal,A.P., DowdleW.R., and Noble G.R. 1985. Influenza viruses, p. 755-762. In Lennette E.H., Balows A., Hausler W.J., Jr., and Shadomy H.J. (eds.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology,Washington, D.C.
32. Kim, H.W., Arrobio J.O., Brandt C.D., Jeffries B.C., Pyles G., Reid J.L., Chanock R.M., and Parrott R.H. 1973. Epidemiology of respiratory syncytial virus infection in Washington, D.C. Am. J. Epidemiol. 98:216-225.
33. Lennette, D.A. 1985. Collection and preparation of specimens for virological examination, p. 687-693. In Lennette E.H., Balows A., Hausler W.J., Jr., and Shadomy H.J. (eds.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology,Washington, D.C.
34. McIntosh, K., and Clark J.C. 1985. Parainfluenza and respiratory syncytial viruses, p. 763-768. In Lennette E.H., Balows A., Hausler W.J., Jr., and Shadomy H.J. (eds.), Manual of Clinical Microbiology, 4th ed. American Society for Microbiology,Washington, D.C.
35. McIntosh, K., and Pierik L. 1983. Immunofluorescence in Viral Diagnosis, p. 57-81. In Coonrod J.D., Kunv L.J., and Ferraro M.J. (eds.), The Direct Detection of Microorganisms in Clinical Samples. Academic Press, Inc., Orlando, FL.
36. Pruneda, R.C., and Almanza I.. 1987. Centrifugation-shell vial technique for rapid detection of herpes simplex virus cytopathic effect in vero cells. J. Clin. Microbiol. 25:423-424.
37. Ray, C.G., and Minnich L.L. 1987. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. J. Clin. Microbiol. 25:355-357.
38. Scheible, J.H., Lennette E.H., and Kase A. 1965. An immunofluorescent staining method for rapid identification of respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. 120:203-208.
39. Schmidt, N.J., Lennette E.H., and King C.J. 1966. Neutralizing, hemagglutination inhibiting and group complement-fixing antibody responses in human adenovirus infections. J. Immunol. 97:64-74.
40. Schmitz, H.,Wigand R., and Heinrich W. 1983.Worldwide epidemiology of human adenovirus infections. Am. J. Epidemiol. 117:455-466.
41. Smith, C.D., Craft D.W., Shiromoto R.S., and Yan P.O. 1986. Alternative Cell Line for Virus Isolation. J. Clin. Microbiol. 24:265-268.
42. Tobita, K., Sugiura A., Enomuto C., and Furuyama M. 1975. Plaque assay and primary isolation of influenza A viruses in an established line of canine kidney cells (MDCK) in the presence of trypsin. Med. Microbiol. Immunol. 162:9-14.
43. Tobita, K. 1975. Permanent canine kidney (MDCK) cells for isolation and plaque assay of influenza B viruses. Med Microbiol. Immunol. 162:23-27.
44. Woods, G.L., and Young A. 1988. Use of A549 cells in a clinical virology laboratory. J. Clin. Microbiol. 26:1026-1028.
45. Mathey, S., Nicholson D., Ruhs S., Alden B., Knock M., Schultz K., Schmuecker A. 1992. Rapid Detection of Respiratory Viruses by Shell Vial Culture and Direct Staining by Using Pooled and Individual Monoclonal Antibodies. J. Clin. Microbiol. 30:540-544.
46. Lee, S.H.S., Boutilier J.E., MacDonald M.A., Forward K.R. 1992. Enhanced detection of respiratory viruses using the shell vial technique and monoclonal antibodies. J. Viro. Methods. 39:39-46.

GUIDE TO SYMBOLS

Consult Instructions for Use

Use by

REF

Catalogue number

IVD

For *in vitro* Diagnostic Use

CONT

Contents

Manufacturer

LOT

Batch code

CONTROL	SLIDE	VRK	AG

VRK Antigen Control Slide

REAG	VRK	AVSR

Anti-viral antibody screening reagent

REAG	VRK	ADENO

Adenovirus reagent

REAG	VRK	FLU	A

Influenza A reagent

REAG	VRK	FLU	B

Influenza B reagent

REAG	VRK	PARA	1

Parainfluenza type 1 reagent

REAG	VRK	PARA	2

Parainfluenza type 2 reagent

REAG	VRK	PARA	3

Parainfluenza type 3 reagent

REAG	VRK	RSV

Respiratory syncytial virus reagent

CONJ	VRK	FITC

FITC conjugate

REAG	VRK	NIMA

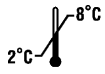
Non-immune mouse antibodies

MOUNT	MEDIUM

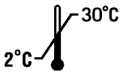
Mounting medium

PBS

Phosphate buffered saline



Store at 2-8°C



Store at 2-30°C

Trinity Biotech Plc
Bray, Co. Wicklow, Ireland
Tel. 353 1 2769800
Fax. 353 1 2769888
www.trinitybiotech.com

CE

USA ENQUIRIES:
Trinity Biotech USA
2823 Girts Road,
Jamestown, NY 14701.
Tel. 1800 325 3424
Fax. 908 898 1539

B1029-86-29 Rev C
06/2015