CE

QUIDEL

D³ Ultra™
DFA Respiratory Virus
SCREENING & IDENTIFICATION KIT

For the qualitative detection and identification of the influenza A, influenza B, respiratory syncytial virus (RSV), adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 virus in respiratory specimens.

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

INTENDED USE

The Diagnostic Hybrids, Inc. D³ Ultra DFA (direct fluorescent antibody) Respiratory Virus Screening & ID Kit is intended for the qualitative detection and identification of the influenza A, influenza B, respiratory syncytial virus (RSV), adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 virus in respiratory specimens, by either direct detection or cell culture method, by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). It is recommended that specimens found to be negative after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL3+ facility is available to receive and culture specimens.[1]

SUMMARY AND EXPLANATION OF THE TEST

With the addition of new antiviral drugs for the treatment of influenza[2], more rapid and sensitive tests for respiratory virus detection,[3,4] and the increasing need to be more discriminating in the use of antibiotics[5], early detection and identification of the infecting viral agent has grown substantially in importance. Viral identification is becoming increasingly important in ruling out bacteria as the cause of respiratory infections. Virus identification by either direct antigen detection or cell culture using fluorescent monoclonal antibodies continues to be the standard method in virology laboratories.

Influenza Virus

Influenza viruses (family *Orthomyxoviridae*) contain a single-stranded RNA genome which is present in 8 separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses and probably

contributes to the rapid development of new influenza strains through interchange of gene segments if two different viruses infect the same cell. There are 3 types of influenza, A, B and C. Type A has counterparts in birds and pigs as well as humans, while types B and C are known only in man.

Due to the possibility of another pandemic caused by influenza A, as occurred in 1918 when 25 to 35 million people worldwide died, the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of influenza strains and make predictions of suitable strains for vaccine production.

Influenza infects an estimated 120 million people in the US, Europe and Japan each year and it is estimated that in the US there are 75,000 deaths annually from pneumonia caused by influenza. Primary viral pneumonia or pneumonia from secondary bacterial infections are the primary causes of morbidity of the viral infection.[6]

Pandemics of influenza A occur about every 10 to 30 years and epidemics of either influenza A or B occur annually. Infections are seasonal, typically extending from November to April in the northern hemisphere. Complications tend to occur in the young, elderly and persons with chronic cardio-pulmonary diseases. Incubation time is 1-3 days with rapid spread by inhalation via aerial droplets and fomites.

It is characterized by fever, myalgia, headache and pharyngitis. Influenza A and B are most commonly isolated in A549/Mv1Lu mixtures (R-Mix™), A549/MDCK mixtures (R-Mix Too™), Rhesus MK, MDCK, MRC-5 and A549 cells.

Adenovirus

Adenoviruses (family *Adenoviridae*) are non-enveloped, double stranded DNA viruses. There are 49 serotypes, further divided into 6 groups, A to F, with most associated with respiratory and ocular infections. Generally, adenovirus infections in adults have a low morbidity with the exceptions of immunocompromised patients and individuals living in cramped quarters where infections can cause atypical pneumonia. Virus spread is commonly via aerial droplets and fomites where they infect the mucous membranes of the eye, respiratory tract and gut.[7]

Adenovirus can be isolated in A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEp2, HEK, A549 and MRC-5 cells.[7]

Parainfluenza Virus

Parainfluenza viruses (family *Paramyxoviridae*) are enveloped viruses with a single, negative strand RNA genome. The 4 different types, 1 to 4, cause croup and viral pneumonia in children under the age of 5 years and cause upper respiratory illness in adults. Parainfluenza is the number 2 leading cause of lower respiratory illness in children (after RSV). Outbreaks caused by parainfluenza viruses occur during alternate years in the fall (P1 and P2) or throughout the year, with increased activity in the spring (P3).[8]

Parainfluenza viruses can be isolated in A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), Rhesus MK, MRC-5 and LLC-MK2 cells. Trypsin is helpful in the medium for recovery of types 1 and 2 but not type 3.[7]

Respiratory Syncytial Virus (RSV)

RSV (family *Paramyxoviridae*) is an enveloped virus with a single, negative strand RNA genome. RSV infections cause viral bronchiolitis and pneumonia in infants and the common cold in adults.[9] RSV is usually a seasonal (winter and early spring) infection with epidemics lasting up to 5 months. Peak mortality due to RSV occurs in 3-4 month old infants. There are two major subtypes, A and B; Subtype B is characterized as the asymptomatic strain that the majority of the population experiences. The more severe clinical illnesses involve Subtype A

strains which tend to predominate in most outbreaks.[10] RSV is the primary viral cause of lower respiratory disease in infants and young children. Re-infections do occur but tend to be limited to minor upper respiratory infections.[11] RSV is also now recognized as a significant problem in certain adult populations. These include the elderly, persons with cardiopulmonary diseases, and immunocompromised hosts.[12]

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents[10] although it can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEp2, Vero, LLC-MK2 and MRC-5 cells.[7]

PRINCIPLE OF THE PROCEDURE

The D^3 Ultra DFA Respiratory Virus Screening & ID Kit uses viral antigen-specific murine monoclonal antibodies that are directly labeled with fluorescein for the rapid detection and identification of respiratory viruses. The kit includes a DFA Screening Reagent that contains a blend of murine monoclonal antibodies (MAbs) directed against seven respiratory viruses (influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza 1, parainfluenza 2, and parainfluenza 3) plus seven separate DFA Reagents, each consisting of MAb blends directed against a single respiratory virus. The kit can be used for direct specimen or cell culture screening and final virus identification.

The cells to be tested, either derived from a clinical specimen or cell culture, are fixed in acetone. The DFA Screening Reagent is added to the cells to determine the presence of viral antigens. After incubating at 35°C to 37°C, the stained cells are rinsed with the diluted Wash Solution (1X). A drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. Virus infected cells will be stained with viral specific apple-green fluorescence when stained with the DFA Screening Reagent while non-infected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain. If the specimen contains fluorescent cells, the particular virus is identified using the separate DFA Reagents on new, separate cell preparations.

If on examination of a direct stained specimen, no fluorescent-stained cells are found and all the cells stain red from the Evans Blue, it is recommended that the specimen be cultured and stained using the DFA Screening Reagent. If fluorescent cells are seen, the identification of the virus is determined as described above. Cell preparations are fixed in acetone. The individual DFA Reagents are added to the cell preparations. After incubating at 35°C to 37°C, the stained cells are rinsed with the diluted Wash Solution (1X). A drop of the supplied Mounting Fluid is added and a coverslip is placed on the stained cells. The cells are examined using a fluorescence microscope for the presence of viral specific apple-green fluorescence. The unknown respiratory virus is then identified and reported.

REAGENTS AND MATERIALS PROVIDED

The D^3 Ultra DFA Respiratory Virus Screening & ID Kit contains the following:

Respiratory Virus Screening Reagent **10 mL**

One dropper bottle containing a blend of fluorescein labeled murine monoclonal antibodies directed against respiratory viral antigens of influenza A, influenza B, respiratory syncytial virus (RSV), adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Influenza A DFA Reagent **2 mL**

One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by influenza A virus (Texas 1/77, H3N2 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Influenza B DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by influenza B virus (Hong Kong 5/72 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

RSV DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by RSV (Long strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Adenovirus DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by adenovirus (Type 3-GB strain and Type 6-tonsil 99 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Parainfluenza 1 DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza virus type 1 (VP-1 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Parainfluenza 2 DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza virus type 2 (Greer strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Parainfluenza 3 DFA Reagent **2 mL**
One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza 3 (C243 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Respiratory Virus Antigen Control Slides **5 slides**
Five individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each positive well is identified as to the virus infected cells present, i.e., influenza A, influenza B, respiratory syncytial virus (RSV), adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3. The negative well contains non-infected cells. Each slide is intended to be stained only one time.

Normal Mouse Gamma Globulin DFA Reagent **10 mL**
One dropper bottle containing a mixture of fluorescein labeled murine gamma globulin that has been shown to be un-reactive with any of the listed respiratory viruses. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

40X Wash Solution Concentrate **25 mL**
One bottle of 40X PBS concentrate consisting of Tween 20 and 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water).

Mounting Fluid **15 mL**
One dropper bottle containing an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X
- Cell culture for respiratory virus isolation. Suggested cell lines include LLC-MK2, HEp-2, A549 cells, R-Mix and R-Mix Too MixedCells™, and primary Rhesus monkey kidney cells (all are available from Quidel)
- Coverslips (22 x 50 mm) for Antigen Control Slides and for specimen slides

- Universal Transport Medium (available from Quidel)
- R-Mix Refeed Medium (for use with R-Mix and R-Mix Too MixedCells) or other standard refeed medium (available from Quidel)
- Reagent-grade acetone (> 99% pure) chilled at 2°C to 8°C for fixation of direct specimen slides, shell-vials and cultured cell preparations

  NOTES:
  - Keep the reagent-grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, non-specific, background fluorescence.
  - A mixture of 80% acetone/20% de-mineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature (20°C to 25°C).
- Sterile graduated pipettes: 10 mL, 5 mL, and 1 mL
- Sterile Pasteur pipettes or other transfer pipettes

  CAUTION: One should not use solvents such as acetone with polyethylene transfer pipettes.
- Fine-tipped forceps
- Wash bottle, 200 mL
- Bent-tip teasing needle (for removal of coverslip from a shell-vial for the typing portion of the procedure); fashion the teasing needle by bending the tip of a syringe needle or similar object (e.g., mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.
- Sodium hypochlorite solution (1:10 final dilution of household bleach)
- Humid chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom)
- Glass microscope slides
- Acetone-cleaned multi-well glass microscope slides (2-well and 8-well masked slides)
- Blotters for multi-well glass microscope slides – 2- and 8-well absorbent blotters, used to blot excess liquid from the mask to prevent spread of liquid or stained cells from one well to the other
- Sterile, nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell cultures
- Incubator, 35°C to 37°C (5% CO_2 or non-CO_2, depending on the cell culture format used)
- Centrifuge with free-swinging bucket rotor
- De-mineralized water for dilution of 40X Wash Solution Concentrate and for dilution of the reagent-grade acetone for use in polystyrene multi-well plates
- PBS, sterile, for use in rinsing and suspending cells
- Live control viruses for positive culture controls – Known strains of the 7 respiratory viruses for use in monitoring the cell culture and staining procedures; such control virus strains can be obtained from Quidel.
- Aspirator Set-up – Vacuum aspirator with disinfectant trap containing sufficient household bleach (5%) that the concentration is not decreased by more than 10-fold as it is diluted with discarded fluids
- Wash Container – Beaker, wash bottle or Coplin jar for washing slides
- Fixing Container – Coplin jar, slide dish or polyethylene holder for slides for use in fixing the cells on the slides
- Inverted Light Microscope – Used for examining the monolayers of cells prior to inoculation and examination for toxicity, confluency and for cytopathic effects (CPE); it should have between 40X to 100X magnification capability.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens are handled in accordance with the OSHA Standard on Bloodborne Pathogens.

- Cell culture isolation may have some potential to be hazardous. Personnel working with cell cultures must be properly trained in safe handling techniques[13,14,15] and have experience with cell culture before attempting this procedure.
- All procedures must be conducted in accordance with the CDC 5th Edition Biosafety in Microbiological and Biomedical Laboratories, 2007, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.

- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.
  - Biosafety Level 2 or other appropriate biosafety practices should be used when handling these materials.
  - Decontamination of specimens and cultures is most effectively accomplished using a solution of sodium hypochlorite (1:10 final dilution of household bleach).
  - Although Antigen Control Slides have been shown to be non-infectious, the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
- Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
- Avoid splashing and the generation of aerosols with clinical samples.
- Use aseptic technique and sterile equipment and materials for all tissue culture procedures.
- Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.
- Sodium azide is included in the 40X PBS Concentrate at a concentration of 4% (w/v), and in the other solutions in this kit at 0.1% concentration.
  - Sodium azide is considered poisonous. If the 40X PBS Concentrate is swallowed, seek medical advice immediately.
  - Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.
  - Avoid disposal of these solutions down sanitary or industrial plumbing systems. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
  - Avoid release to the environment.
- Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
- The DFA Reagents are supplied at working strength. Any dilution of the reagents will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Each Respiratory Virus Antigen Control Slide should be used only once. Do not re-use a control slide.
- Microbial contamination of the DFA Reagents may cause a decrease in sensitivity.
- Store 1X Wash Solution and PBS in a clean container to prevent contamination.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Do not expose the DFA Reagents to bright light during staining or storage.
- Use of reagents other than those specified with the components of this kit may lead to erroneous results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

PREPARATION OF 1X WASH SOLUTION

- After storage at 2°C to 8°C, some salts in the 40X Wash Solution Concentrate may have crystallized. Warm the solution to room temperature (20°C to 25°C) to re-dissolve the crystals, then mix.

- Add contents of the fully dissolved 25 mL 40X Wash Solution Concentrate to 975 mL of de-mineralized water.
- Label the 1X Wash Solution with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

STORAGE

Table 1. Reagent Storage Conditions

Respiratory Virus DFA Screening Reagent	Store at 2°C to 8°C in the dark.
Influenza A DFA Reagent	
Influenza B DFA Reagent	
RSV DFA Reagent	
Adenovirus DFA Reagent	
Parainfluenza 1 DFA Reagent	
Parainfluenza 2 DFA Reagent	
Parainfluenza 3 DFA Reagent	
Mounting Fluid	
Normal Mouse Gamma Globulin DFA Reagent	
Respiratory Virus Antigen Control Slides	Store at 2°C to 8°C.
40X Wash Solution Concentrate NOTE: The Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when the Concentrate is warmed to room temperature.	Store liquid at 2°C to 8°C prior to dilution.
1X Wash Solution	Store at room temperature (20°C to 25°C).

STABILITY

Reagents and components will retain their full potency through the expiration date shown on the label of each bottle when stored at recommended temperatures. Light exposure of the DFA Reagents should be kept to a minimum.

Discard 1X Wash Solution if it becomes cloudy.

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful respiratory virus detection. Specimen collection, specimen processing, and cell culture of viruses should be attempted only by personnel that have been trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

For specimen collection and processing recommendations, refer to CLSI Approved Viral Culture Guidelines.[16]

Collection[17]

Aspirates and Washes containing secretions from the nasopharyngeal epithelium provide the best specimens for direct specimen testing since they will contain large numbers of epithelial cells.

Aspirates can be collected using a sterile, soft polyethylene #8 infant feeding tube attached to a disposable aspiration trap connected to a suction device. Washes can be collected by instilling and aspirating 1 to 2 mL of saline in the patient's nostril while the patient is in a supine position.

Aspirates and washes should be diluted with equal volumes of transport medium contained in a centrifuge tube with several sterile glass beads. Swabs from nasal, throat and nasopharyngeal areas often do not contain sufficient numbers of columnar epithelial cells to allow for direct specimen detection of respiratory viruses.

Specimen Transport and Storage

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the US Code of Federal Regulations, or other regulatory requirements, as may be applicable.

Specimens should be transported to the laboratory at 2°C to 8°C. This temperature can be attained by using cold packs, wet ice, foam refrigerant, or other coolants.[18] The specimens should be processed and tested as soon as possible and then stored at 2°C to 8°C.

Specimens should be stored at 2°C to 8°C for no longer than 2 days before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

Freezing and thawing of specimens should be avoided since this will result in a loss of viability of viruses, leading to decreased sensitivity of the test.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
- For specimen swabs received in transport medium with glass beads, vortex vigorously for about 15 seconds to dissociate adhered cells. For swabs not received in transport medium, transfer them to a tube of transfer medium containing glass beads and vortex vigorously for about 15 seconds to dissociate adhered cells.
- When staining with fluorescent reagents and examining cells microscopically for fluorescence, it is very important to include controls, both positive and negative, to monitor the procedure and performance of the reagents. It is recommended that such controls be run with each batch of patient specimens.
- Place the closed, humidified chamber for holding slides during staining into the incubator for equilibration to 35°C to 37°C prior to staining. By doing this, the test slides and reagents will come to temperature quickly, yielding more rapid, intense staining.
- Bring DFA Reagents to ambient temperature (20°C to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2°C to 8°C.

Regarding Cell Culture Testing

- Good Laboratory Practice dictates that positive and negative virus controls be run with each new batch of cells to confirm their performance in culturing specific viruses.
- It is good practice to retain the medium removed from the monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer and incubated for the appropriate time period for repeat testing.
- When using cell cultures in polystyrene multi-well plates, dilute the acetone fixative to 80% by adding 20 mL of de-mineralized water to 80 mL of acetone. (See *MATERIALS REQUIRED BUT NOT PROVIDED*.)
- Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased sensitivity.
- Do not allow the DFA Reagents to dry on the monolayers; this can lead to high background.

Regarding Immunofluorescence Microscopy

- Examine the positive and negative controls before examining the test specimens. If one of these fails to perform as expected, review the steps and conditions under which the test was performed to determine the root cause(s). Do not report results for patient samples until controls perform as expected.
- Three aspects of the fluorescence microscope must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
  - The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the DFA Reagent.
  - The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
  - The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.
- Fluorescent artifacts may be observed in the cell monolayers:
  - Cell debris, lint, etc. can non-specifically adsorb the DFA Reagent, resulting in highly intense fluorescence. These can be identified by their morphology, i.e., they don't have the appearance of a complete cell and typically are not seen on the same plane of the monolayer as the other cells would be.
  - A low grade, yellow-green fluorescence may sometimes be seen, particularly in areas that have piled cells or are near holes in the cell monolayer. In both cases, the diffusion of the entrapped DFA Reagent is retarded during the wash step, resulting in the non-specific fluorescence.
  - Intense fluorescence around the periphery of slide wells is indicative of drying of the DFA Reagent during incubation, suggesting that it was incubated too long or the humidity was not well controlled.
  - Non-specific fluorescence caused by adsorption of the DFA Reagent trapped by inadequate removal of mucus from direct specimens.
  - Trapping of fluorescence by leukocytes and monocytes may occur on direct specimens. Likewise, RBCs in the specimen may leave a green haze on the sample.
  - Inadequate washing can lead to general low-grade fluorescence due to residual DFA Reagent remaining on the monolayer of cells
- Protect stained slides and monolayers from light as much as possible during testing.
  - Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
  - This bleaching can occur when a stained cell is viewed in a fluorescence microscope for an extended period of time.

Specimen Preparation

For specimen processing recommendations, refer to CLSI Approved Viral Culture Guidelines.[19]

1. Vortex the specimen vigorously for 10 to 15 seconds.
2. Centrifuge at 400 to 600xg for 5 to 10 minutes.
3. Collect and set aside the supernatant for viral isolation. (See step #10 below.)
4. Add 5 mL of PBS and vortex vigorously for 10 to 15 seconds.
5. Centrifuge at 400 to 600xg for 5 to 10 minutes.
6. Remove the supernatant and the mucus layer above the cell pellet taking care not to disturb the cell pellet.
7. Repeat steps 4 through 6 until the mucus layer has been completely removed.
   NOTE: It is important to remove all the mucus since it can cause non-specific fluorescence.
8. Add 0.5 to 1 mL of PBS.
9. Mix the suspension by pipetting up and down to re-suspend the cell pellet, forming a slightly cloudy suspension. This cell suspension will be used for Direct Specimen Testing.
   NOTE: The quality of the slide preparation is dependent on the concentration of cells in the suspension; too many cells make it difficult to read the result and too few decrease the sensitivity of the procedure. Specimens may also be centrifuged if a monolayer is preferred.

10. For use in Cell Culture Testing, add the supernatant that was reserved in Step #3, to the cell suspension that remains after Direct Specimen Testing. Add a few sterile glass beads to the tube and vortex for about 15 seconds to break up the cells and release any virus. Repeat this step for each specimen.

Direct Specimen Testing

1. Spot 25 µL of the prepared cell suspension[20] on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.
2. Air-dry the wells completely.
3. Fix the cells to the slides using fresh, chilled 100% acetone for 5 to 10 minutes at 20°C to 25°C**.**
   CAUTION: Acetone is volatile and flammable; keep away from open flames.
4. Remove the slides from the fixative and allow to air dry.
5. Add one drop of the DFA Screening Reagent to completely cover the dried, fixed cells on one well of each of the 2-well slides.
6. Also, to each of the wells of a fresh Respiratory Virus Antigen Control Slide add one drop of the DFA Screening Reagent. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
7. Add one drop of the Normal Mouse Gamma Globulin DFA Reagent to completely cover the dried, fixed cells on the other well of each of the 2-well slides.
8. Place the slides in a covered humidified chamber at 35°C to 37°C for 15 to 30 minutes.
9. Rinse the stained cells using the 1X Wash Solution. For only a few slides, this can be done using a beaker of the 1X Wash Solution. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container of 1X Wash Solution. For effective rinsing, dip the slide(s) up and down a minimum of four times.
10. Discard the used wash and repeat the washing step using new 1X Wash Solution.
11. Rinse the stained cells using de-mineralized water. For only a few slides, this can be done using a beaker of the de-mineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
12. Gently blot the excess de-mineralized water.
13. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
14. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*)
15. Refer to *INTERPRETATION OF RESULTS*.
16. If the result is positive for respiratory virus, the staining procedure may be repeated using the reserved 8-well specimen slide in order to identify which respiratory virus may be present.
    - Add one drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Leave one well as a negative.
    - For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well.
      NOTE: An Antigen Control Slide should be stained only once, do not re-stain.
    - Continue with steps 8 through 15, above.

Cell Culture Testing – Tube Culture

1. Examine the monolayers for proper morphology prior to inoculation.
2. Aspirate maintenance medium from the monolayers and add 0.2 to 0.4 mL of each prepared specimen (see *Specimen Preparation*) to each of the cell lines used for respiratory virus culture.
3. Place the tubes at an angle sufficient for the monolayers to be covered by the inoculum; then place tubes in an incubator for 1 hour at 35°C to 37°C to allow virus adsorption to occur.
4. After adsorption, add 2 mL of appropriate refeed medium.

5. Incubate the tubes at 35°C to 37°C in a roller drum at 1 to 3 rpm. Examine the monolayers daily for evidence of toxicity or viral CPE or test for hemadsorption.
6. When the monolayers are ready to be stained, remove the medium by aspiration and gently rinse the monolayer two times with 1 to 2 mL PBS.
7. Add 0.5 mL of PBS to the tube and prepare a suspension of the cells by scraping the monolayer using a pipette and breaking the cell aggregates up by pipetting the PBS up and down several times.
8. Prepare cell spots using about 25 µL of the suspension on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.
9. Air dry the wells completely.
10. Fix the cells to the slides using fresh, chilled 100% acetone. Let stand for 5 to 10 minutes, at 20°C to 25°C. **CAUTION: Acetone is volatile and flammable; keep away from open flames.**
11. Remove the slides from the fixative and allow to air dry.
12. Add one drop of the DFA Screening Reagent to completely cover the dried, fixed cells on one well of each of the 2-well slides.
13. Also, to each of the wells of a fresh Respiratory Virus Antigen Control Slide, add one drop of the DFA Screening Reagent. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
14. Place the slides in a covered chamber at 35°C to 37°C for 15 to 30 minutes.
15. Rinse the stained cells using the 1X Wash Solution. For only a few slides, this can be done using a beaker of the 1X Wash Solution. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with 1X Wash Solution. For effective rinsing, dip the slide(s) up and down a minimum of four times.
16. Discard the used wash and repeat the washing step using new 1X Wash Solution.
17. Rinse the stained cells using de-mineralized water. For only a few slides, this can be done using a beaker of the de-mineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
18. Remove the de-mineralized water by aspiration.
19. Gently blot the excess liquid.
20. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
21. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy.*)
22. Refer to *INTERPRETATION OF RESULTS*.
23. If the result is positive for respiratory virus, the staining procedure may be repeated using the reserved 8-well specimen slides in order to identify which respiratory virus may be present.
    - Add one drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Leave one well as a negative.
    - For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well.
      NOTE: An Antigen Control Slide should be stained only once, do not re-stain.
    - Continue with steps 14 through 21 above.

Cell Culture Testing – Shell-Vial

1. Calculate the number of shell-vials needed based on the staining protocol to be used (this staining protocol requires 3 shell-vials):
    - One shell-vial is required for each day the culture will be screened with the DFA Screening Reagent (i.e., staining at 16 to 24 hours, and again at 48 to 72 hours, requires 2 shell-vials).
    - One additional shell-vial is required for preparing 8-well slides used to identify the viruses from positive screens.
2. Examine the monolayers for proper morphology prior to inoculation.

3. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each shell-vial.
4. Add 0.2 to 0.4 mL of prepared specimen to each shell-vial.
5. Centrifuge the shell-vials at 700xg for 1-hour at 20°C to 25°C.
6. Place stoppered shell-vials in an incubator at 35°C to 37°C.
7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium by aspiration and add 1 mL of PBS.
8. Swirl to mix and then aspirate.
9. Repeat this wash with another 1 mL of PBS and then aspirate.
10. Add 1 mL of fresh, chilled 100% acetone and allow to stand for 5 to 10 minutes at 20°C to 25°C.
    CAUTION: Acetone is volatile and flammable; keep away from open flames.
11. Remove the fixative by aspiration.
12. Add 0.5 mL of PBS to wet the monolayer.
13. Swirl and then aspirate completely.
14. Add 4 drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples, and rock to **ensure complete coverage** of the monolayer by the Reagent.
15. Place stoppered shell-vials in a 35°C to 37°C incubator for 15 to 30 minutes.
16. Aspirate the DFA Screening Reagent from the monolayers.
17. Add 1 mL of the 1X Wash Solution.
18. Remove the 1X Wash Solution by aspiration, repeat the wash step, and again remove by aspiration.
19. Add 1 mL of de-mineralized water.
20. Remove the de-mineralized water by aspiration.
21. Lift the coverslip from the bottom of the shell-vial using a bent-tip needle on a syringe barrel. Grasping it with the fine-tipped forceps, transfer it, monolayer-side down, to a small drop of Mounting Fluid on a standard microscope slide.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*.)
23. Refer to *INTERPRETATION OF RESULTS*.
24. If the result is positive for respiratory virus, process a reserved replicate culture as a cell suspension and spot onto an 8-well specimen slide in order to identify which respiratory virus may be present (See *Cell Culture Testing – Tube Culture* steps 6 through 11, for procedure to prepare a specimen slide), then:
    - Add one drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Leave one well as a negative.
    - For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well.
      NOTE: An Antigen Control Slide should be stained only once, do not re-stain.
    - Continue with steps 14 through 21 from *Cell Culture Testing – Tube Culture* procedure.

Cell Culture Testing – Multi-Well Plate

1. Calculate the number of wells needed for the staining protocol to be used (this staining protocol requires 3-wells):
    - One well is required for each day the culture will be screened with the DFA Screening Reagent (i.e., staining at 16 to 24 hours, and again at 48 to 72 hours, requires 2-wells).
    - One additional well is required for preparing 8-well slides used to identify the viruses from positive screens.
    - It is recommended that each replicate well be on a different multi-well plate. This allows each plate to be processed on the appropriate day.
2. Examine the monolayers for proper morphology prior to inoculation.
3. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate re-feed medium to each 24-well multi-well plate monolayer; add 0.8 mL to each 48-well plate monolayer.

4. Add 0.2 to 0.4 mL of prepared specimen to the appropriate wells of a multi-well plate.
5. Centrifuge the multi-well plates at 700xg for 1 hour at 20°C to 25°C.
6. Place the covered multi-well plates in a 35°C to 37°C incubator with a humidified, 5% CO_2 atmosphere.
7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium by aspiration and add 1 mL of PBS.
8. Swirl to mix and then aspirate.
9. Repeat this wash with another 1 mL of PBS and then aspirate.
10. Add 1 mL of 80% aqueous acetone and let stand 5 to 10 minutes at 20°C to 25°C.
    NOTE: Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10 minutes since it may craze and cloud the plastic, making it difficult to examine the monolayers.
    CAUTION: Acetone is volatile and flammable; keep away from open flames.
11. Remove the fixative by aspiration.
12. Add 0.5 mL of the PBS to wet the monolayer.
13. Swirl and then aspirate completely.
14. Add 4 drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples in each well of a 24-well plate; add 3 drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples in each well of a 48-well plate. Rock to **ensure complete coverage** of the monolayer by the Reagent.
15. Place the covered multi-well plate in a 35°C to 37°C, humidified incubator for 15 to 30 minutes.
16. Aspirate the DFA Screening Reagent from the monolayers.
17. Add 1 mL of the 1X Wash Solution and mix to wash.
18. Remove the 1X Wash Solution by aspiration, repeat the wash step, and again remove by aspiration.
19. Add 1 mL of de-mineralized water.
20. Remove the de-mineralized water by aspiration.
21. Add 2 to 3 drops of Mounting Fluid to each monolayer, then cover the plate.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*.)
23. Refer to *INTERPRETATION OF RESULTS*.
24. If the result is positive for respiratory virus, process a reserved replicate culture as a cell suspension and spot onto an 8-well specimen slide in order to identify which respiratory virus may be present (See *Cell Culture Testing – Tube Culture* steps 6 through 11, for procedure to prepare a specimen slide), then:
    - Add one drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Leave one well as a negative.
    - For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well.
      NOTE: An Antigen Control Slide should be stained only once, do not re-stain.
    - Continue with steps 14 through 21 from *Cell Culture Testing – Tube Culture* procedure.

Quality Control

Reagents

- A fresh Respiratory Virus Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
- The positive wells will show multiple infected cells of bright apple-green fluorescence with negative cells staining a dull red due to the included Evans Blue counter-stain.
- The negative well will show only negative cells staining a dull red.
- Positive and negative controls must demonstrate appropriate fluorescence for specimen results to be considered valid. Antigen Control Slides may also aid in the interpretation of patient specimens.

- The Normal Mouse Gamma Globulin DFA Reagent is used to rule out those rare instances where patient cells are present that non-specifically bind the Fc portion of the mouse gamma globulin in direct specimens, which could lead to a false positive result.

Cell Culture

- Positive and negative virus controls should be run with each new batch of cells to confirm their performance in culturing specific viruses.
- To ensure viral sensitivity, virus-inoculated control monolayers should be included each time a new lot of cell culture is used.
- A non-inoculated monolayer from each lot should be stained to serve as a negative control. Adverse storage conditions or handling procedures will also be reflected in the negative control.
- If control cultures fail to perform correctly, results are considered invalid.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

- Examine controls first to ensure proper test performance before examining patient specimens.
- A positive reaction is one in which bright apple-green fluorescence is observed in the infected cells.
- Non-infected cells will fluoresce dull red due to the Evans Blue counter-stain included in the DFA Reagent.
- Examine the entire cell spot or monolayer of cells before reporting final results.
- Do not report results for patient samples unless controls perform as expected.

Artifacts of Staining

- Dried edges of the monolayer or cell clumps may non-specifically fluoresce due to antibody trapping.
- Dead, rounded cells may non-specifically fluoresce dull olive-green due to specimen toxicity or improper cell storage.
- Properly controlled humidity during staining and adequate washing between steps helps prevent non-specific staining.

Fluorescent Staining Pattern of Respiratory Virus Infected Cells

The following describes typical fluorescent patterns and should be used as a guide to identify specific viruses. Note that specific viral identification requires the demonstration of characteristic staining with MAbs.

The “typical” apple-green fluorescence staining pattern for each virus is as follows:

- Influenza A and B Virus – The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.
- Respiratory Syncytial Virus – The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.
- Adenovirus – The fluorescence is cytoplasmic and punctate or bright nuclear or both.
- Parainfluenza virus types 1, 2, and 3 – The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.

Co-infection with more than one infecting virus present in the specimen has been reported in a number of studies. The presence of multiple viruses is indicated when more than one well of the 8-well slide has fluorescent cells. The identification of the viruses is based on the individual virus DFA Reagents showing fluorescence. In such a case, it should be reported as “… and … detected by direct specimen testing.” or “… and … isolated by cell culture.”

Results of Direct Specimen Staining

- Evaluation of sample suitability
  - **Each stained patient specimen** should be reviewed for the presence of columnar epithelial cells (cells that are taller than they are wide). The quality of the specimen with respect to the number of epithelial cells in the sample can be assessed by examining different fields at a magnification of 200X.
  - **A satisfactory specimen** should have at least 2 columnar epithelial cells per field. A negative result is indicated by the absence of fluorescence in a minimum sampling of 20 columnar epithelial cells.
  - **An inadequate specimen** is indicated by fewer than 20 columnar epithelial cells present in the sample, in which case the test is considered invalid. A new specimen should be obtained and tested or cell culture for virus isolation should be initiated from the remaining specimen.

Reporting Results of Direct Specimen Staining

- The entire cell spot must be examined for virus-infected, apple-green fluorescent cells.
  - A satisfactory specimen with **no fluorescent cells observed** should be reported as "No influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza type 1, parainfluenza type 2, or parainfluenza type 3 viral antigens detected by direct specimen testing". However, such negative results should be confirmed using cell culture.
  - Specimens negative by direct specimen testing but yielding positive culture results should be reported as "… isolated by cell culture", where '…' is the appropriate virus, e.g., influenza A, influenza B, adenovirus, respiratory syncytial virus, parainfluenza virus type 1, 2, or 3 (see *Results from Culture Isolation/Confirmation*).
  - If apple-green fluorescent cells are found, the identification of the virus(es) may be based on the individual DFA Reagents (see *PROCEDURE – Direct Specimen Testing*). The individual virus DFA Reagent that yields fluorescent cells represents the identification of the respiratory virus. In such a case, it should be reported as "… detected by direct specimen testing", where '…' is the appropriate virus, e.g., influenza A, influenza B, adenovirus, respiratory syncytial virus, parainfluenza virus type 1, 2, or 3.

Results from Culture Isolation/Confirmation

- The entire cell spot or monolayer of cells must be examined for virus-infected, apple-green fluorescent cells. If no fluorescent cells are found, the results should be reported as, "No influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3 isolated in cell culture."
- If apple-green fluorescing cells are found, the identification of the virus(es) may be based on the individual DFA Reagents (see the appropriate *PROCEDURE* sections). In such cases, identification of the viral antigen(s) should be reported as "___ isolated in cell culture", where '___' is the appropriate virus, e.g., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3.

LIMITATIONS OF THE PROCEDURE

- Inappropriate specimen collection, storage, and transport may lead to false negative culture results.[21]
- Assay performance characteristics have not been established for direct specimen staining on specimens other than respiratory specimens. It is the user's responsibility to establish assay performance for specimens other than respiratory specimens.
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of viruses will vary greatly depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of virus 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.

- The effects of antiviral therapy on the performance of this kit have not been established.
- The MAbs used in this kit are from hybridomas created using viral infected cells as the immunogen. The specific viral antigens detected by the antibodies are undetermined.
- Since the MAbs have been prepared using defined virus strains, they may not detect all antigenic variants or new strains of the viruses, should they arise. MAbs may fail to detect strains of viruses which have undergone minor amino acid changes in the target epitope region.
- The MAbs used in this kit are not group-specific and therefore cannot be used to differentiate among the different types of adenovirus and RSV.
- The viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture. This is particularly true of RSV which is known for its instability and loss of viability.
- A negative direct specimen should be inoculated into an appropriate cell culture and incubated to isolate and identify any respiratory virus that may be present in the specimen.
- A negative result on a direct or cultured specimen does not rule out the presence of virus.
- Performance of the kit can only be assured when components used in the assay are those supplied by Quidel.
- Prolonged storage of the DFA Reagents under bright light will decrease the staining intensity.
- Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will bind to the F_c portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution.

EXPECTED VALUES

Respiratory virus infections are often seasonal, with influenza typically extending from November to April in the northern hemisphere, and adenovirus infections occurring more often during late winter to early summer. RSV is usually a seasonal (winter and early spring) infection as well, with epidemics lasting up to five months, while outbreaks caused by parainfluenza viruses may occur throughout a year.

The clinical studies described in *SPECIFIC PERFORMANCE CHARACTERISTICS* were comprised of respiratory specimens collected during the winter to early spring months of 2005/2006. Prevalence of the respiratory viruses within the population of specimens that were prospectively collected and tested fresh is noted in Table 2 below (also, see *Study 1-DS* in *SPECIFIC PERFORMANCE CHARACTERISTICS*).

Table 2. Prevalence of the Respiratory Viruses Within the Study Population

Expected Values	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Fresh Specimens (n=326)	18	32	19	2	0	5	18
Prevalance	5.5%	9.8%	5.8%	0.6%	0%	1.5%	5.5%

SPECIFIC PERFORMANCE CHARACTERISTICS

This study included eight hundred and forty-nine (849) original specimens evaluated by this product ("Subject" device) and a currently-marketed DFA Screening & ID Kit ("Comparator" device). All 849 specimens were studied by Direct Specimen (DS) testing with 22 of these specimens having insufficient cell numbers to be evaluated, and one other which could not be evaluated because it exhibited non-specific staining from the Normal Mouse Gamma Globulin DFA Reagent; 520 of the specimens also were studied by Cell Culture (CC) method with one specimen not evaluated because it produced a toxic cell culture monolayer. All but 30 of the

specimens were prospectively collected during the 2005 to 2006 season; those 30 specimens had been archived as parainfluenza-positive. In addition, a set of 81 clinical isolates were tested by CC methods only. The evaluations were conducted at three laboratory sites: (1) A reference laboratory in northeast United States; (2) A hospital laboratory in northeast United States; and (3) An internal reference laboratory using specimens collected from an external hospital laboratory.

Percent Agreement between the Subject and Comparator devices was calculated for prospectively collected specimens. For the Respiratory Virus DFA Screening Reagent:

- By DS method using fresh specimens, positive percent agreement is 95.5% and negative percent agreement is 98.3% (see Table 3). By DS method using frozen specimens, both positive percent agreement and negative percent agreement are 100% (see Table 4).
- By CC method using frozen specimens, both positive percent agreement and negative percent agreement are 100% (see Table 5).
- [See individual study results, below]

Table 3. Overall Direct Specimen Results (Fresh Specimens)

DS – Fresh 326 Specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	236	90	18	32	18	2	0	2	18
Subject Results	232	94	18	32	19	2	0	5	18
Positive Percent Agreement* (PPA)		95.5%	100%	100%	100%	100%	–	100%	100%
95% CI** – PPA		89.0% to 98.2%	82.4% to 100%	89.3% to 100%	82.4% to 100%	34.2% to 100%	–	34.2% to 100%	82.4% to 100%
Negative Percent Agreement** (NPA)	98.3%		100%	100%	98.7%	100%	100%	96.7%	100%
95% CI – NPA	95.7% to 99.3%		95.2% to 100%	94.2% to 100%	92.9% to 99.8%	96.0% to 100%	96.1% to 100%	90.8% to 98.9%	95.2% to 100%

* "Positive Percent Agreement", or "PPA", values were calculated according to **{[**(Total Number of Positive Results in Agreement by both Subject and Comparator Devices) divided by [(Total Number of Positive Results in Agreement by both Subject and Comparator Devices) plus (Number of Results Positive by Comparator but Negative by Subject)]**}** multiplied by 100%.

** "Negative Percent Agreement", or "NPA", values were calculated according to **{[**(Total Number of Negative Results in Agreement by both Subject and Comparator Devices) divided by [(Total Number of Negative Results in Agreement by both Subject and Comparator Devices) plus (Number of Results Negative by Comparator but Positive by Subject)]**}** multiplied by 100%.

Table 4. Overall Direct Specimen Results (Frozen Specimens)

DS – Frozen 474 specimens	**Negative**	**Screen +**	**Adeno**	**Flu A**	**Flu B**	**Para 1**	**Para 2**	**Para 3**	**RSV**
Comparator Results	306	168	8	85	19	3	3	9	51
Subject Results	306	168	8	85	19	3	3	9	51
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		97.8% to 100%	63.1% to 100%	95.7% to 100%	82.3% to 100%	38.3% to 100%	38.3% to 100%	70.1% to 100%	93.0% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	98.8% to 100%		97.7% to 100%	95.6% to 100%	97.6% to 100%	97.8% to 100%	97.8% to 100%	97.6% to 100%	96.7% to 100%

Table 5. Overall Cell Culture Results (Frozen Specimens)

CC – Frozen 490 specimens	**Negative**	**Screen +**	**Adeno**	**Flu A**	**Flu B**	**Para 1**	**Para 2**	**Para 3**	**RSV**
Comparator Results	309	181	13	93	23	6	4	9	49
Subject Results	309	181	13	93	23	6	4	9	49
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		98.0% to 100%	73.4% to 100%	95.2% to 100%	83.1% to 100%	55.7% to 100%	45.4% to 100%	65.5% to 100%	91.3% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	98.5% to 100%		97.3% to 100%	95.0% to 100%	97.1% to 100%	97.4% to 100%	97.4% to 100%	96.6% to 100%	96.6% to 100%

Specimens and culture isolates used in these studies came from nasopharyngeal (NP) aspirates, washes, swabs, bronchial alveolar lavages (BAL) and/or tracheal aspirates.

Table 6 summarizes the participant age demographics according to test results for a population of 326 fresh specimens, prospectively collected and evaluated for performance using the Comparator assay (see *Study 1-DS – Direct Specimen Method* below).

Table 6. Age Distribution

	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV	Negative
Totals	*18*	*32*	*18*	*2*	*0*	*2*	*18*	*236*
< 1m*	0	0	0	0	0	0	2	1
1m to 2y	8	9	4	1	0	2	8	80
2y to12y	8	7	6	0	0	0	1	42
12y to18y	1	1	5	0	0	0	0	8
18y to 21y	0	0	1	0	0	0	0	2
> 21y	0	12	1	0	0	0	1	78
Not reported	1	3	1	1	0	0	6	25

* m = months, y = years

Prospectively Collected Specimens

Clinical Study Sites 1, 2, and 3 generated data for Direct Specimen (DS) Testing according to the study design briefly summarized for each site.

Clinical Study Sites 2 and 3 generated data for Cell Culture (CC) Testing according to study design as summarized for each site.

Study 1-DS - Direct Specimen Method

The study consisted of a total of 329 fresh specimens submitted February through May, 2006, to the laboratory for respiratory virus testing. Slides were prepared from PBS-washed cells from the fresh specimens and fixed according to the prescribed protocol.

The slides were stored at –70°C until testing was performed. The slides were brought to ambient temperature and stained in accordance with the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices). Three (3) specimens were found to contain insufficient numbers of cells for interpretation of DS stain results, leaving 326 specimens for evaluation. The results of this testing are summarized in Table 7 below.

Table 7. Study 1-DS – Direct Specimen Results

326 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	236	90	18	32	18	2	0	2	18
Subject Results	232	94	18	32	19	2	0	5	18
PPA		100%	100%	100%	100%	100%	–	100%	100%
95% CI – PPA		95.1% to 100%	79.3% to 100%	87.3% to 100%	79.3% to 100%	29.0% to 100%	–	29.0% to 100%	79.3% to 100%
NPA	98.3%		100%	100%	98.7%	100%	100%	96.7%	100%
95% CI – NPA	95.4% to 99.5%		94.2% to 100%	93.0% to 100%	92.2% to > 99.9%	95.2% to 100%	95.3% to 100%	90.5% to 99.3%	94.2% to 100%

With the exception of 4 specimens, the DS test results were concordant for both the screen and the identification of the individual viruses; the Comparator device identified 4 specimens as being negative while the Subject device identified one as Flu B and three as Para 3 infections. All but one of the Para 3 specimens were confirmed by culture; the one Para 3, although strongly positive by the Subject assay, could not be cultured to confirm it as a Para 3. The culture method was not performed on the rest of the specimens from this site.

Study 2-DS – Direct Specimen Method

The study consisted of 192 specimens submitted to the laboratory for respiratory virus testing during December 2005 through February 2006, with residual specimen material stored at –70°C from a few days to 2 months. The frozen specimens were thawed and processed between 13 February to 17 February 2006 according to the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices).

Slides were prepared from the specimens according to instructions detailed in the Comparator device's product insert. These slides were stained with both the Comparator and Subject devices and interpreted according to the Comparator device's product insert procedure (same procedure for both Subject and Comparator devices). All of the frozen/thawed specimens had sufficient intact cells for interpretation. The results of this testing are summarized in Table 8 below.

Table 8. Study 2-DS – Direct Specimen Results

192 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	142	50	2	26	3	1	1	0	17
Subject Results	142	50	2	26	3	1	1	0	17
PPA		100%	100%	100%	100%	100%	100%	–	100%
95% CI – PPA		91.5% to 100%	29.0% to 100%	84.% to 100%	38.3% to 100%	16.8% to 100%	16.8% to 100%	–	80.5% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	96.8% to 99.5%		92.6% to 100%	96.2% to 100%	96.8% to >99.9%	96.8% to 100%	96.8% to 100%	96.8% to 100%	89.4% to 100%

The DS test results were concordant for both the Screen and the ID reagents.

Study 2-CC – Cell Culture Method

The same 192 specimens that were evaluated by DS testing were also processed according to the Comparator device's product insert procedure for cell culture (same procedure for both Subject and Comparator devices). Briefly, 200 µL from the specimens were inoculated onto each of 4 monolayers of R-Mix Too FreshCells contained in shell-vials which were centrifuged for 60 minutes at 700xg and incubated for 24 hours at 35°C to 37°C. The shell-vials were processed according to instructions detailed in the Comparator device's product insert. The results of this testing are summarized in Table 9 below.

Table 9. Study 2-CC – Cell Culture Results

192 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	142	50	3	26	3	1	1	0	16
Subject Results	142	50	3	26	3	1	1	0	16
PPA		100%	100%	100%	100%	100%	100%	–	100%
95% CI – PPA		91.5% to 100%	38.3% to 100%	84.8% to 100%	38.3% to 100%	16.8% to 100%	16.8% to 100%	–	77.3% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI– NPA	96.8-99.5%		96.8% to 100%	96.2% to 100%	96.8% to > 99.9%	96.8% to 100%	96.8% to 100%	96.8% to 100%	96.4% to 100%

The CC test results were concordant for both the Screen and the ID of the specific viruses.

Study 3-DS – Direct Specimen Method

The study consisted of 298 specimens originally received by a hospital laboratory in the eastern US for respiratory virus testing during January through March 2006, with residual specimen material stored at –70°C from 3 to 6 months. The frozen specimens were sent to Quidel, where they were thawed and processed between 30 May and 1 June 2006, according to the Comparator device's product insert. All specimens used in the studies were tested by both the DS and CC procedures as detailed in the Comparator device's product insert; however, a total of 16 specimens were inadequate for interpretation of DS stain results (15 were found to contain insufficient numbers of cells, and one other specimen exhibited non-specific staining with the Normal Mouse Gamma Globulin DFA Reagent), leaving 282 specimens for evaluation.

The DS results for these specimens tested using the Comparator and Subject devices are summarized in Table 10 below.

Table 10. Study 3-DS – Direct Specimen Results

282 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	164	118	6	59	16	2	2	9	34
Subject Results	164	118	6	59	16	2	2	9	34
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA	96.2% to 100%	55.7% to 100%	92.7% to 100%	77.3% to 100%	29.0% to 100%	29.0% to 100%	65.5% to 100%	87.9% to 100%	96.2% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI– NPA	97.3-100%		96.0% to 100%	92.7% to 100%	95.6% to > 99.9%	96.2% to 100%	96.2% to 100%	95.9% to 100%	91.3% to 100%

The DS test results were concordant for both the Screen and the ID reagents. There were ten (10) specimens identified with co-infections as follows: three (3) Flu A+Para 3, one (1) Flu B+Para 2, one (1) Flu B+Para 3, one (1) RSV+Para 1, three (3) RSV+Para 3 and one (1) Adeno+Para 3. Because of the ten (10) co-infections, the Negatives and Positives add up to 292 ID results.

Study 3-CC – Cell Culture Method

The same 298 specimens that were evaluated by DS testing were also processed for CC testing according to the Comparator device's product insert for cell culture using R-Mix Too FreshCells in 48/24-fill cluster plates. The results of this testing are summarized in Table 11 below.

Table 11. Study 3-CC – Cell Culture Results

298 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	167	131	10	67	20	5	3	9	33
Subject Results	167	131	10	67	20	5	3	9	33
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		96.6% to 100%	67.9% to 100%	93.5% to 100%	81.0% to 100%	51.1% to 100%	38.3% to 100%	65.5% to 100%	87.6% to 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	97.3% to 100%		96.3% to 100%	93.2% to 100%	96.0% to >99.9%	96.4% to 100%	96.5% to 100%	96.3% to 100%	95.5% to 100%

The CC test results were concordant for both the Screen and the ID reagents. There were sixteen (16) co-infections as follows: three (3) Flu A+Para 3, one (1) Flu A+Para 1, one (1) Flu A+Para 2, two (2) Flu A+RSV, one (1) Flu A+Adeno, one (1) Flu B+Para 2, one (1) Flu B+Para 3, one (1) Flu B+RSV, one (1) RSV+Para 1, two (2) RSV+Para 3, one (1) Adeno+Para 1 and one (1) Adeno+Para 3. Because of the sixteen (16) co-infections, the Negatives and Positives in the table add up to 314 ID results.

Non-prospective archival specimens

Due to relative low prevalence of parainfluenza infections in populations of respiratory specimens, few specimens in the studies detailed above were reactive with the Parainfluenza DFA Reagents. In order to better demonstrate performance characteristics of the Parainfluenza DFA Reagents, frozen original specimens previously determined to contain parainfluenza (types 1, 2, or 3) during the 2006 "respiratory season" were obtained from an additional laboratory, and were tested in an internal reference laboratory using the Subject and Comparator devices by Direct Specimen method (*Study 3a-DS*; see Table 12, below). The same specimens were tested by Cell Culture method (see Table 13). Original results reported by the laboratory were unknown to the study investigator. Although the study design has a selection bias, this study offers further analytical information on the assay's ability to detect parainfluenza viruses.

Study 3a-DS – Direct Specimen Method

The study consisted of 30 specimens originally received by a hospital laboratory in Italy for respiratory virus testing during the period from October 2005 through April 2006, with residual specimen material stored at -70°C from 2 to 6 months. The frozen specimens were sent to Quidel, where they were thawed and processed

between June 7 and 8, 2006, according to the prescribed protocol. All specimens used in the studies were tested by both the DS and CC procedures as detailed in the Comparator device's product insert; however, a total of four specimens were found to contain insufficient numbers of cells for interpretation of DS stain results, leaving 26 specimens.

The DS results for these specimens tested using the Comparator and Subject devices are summarized in Table 12 below.

Table 12. Study 3a-DS – Direct Specimen Method

26 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	9	17	0	0	0	1	5	11	0
Subject Results	8	18	0	0	0	1	5	12	0
PPA		100%	–	–	–	100%	100%	100%	–
95% CI – PPA		78.4% to 100%	–	–	–	16.8% to 100%	51.1% to 100%	70.0% to 100%	–
NPA	88.9%		100%	100%	100%	100%	100%	85.7%	100%
95% CI – NPA	54.3% to > 99.9%		79.3% to 100%	79.3% to 100%	79.3% to 100%	78.4% to 100%	73.4% to 100%	46.7% to 99.5%	79.3% to 100%

With the exception of one specimen, the DS test results were concordant for both the Screen and the ID of individual viruses; the Subject device identified one specimen as positive for Para 3 while the Comparator device was negative for this specimen.

Study 3a-CC – Cell Culture Method

The same 30 frozen specimens that were evaluated by DS testing were also processed for CC testing according to the Comparator device's product insert for cell culture using R-Mix FreshCells in 48/24-fill cluster plates. One specimen was found to be unsuitable for CC testing because it was toxic to the monolayer of cells. The results of this testing are summarized in Table 13 below.

Table 13. Study 3a-CC – Cell Culture Results

29 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	8	21	0	0	0	3	5	13	0
Subject Results	8	21	0	0	0	3	5	13	0
PPA		100%	–	–	–	100%	100%	100%	–
95% CI – PPA		81.8% to 100%	–	–	–	38.3% to 100%	51.1% to 100%	73.4% to 100%	–
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	62.8% to 100%		81.8% to 100%	81.8% to 100%	81.8% to 100%	79.3% to 100%	77.3% to 100%	62.8% to 100%	81.8% to 100%

The CC test results were concordant for both the Screen and the ID reagents.

Non-prospective archival clinical isolates

To further demonstrate the proficiency of the Screening and Typing Reagents in the Subject Device, a study was conducted using a collection of banked clinical isolates known to contain respiratory viruses that had been frozen from the 2005/2006 respiratory season. These specimens were selected because they were previously shown to contain at least one of the seven virus analytes detected by the Subject Device.

Study 3b-CC – Cell Culture Method

A total of 81 clinical isolates from a frozen archival repository were processed according to the Comparator device's product insert for cell culture using R-Mix FreshCells cultures in shell-vials. The results of this testing are summarized in Table 14 below.

Table 14. Study 3b-CC – Cell Culture Method

81 specimens	Negative	Screen +	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Comparator Results	0	81	11	18	17	4	1	26	5
Subject Results	0	81	11	18	17	4	1	26	5
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		94.6% to 100%	70.% to 100%	79.3% to 100%	78.4% to 100%	45.4% to 100%	16.8% to 100%	84.8% to 100%	51.1% to 100%
NPA			100%	100%	100%	100%	100%	100%	100%
95% CI – NPA		97.3% to 100%	93.8% to 100%	93.1% to 100%	93.2% to 100%	94.3% to 100%	94.5% to 100%	92.2% to 100%	94.2% to 100%

The CC test results were concordant for both the Screen Reagent and the specific virus ID Reagents. Because of the one co-infection, Para 1+ Para 3, the positive ID results added up to 82.

Cross-reactivity Testing

D^3 Ultra DFA Respiratory Virus Screening & ID Kit DFA Reagents were tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 64 virus strains (cultured and processed for staining) or for 18 host culture cell types. Nineteen (19) bacterial cultures were stained and examined for cross-reactivity, including *Staphylococcus aureus*, a protein-A-producing bacterium. Staining of *S. aureus* appeared as small points of fluorescence (see *LIMITATIONS OF PROCEDURE*) while all other bacterial cultures were negative. [See Table 15 for cross-reactivity study results. The table indicates which organisms were reactive with which DFA Reagent.]

Stringent conditions for cross-reactivity testing were achieved by using high concentration of the DFA Reagents and high titers of microorganisms. The DFA Reagents (i.e., directly fluoresceinated monoclonal antibodies) were prepared at 1.5X the concentration that is provided in the kit. Each of the tested viruses was prepared as infected cell monolayers (250 infected cells inoculated into a shell-vial culture and incubated for 24 to 48 hours, to yield a 3+ to 4+ infection), and processed and stained with the 1.5X DFA Reagents according to the procedure detailed in this product insert. Bacterial strains were cultured, processed as suspensions, then

spotted on microscope slides (yielding > 150 bacteria per 400X microscope field), then stained with the 1.5X DFA Reagents according to the procedure in this product insert. Cell cultures were stained as confluent monolayers.

Table 15. Cross-Reactivity Study Results

		DFA Reagent (Results are Positive (+) or Negative (–) for Reactivity						
Organisms	**Strain**	**Adeno**	**Flu A**	**Flu B**	**Para 1**	**Para 2**	**Para 3**	**RSV**
Adenovirus	Type 1	+	–	–	–	–	–	–
	Type 3	+	–	–	–	–	–	–
	Type 5	+	–	–	–	–	–	–
	Type 6	+	–	–	–	–	–	–
	Type 7	+	–	–	–	–	–	–
	Type 10	+	–	–	–	–	–	–
	Type 13	+	–	–	–	–	–	–
	Type 14	+	–	–	–	–	–	–
	Type 18	+	–	–	–	–	–	–
	Type 31	+	–	–	–	–	–	–
	Type 40	+	–	–	–	–	–	–
	Type 41	+	–	–	–	–	–	–
Influenza A	Mexico/4108/2009 (H1N1) from CDC*	–	+	–	–	–	–	–
	California/07/2009 (H1N1) from CDC*	–	+	–	–	–	–	–
	Aichi (H3N2)	–	+	–	–	–	–	–
	Mal (H1N1)	–	+	–	–	–	–	–
	Hong Kong (H3N2)	–	+	–	–	–	–	
	Denver (H1N1)	–	+	–	–	–	–	–
	Port Chalmers (H3N2)	–	+	–	–	–	–	
	Victoria (H3N2)	–	+	–	–	–	–	–
	New Jersey ($H_{SW}N1$)	–	+	–	–	–	–	–
	WS (H1N1)	–	+	–	–	–	–	–
	PR (H1N1)	–	+	–	–	–	–	–
Influenza B	Hong Kong	–	–	+	–	–	–	–
	Maryland	–	–	+	–	–	–	–
	Mass	–	–	+	–	–	–	–
	Taiwan	–	–	+	–	–	–	–
	GL	–	–	+	–	–	–	–
	Russia	–	–	+	–	–	–	–
RSV	Long	–	–	–	–	–	–	+
	Wash	–	–	–	–	–	–	+
	9320	–	–	–	–	–	–	+
Parainfluenza 1	C-35	–	–	–	+	–	–	–
Parainfluenza 2	Greer	–	–	–	–	+	–	–
Parainfluenza 3	C 243	–	–	–	–	–	+	–
Parainfluenza 4a	M-25	–	–	–	–	–	–	–
Parainfluenza 4b	CH19503	–	–	–	–	–	–	–
Metapneumovirus	A1	–	–	–	–	–	–	–

		DFA Reagent (Results are Positive (+) or Negative (–) for Reactivity						
Organisms	**Strain**	**Adeno**	**Flu A**	**Flu B**	**Para 1**	**Para 2**	**Para 3**	**RSV**
Metapneumovirus	A2	–	–	–	–	–	–	–
	B3	–	–	–	–	–	–	–
	B4	–	–	–	–	–	–	–
Coronavirus	OC43	–	–	–	–	–	–	–
	229E	–	–	–	–	–	–	–
Rhinovirus	209 Picornavirus	–	–	–	–	–	–	–
Herpes simplex virus Type 1	1F	–	–	–	–	–	–	–
	MacIntyre	–	–	–	–	–	–	–
Herpes simplex virus Type 2	MS	–	–	–	–	–	–	–
	Strain G	–	–	–	–	–	–	–
Cytomegalovirus	Towne	–	–	–	–	–	–	–
	Davis	–	–	–	–	–	–	–
	AD169	–	–	–	–	–	–	–
Varicella-zoster	Webster	–	–	–	–	–	–	–
	Ellen	–	–	–	–	–	–	–
Echovirus	9	–	–	–	–	–	–	–
	11	–	–	–	–	–	–	–
	30	–	–	–	–	–	–	–
	34	–	–	–	–	–	–	–
Coxsackievirus	B1	–	–	–	–	–	–	–
	B2	–	–	–	–	–	–	–
	B3	–	–	–	–	–	–	–
	B4	–	–	–	–	–	–	–
	B5	–	–	–	–	–	–	–
	B6	–	–	–	–	–	–	–
Mumps		–	–	–	–	–	–	–
Rubeola		–	–	–	–	–	–	–
BACTERIA								
Acholeplasma laidlawii		–	–	–	–	–	–	–
Bordetella bronchiseptica		–	–	–	–	–	–	–
Bordetella pertussis		–	–	–	–	–	–	–
Chlamydia pneumoniae		–	–	–	–	–	–	–
Clostridium diphtheriae		–	–	–	–	–	–	–
Haemophillus influenzae type A		–	–	–	–	–	–	–
Klebsiella pneumoniae		–	–	–	–	–	–	–
Listeria pneumophila		–	–	–	–	–	–	–
Moraxella cartarrhalis		–	–	–	–	–	–	–
Mycobacterium avium		–	–	–	–	–	–	–
Mycobacterium intracellulare		–	–	–	–	–	–	–
Mycoplasma hominis type 1		–	–	–	–	–	–	–
Mycoplasma orale		–	–	–	–	–	–	–
Mycoplasma pneumoniae		–	–	–	–	–	–	–
Mycoplasma salivarium		–	–	–	–	–	–	–
Pseudomonas aeruginosa		–	–	–	–	–	–	–
Streptococcus pneumoniae		–	–	–	–	–	–	–

Organisms	Strain	DFA Reagent (Results are Positive (+) or Negative (–) for Reactivity) Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Streptococcus pyogenes		–	–	–	–	–	–	–
Ureaplasma urealyticum		–	–	–	–	–	–	–
CELL CULTURES								
A549		–	–	–	–	–	–	–
BGMK		–	–	–	–	–	–	–
HEp-2		–	–	–	–	–	–	–
LLC-MK2		–	–	–	–	–	–	–
MDCK		–	–	–	–	–	–	–
MRC-5		–	–	–	–	–	–	–
MRHF		–	–	–	–	–	–	–
Mv1Lu		–	–	–	–	–	–	–
NCI-H292		–	–	–	–	–	–	–
pCMK		–	–	–	–	–	–	–
pRhMK		–	–	–	–	–	–	–
pRK		–	–	–	–	–	–	–
RD		–	–	–	–	–	–	–
RhMK II		–	–	–	–	–	–	–
R-Mix		–	–	–	–	–	–	–
R-Mix Too		–	–	–	–	–	–	–
Vero		–	–	–	–	–	–	–
WI-38		–	–	–	–	–	–	–

* Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D³ Ultra DFA Respiratory Virus Screening and ID Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. FDA Guidance Document: *In vitro* Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; Issued 4/10/2006.
2. Englund, J.A., (2002). Antiviral therapy of influenza. *Semin. Pediatr. Infect. Dis.*, 13(2):120-128.
3. Patel, N., Hartwig, R., Kauffmann, L. and Evans, M. (2000). Rapid influenza A and B culture 20-hour detection using R-Mix: A new gold standard. Presented at The Sixteenth Annual Clinical Virology Symposium, April 30-May 3, Clearwater Beach, FL.
4. Rodriguez, W.J., Schwartz, R.H. and Thorne, M.M. (2002). Evaluation of diagnostic tests for influenza in a pediatric practice. *Pediatr. Inf. Dis. J.,* 3:193-6.
5. Gould, I.M. (2002). Antibiotic Policies and control of resistance. *Curr. Opin. Infect. Dis*., 15(4):395-400.
6. Bischofberger, N., Webster, R.G. and Laver, G. (1999). Disarming Flu Viruses. *Scientific American,* January.

7. Foy, H.M. (1997). Adenoviruses. In: Evans, A., Kaslow, R., eds. Viral Infections in Humans: Epidemiology and Control. 4th ed., New York, Plenum, 119-138.
8. Easton, A.J., Eglin, R.P. (1989). Epidemiology of Parainfluenza virus type 3 in England and Wales over a 10 year period. *Epidemiol. Infect.*, 102:531-535.
9. Fete, T.J., Noyes, B. (1996). Common (but not always considered) viral infections of the lower respiratory tract. *Pediatr. Ann.,* 25:10), 577-584.
10. Hall, C.B. (1981). Respiratory Syncytial Virus. In: Feigin, R. D., Cherry, J.D., eds. *Textbook of Pediatric Infectious Diseases*, Phila., W.B. Saunders, 1247-1267.
11. Hall, C.B., Hall, W.J., Gala, C.L., MaGill, F.B., Leddy, J.P. (1984). Longterm prospective study in children after Respiratory Syncytial Virus infection. *J. Pediatr*., 105:358-364.
12. Falsey, Ann R. and Walsh, E.E. (2000). Respiratory Syncytial Virus Infection in Adults. Clinical Microbiology Reviews 13(3):371-384.
13. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th edition, 2007, CDC-NIH manual. http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm]
14. *Biosafety Manual*, 3rd edition, 2004. World Health Organization [Manual may be available in additional languages; refer to WHO web page http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/]
15. *Laboratory Biosafety Guidelines*, 3rd edition, 2004. Published by authority of the Minister of Health, Population and Public Health Branch, Centre for Emergency Preparedness and Response [Guideline is available in French or English; refer to web page [http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index.html]
16. Clinical and Laboratory Standards Institute. *Viral Culture; Approved Guidelines.* CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA 2006.
17. Eisenberg, Henry D. (1992). *Clinical Microbiology Procedures Handbook,* published by American Society for Microbiology, Washington DC, pg. 8.2.3.
18. Clinical and Laboratory Standards Institute. *Viral Culture; Approved Guidelines.* CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006, Section 7.4.
19. Clinical and Laboratory Standards Institute. *Viral Culture; Approved Guidelines.* CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006,
20. Isenberg, Henry D., 2004. *Clinical Microbiology Procedures Handbook,* published by American Society for Microbiology, Washington DC, 10.7.1 – 10.7.10.
21. Leland, Diane S. (1996). *Clinical Virology,* published by W.B. Saunders, Philadelphia, PA.

REF 01-010000.v2 – D^3 Ultra DFA Respiratory Virus Screening & ID Kit

IVD

CE

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Diagnostic Hybrids, Inc. – a subsidiary of Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com **PI1670001EN00 (05/19)**

GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
Rx ONLY Prescription use only	 Consult e-labeling instructions for use
 Do not reuse	IVD For *In Vitro* diagnostic use
 160 to 250 Contains sufficient for 160 to 250 determinations	CONT NaN_3 Contains sodium azide
NaN_3 4% Contains 4% sodium azide when undiluted	