CE

QUIDEL

D³ Duet™ DFA Influenza A/Respiratory VIRUS SCREENING KIT

For the qualitative detection and identification of influenza A, while screening for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viral antigens.

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

INTENDED USE

The Diagnostic Hybrids, Inc. D³ Duet DFA Influenza A/Respiratory Virus Screening Kit, is intended for the qualitative detection and identification of influenza A, while screening for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viral antigens, in nasal and nasopharyngeal swabs and aspirates or in cell culture. The assay detects viral antigens by immunofluorescence using monoclonal antibodies (MAbs), from patients with signs and symptoms of respiratory infection.

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 influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Performance characteristics for influenza A virus detection and identification were established when influenza A H3N2 and influenza A H1N1 were the predominant influenza A strains circulating in the United States. 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 a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.[1]

SUMMARY AND EXPLANATION OF THE TEST

With the development of drug treatments for influenza,[2] rapid and sensitive laboratory tests for virus identification can impact the choice of specific therapy, eliminating the inappropriate use of antibiotics and other agents. Virus identification using specific, fluorescent MAbs for direct antigen detection in respiratory specimens or in cell culture continues to be a diagnostic procedure used in clinical virology laboratories.

Influenza Types A and B

Influenza viruses (family *Orthomyxoviridae*) contain a single-stranded RNA genome which is present in eight separate segments of ribonucleoprotein. Segmentation of the genome is rare among viruses and contributes to the development of new influenza strains through interchange of gene segments when two different influenza strains infect the same cell. There are three influenza types: A, B, and C. Type A has counterparts in birds, horses, sea mammals, and pigs as well as in humans, while types B and C are primarily known in humans.

With the potential for an additional influenza A pandemic such as occurred in 1918 when 25-35 million people died worldwide, the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of emerging influenza strains and make recommendations for suitable strains for vaccine production.

Influenza infects an estimated 120 million people in the US, Europe and Japan each year, and it is estimated there are 75,000 deaths annually in the US from pneumonia caused by influenza. Primary viral pneumonia or pneumonia from secondary bacterial infections are the primary causes of morbidity associated with influenza infection.[3] Complications tend to occur in the young, the elderly and persons with chronic cardio-pulmonary diseases.

Pandemics of influenza A occur about every 10 to 30 years while annual epidemics are usually of either influenza A or B; however, both types may circulate concurrently. Infections are seasonal, typically extending from November to April in the northern hemisphere. Disease incubation is 1-3 days with rapid transmission through aerosolized droplets and fomites. The disease is characterized by sudden onset, fever, myalgia, headache and pharyngitis.

Influenza A and B are most commonly isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix™[1]), A549/MDCK mixtures (R-Mix Too™), Rhesus MK, MDCK, MRC-5 and A549 cells.[4]

Adenovirus

Adenoviruses (family *Adenoviridae*) are non-enveloped, double stranded DNA viruses. At the present time there are 51 serotypes, further divided into 6 groups, A to F. Most adenoviruses are associated with respiratory and ocular infections. Generally, adenovirus infections in adults have a low morbidity with the exceptions of immunocompromised individuals and those living in overcrowded conditions, in which infections can cause atypical pneumonia. Virus spread is commonly through aerosolized droplets and fomites with infection of mucous membranes of the eye, respiratory tract and gut.[5]

Adenoviruses can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCK mixtures (R-Mix Too), HEp2, HEK, A549 and MRC-5 cells.[4]

Parainfluenza Virus Types 1, 2, 3 and 4

Parainfluenza viruses (family *Paramyxoviridae*) are enveloped viruses with a single, negative strand RNA genome. The four different types cause croup and viral pneumonia in children under the age of 5 years and upper respiratory illness in adults. Parainfluenza is the second leading cause of lower respiratory illness in children after RSV. Outbreaks caused by parainfluenza viruses usually occur in the fall during alternate years (P1 and P2) or throughout the year, with increased activity in the spring (P3).[6]

Parainfluenza viruses can be isolated in cell cultures of 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.[4]

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.[7] RSV is the primary viral cause of lower respiratory disease in infants and young children with peak mortality due to RSV in 3-4 month old infants. RSV is usually a seasonal (winter and early spring) infection with epidemics lasting up to 5 months. There are two major subtypes, A and B: subtype B is characterized as the asymptomatic strain that the

[1]The use of R-Mix and R-Mix Too cells is covered by U.S. Patents with additional patents pending.

majority of the population experiences. More severe clinical illness involves subtype A strains which tend to predominate in most outbreaks.[8] Re-infections do occur but tend to be limited to minor upper respiratory infections.[9] RSV is also recognized as a significant problem in certain adult populations including the elderly, individuals with cardiopulmonary diseases, and immunocompromised hosts.[10]

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents[8] 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.[4]

PRINCIPLE OF THE PROCEDURE

The D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit, uses a blend of viral antigen-specific murine MAbs. MAbs for influenza A virus are directly labeled with R-phycoerythrin (R-PE) for the rapid detection and identification of influenza A virus. MAbs for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 are directly labeled with fluorescein isothiocyanate (FITC), for rapid detection of these agents.

The cells to be tested, derived from a clinical specimen or cell culture, are placed onto a glass slide and allowed to air dry. The cells are fixed in acetone. The D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent is added to the cells which are then incubated for 15 to 30 minutes at 35°C to 37°C in a humidified chamber or humidified incubator. The stained cells are then washed with the diluted wash solution, 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. The influenza A virus infected cells will fluoresce golden-yellow, while cells infected with any of the other six viruses will fluoresce apple-green. Non-infected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain. If only golden-yellow fluorescent cells are present the specimen can be reported as positive for influenza A antigen. If only apple-green fluorescent cells are present, the particular virus is identified using the individual reagents from the D^3 Ultra™ DFA Respiratory Virus Screening and ID Kit (D^3 Ultra) on new, separate cell preparations. If both golden-yellow and apple-green are present, the additional virus is identified using the individual reagents from the D^3 Ultra on new, separate cell preparations.

It is recommended that results for specimens found to contain no fluorescent cells after examination of the direct specimen result be confirmed by cell culture.

REAGENTS AND MATERIALS PROVIDED

The D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit contains the following:

D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent 10 mL

One dropper bottle containing R-phycoerythrin-labeled murine MAbs directed against influenza A virus and a mixture of fluorescein-labeled murine MAbs directed against influenza B, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3. The buffered, stabilized, aqueous solution also contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

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 non-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.

Respiratory Virus Antigen Control Slides **5 slides**

Individually packaged control slides containing wells with cell culture-derived positive and negative control cells. Each positive well is identified with the virus infected cells present, i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2 and 3. The negative well contains non-infected cultured cells. Each slide is intended to be stained only one time.

40X Wash Solution Concentrate **25 mL**

One bottle containing a 40X concentrate consisting of Tween 20 and 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water) in a 40X phosphate buffered saline solution.

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) and for R-PE; magnification 200 to 400X
- Cell culture for respiratory virus isolation according to the laboratory's method of choice (Suggested cell lines that are susceptible to respiratory viruses include LLC-MK2, HEp-2, A549, R-Mix and R-Mix Too MixedCells™, and primary Rhesus monkey kidney cells.)
- Live control viruses for positive culture controls; Known viral strains for monitoring cell culture susceptibility and staining procedures
- Cover slips (22 x 50 mm) for Antigen Control Slides and for specimen slides
- Universal Transport Medium
- Tissue culture refeed medium – R-Mix Refeed Medium (for use with R-Mix and R-Mix Too MixedCells) or other standard refeed medium
- Reagent grade acetone (> 99% pure) chilled at 2°C to 8°C for fixation of direct specimen slides and shell vials

  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 room temperature (20°C to 25°C).
- Sterile graduated pipettes: 10 mL, 5 mL, and 1 mL
- Sterile glass Pasteur pipettes or other "transfer"-type 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 bench top or with a pair of forceps taking care to avoid injury.
- Sodium hypochlorite solution (1:10 final dilution of household bleach)
- Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom) or humidified incubator
- 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 swab or polyester swab which is non-inhibitory to respiratory viruses and tissue culture
- Incubator, 35°C to 37°C (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 Concentrate Solution and for dilution of the reagent grade acetone for use in polystyrene multi-well plates
- PBS (Phosphate Buffered Saline), sterile, for use in rinsing and suspending cells.
- Control viruses – Known strains of the 7 respiratory viruses for use in monitoring the cell culture and staining procedures
- 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 with 40X to 100X magnification – Used for examining the monolayers prior to inoculation and for cytopathic effects (CPE)

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[11,12,13] 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 Wash Solution 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 Wash Solution 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 and mix. Add the 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 room temperature.

Preparation of 80% Acetone Solution

- Add 20 mL of distilled or de-mineralized water to a 100 mL container.
- Add 80 mL of acetone to the container and mix by inversion.
- Label the container as to contents, the date diluted, and technologist's initials. Store the Acetone Solution at room temperature.

Storage

Table 1. Reagent Storage Conditions

D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent	Store at 2°C to 8°C in the dark.
Normal Mouse Gamma Globulin DFA Reagent	
Mounting Fluid	
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 under recommended conditions. Light exposure of the DFA reagent 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 isolation of viruses should be attempted only by personnel trained in performing 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.[14]

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. These temperatures can be attained using cold packs, wet ice, foam refrigerant, or other coolants. Specimens should be processed and tested as soon as possible but may be stored at 2°C to 8°C for up to 72 hours 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 for cell culture isolation.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times stated in the following procedure to ensure that accurate results are obtained.
- When staining with fluorescent reagents and examining cells microscopically for fluorescence, include both positive and negative controls, to monitor the procedure and performance of the reagents. Run controls with each batch of patient specimens.
- Place the closed, humidified slide chamber in the incubator for equilibration to 35°C to 37°C prior to staining. By doing so, the test slides and reagents will come to temperature more quickly, yielding more rapid, intense staining in shorter periods of time.
- Bring DFA Reagents to room temperature prior to use, and immediately return to refrigerator after use for storage at 2°C to 4°C.

Regarding Cell Culture Testing

- When staining cell monolayers in polystyrene multi-well plates, dilute the acetone fixative to 80% by adding 20 mL of water to 80 mL of acetone. See *REAGENT PREPARATION*.
- Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased assay sensitivity.
- Do not allow the antibody 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 a control fails to perform as expected, review the steps and conditions under which the test was performed to determine the root cause(s) of failure. Do not report results for patient samples unless controls perform as expected.
- Three aspects of the fluorescence microscope that must be functioning properly and optimally 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 Reagents. Change the fluorescent bulb according to the manufacturer's recommendations.
- The light source is focused by a number of lenses and mirror(s). For maximum intensity, ensure that the lenses and mirrors are properly aligned.
- The filters used in the light path must be appropriate for fluorescein.

- Several fluorescent artifacts may be observed in the cell monolayers.
  - Cell debris, lint, etc. can non-specifically adsorb DFA Reagents, resulting in highly intense fluorescence. Staining artifacts do not have the appearance of a complete cell and typically do not appear to be on the plane of the monolayer.
  - Intense fluorescence around the periphery of slide wells indicates drying of the DFA Reagent, suggesting that incubation was too long or the humidity was not controlled.
  - Non-specific fluorescence caused by adsorption of DFA Reagents trapped by inadequate removal of mucus from direct specimens.
  - Low grade fluorescence due to insufficient washing with residual DFA Reagents remaining on the cell monolayer.
  - 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.
- Protect stained 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 microscopically examined for an extended period.

Specimen Preparation

For specimen processing recommendations, refer to CLSI Approved Viral Culture Guidelines.[15]

Direct Specimen Testing

1. Spot 25 µL of a prepared cell suspension[16] onto 2 wells of a 2-well slide and 8 wells of an 8-well slide. Repeat this process for each specimen.
2. Air dry the slides completely.
3. Fix the cells to the slides using fresh, chilled acetone for 5 to 10 minutes at room temperature (20°C to 25°C).
4. Remove the slides from the fixative and allow to air dry.
   CAUTION: Acetone is volatile and flammable; keep away from open flames.
5. Add one drop of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to completely cover the dried, fixed cells on one of the two wells of each 2-well slide. Reserve the prepared 8-well slide for subsequent virus identification (see Step #16).
6. Add one drop of the Normal Mouse Gamma Globulin DFA Reagent to completely cover the dried, fixed cells on the second well of each 2-well slide.
7. Add one drop of D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to each well of a fresh Respiratory Virus Antigen Control Slide. An Antigen Control slide should be stained only once. Do not re-stain.
8. Place the slides in a pre-warmed (See *Preliminary Comments and Precautions*), humidified, covered chamber or a humidified incubator at 35°C to 37°C for 15 to 30 minutes.
9. Rinse the stained slides using the 1X Wash Solution. For only a few slides, this can be done using a wash bottle, beaker, or Coplin jar of 1X Wash Solution. For many slides, a slide carrier that holds 10 to 20 slides can be placed in a 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 solution and repeat the washing step using fresh 1X Wash Solution.

11. Rinse the stained slides using de-mineralized water. For only a few slides, this can be done using a wash bottle, beaker, or Coplin jar of de-mineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in a 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 from the slides.
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 slides using a fluorescence microscope with 200X to 400X-magnification (See *Regarding Immunofluorescence Microscopy*).
15. Refer to *INTERPRETATION OF RESULTS*.
16. If the result is positive for a respiratory virus other than influenza A, you can identify the virus using individual identification reagents from Quidel's D^3 Ultra Kit. Use the reserved, 8-well specimen slide (see Step #1) with the D^3 Ultra Kit as follows:
    - Add one drop of each individual virus DFA Reagent to a corresponding well on the 8-well specimen slide. Stain one well as a reagent control with the Normal Mouse Gamma Globulin DFA Reagent.
    - Add one drop of each individual virus DFA Reagent to its corresponding labeled well on the Respiratory Virus Antigen Control Slide.
    - Stain the negative well with the Normal Mouse Gamma Globulin DFA Reagent.
      NOTE: Stain control slides only one time. Do not re-stain.
17. Continue with steps 8 through 15.

Cell Culture Testing – Tube Culture

1. Examine the monolayers for confluency and proper cell morphology prior to inoculation.
2. Aspirate maintenance medium from the monolayers and add 0.2 to 0.5 mL of prepared specimen to each of the cell lines used for respiratory virus isolation.
3. Place the tubes at an angle sufficient for the monolayers to be covered by the inoculum, and allow virus adsorption to occur for 1 hour at 35°C to 37°C.
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 cytopathic effect (CPE) or test for hemadsorption. Monolayers exhibiting CPE or hemadsorption should be processed as follows.
6. Remove the medium by aspiration and gently rinse the monolayer 2 times with 1 to 2 mL PBS.
7. Add 0.5 mL of fresh PBS to the tube and prepare a suspension of the cells by scraping the monolayer using a pipette and by pipetting up and down several times to break up cell aggregates.
8. Prepare cell spots using about 25 µL of the suspension on each well of a 2-well and 8 wells of an 8-well slide. Repeat this step for each specimen.
9. Air dry the slides completely.
10. Fix the cells to the slides using fresh, chilled acetone 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 D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to completely cover the dried, fixed cells on one of the two wells of each 2-well slide. Reserve the prepared 8-well slide for subsequent virus identification if needed (see Step #22).
13. Add one drop of D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to each well of a fresh Respiratory Virus Antigen Control Slide. An Antigen Control Slide should be stained only once. Do not re-stain.
14. Place the slides in a pre-warmed (see *Preliminary Comments and Precautions*), humidified, covered chamber or in a humidified incubator at 35°C to 37°C for 15 to 30 minutes.
15. Rinse the stained slides using the 1X Wash Solution. For only a few slides, this can be done using a wash bottle, beaker, or Coplin jar 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 fresh 1X Wash Solution.
17. Rinse the stained slides using de-mineralized water. For only a few slides, this can be done using a wash bottle, beaker, or Coplin jar of the de-mineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in a container with de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
18. Gently blot the excess de-mineralized water from the slides.
19. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
20. Examine the stained, mounted slides using a fluorescence microscope with 200X to 400X magnifications (See *Regarding Immunofluorescence Microscopy*).
21. Refer to *INTERPRETATION OF RESULTS*.
22. If the result is positive for a respiratory virus other than influenza A (i.e., apple-green fluorescent cells), you can identify the virus using individual identification reagents from Quidel's D^3 Ultra Kit. Use the reserved 8-well specimen slide (see Step #1) with the D^3 Ultra Kit as follows:
    - Add one drop of each individual virus DFA Reagent to a corresponding well on the 8-well specimen slide. Stain one well as a reagent control with the Normal Mouse Gamma Globulin DFA Reagent.
    - Add one drop of each individual virus DFA Reagent to its corresponding labeled well on the Respiratory Virus Antigen Control Slide.
    - Stain the negative well with the Normal Mouse Gamma Globulin DFA Reagent.
      NOTE: Stain control slides only one time. Do not re-stain.
    - Continue with steps 8 through 15.

Cell Culture Testing – Shell-Vial

1. Calculate the number of vials needed based on your laboratory's preferred staining protocol. (i.e., this staining protocol requires 3 vials.)
    - One shell-vial is required for each day the culture will be screened with the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent (e.g., staining one shell-vial at 16 to 24 hours and a second shell-vial at 48 to 72 hours requires 2 vials).
    - One additional shell-vial is required to identify viruses other than influenza A. An 8-well slide is prepared to identify the viruses from positive screens (apple-green fluorescent cells) using the D^3 Ultra identification reagents (see Step #23).
2. Examine the monolayers for confluency and proper cell morphology prior to inoculation.
3. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate re-feed medium to each shell vial.
4. Add 0.2 to 0.4 mL of prepared specimen or control virus(es) 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. Remove the medium by aspiration after the desired incubation period and add 1 mL of PBS to each vial.
8. Swirl to rinse and aspirate.
9. Add 1 mL of chilled 100% acetone and fix for 5 to 10 minutes at 20°C to 25°C.
   CAUTION: Acetone is volatile and flammable; keep away from open flames.
10. Remove the acetone-fixative by aspiration.
11. Add 0.5 mL of PBS to wet the monolayer.
12. Swirl and aspirate completely.
13. Deliver 4 drops of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to the fixed monolayers of patient and control samples, and rock to ensure complete coverage of the monolayer by the Reagent.
14. Place stoppered shell vials in a 35°C to 37°C incubator for 15 to 30 minutes.
15. Aspirate the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent from the monolayers.

16. Add 1 mL of the 1X Wash Solution to wash.
17. Remove the 1X Wash Solution by aspiration; repeat the wash step using 1 mL fresh Wash Solution and remove by aspiration.
18. Add 0.5 to 1 mL of de-mineralized water.
19. Lift the coverslip from the bottom of the shell-vial using a bent-tip needle on a syringe barrel. Grasp the cover slip with the fine tipped forceps and transfer it, monolayer-side down, to a small drop of mounting fluid on a standard, previously cleaned, microscope slide.
20. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*.)
21. Refer to *INTERPRETATION OF RESULTS*.
22. If the result is positive for respiratory virus other than influenza A, you can identify the virus by using individual identification reagents from Quidel's D^3 Ultra Kit. Process the reserved shell-vial as a cell suspension to prepare an 8-well specimen slide. See steps 6 through 11 under *Cell-Culture Testing – Tube Culture*. Stain with D^3 Ultra DFA reagents as follows:
    - Add one drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Stain one well as a reagent control with the Normal Mouse Gamma Globulin DFA Reagent.
    - Add one drop of each individual virus DFA Reagent to its corresponding labeled well on the Respiratory Virus Antigen Control Slide.
    - Stain the negative well with the Normal Mouse Gamma Globulin DFA Reagent.
      NOTE: Stain Antigen Control Slides only one time. Do not re-stain.
    - Continue with steps 14 through 21 from *Cell-Culture Testing – Tube Culture*.

Cell Culture Testing – Multi-Well Plate

1. Calculate the number of wells needed for the staining based on your laboratory's preferred protocol. (i.e., this staining protocol requires 3 wells)
    - One well is required for each day the culture will be screened with the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent (e.g., staining one well at 16 to 24 hours and a second well at 48 to 72 hours, requires 2 wells).
    - One additional well is required to identify viruses other than influenza A. An 8-well slide is prepared to identify the viruses from positive FITC screens using the D^3 Ultra DFA reagents (Step #23 Under *Cell-Culture Testing – Shell Vial*).
      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 confluency and proper cell 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 or control virus(es) to the appropriate well 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 atmosphere of 5% CO_2.
7. Remove the medium by aspiration after the desired incubation period and add 1 mL of PBS.
8. Swirl to mix and aspirate.
9. Repeat this wash with another 1 mL of PBS and aspirate.
10. Add 1 mL of 80% aqueous acetone and fix for 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 aspirate completely.

14. Add 4 drops of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent to the fixed monolayers of patient and control samples in each well of a 24-well multi-well plate; add 3 drops of the 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 D^3 Duet DFA Influenza A/Respiratory Virus 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. Add 2 to 3 drops of Mounting Fluid to each well and cover the monolayer.
22. Examine the stained monolayers using a fluorescence microscope with 100X to 400X magnification. (See *Regarding Immunofluorescence Microscopy.*)
23. Refer to *INTERPRETATION OF RESULTS*.
24. If the result is positive for a respiratory virus other than influenza A and you wish to identify the virus using the D^3 Ultra Kit, process a reserved replicate culture well as a cell suspension and spot onto an 8-well specimen slide (see steps 6 through 11 under *Cell-Culture Testing – Tube Culture* for procedure to prepare a specimen slide). Stain with the D^3 Ultra individual DFA Reagents as follows:
    - Add one drop of each individual virus I Reagent to its corresponding well on the 8-well specimen slide. Stain one well as a reagent control with the Normal Mouse Gamma Globulin Reagent.
    - Add one drop of each individual virus Reagent to its corresponding labeled well on the Respiratory Virus Antigen Control Slide.
    - Stain the negative well with the Normal Mouse Gamma Globulin Reagent.
      NOTE: Stain Antigen Control Slides only one time. Do not re-stain.
    - Continue with steps 15 through 23 under *Cell-Culture Testing – Tube Culture*.

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 wells positive for influenza A will show multiple infected cells of bright yellow fluorescence with negative cells staining a dull red due to the Evans Blue counter-stain. Cells infected with respiratory viruses other than influenza A will show specific green fluorescence in the nucleus and/or cytoplasm with negative cells staining dull red due to the 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 in the direct specimens 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 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, an influenza-inoculated control monolayer should be included each time a new lot of cell culture is used.
- An un-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 for influenza A virus with the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent is one in which golden-yellow fluorescence is observed in the cytoplasm, nucleus or in both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.
- Green fluorescence observed in the cytoplasm and/or nucleus is suggestive of infection with a respiratory virus other than influenza A virus. (Further identification may be achieved with the D^3 Ultra Kit).
- 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 to limit 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" fluorescence staining pattern for each virus is as follows:

- Influenza A virus – The golden-yellow fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctated with large inclusions while nuclear staining is uniformly bright.
  NOTE: The staining patterns described below are for reference only. Identification of the viral antigens present in the apple-green fluorescent cells must be performed using another FDA cleared device (such as the D^3 Ultra DFA).
  - Influenza 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 influenza A virus and other viruses has been reported in a number of studies. The presence of multiple viruses is indicated when a stained slide well exhibits both golden-yellow and apple-green fluorescent cells.

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, golden-yellow or 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".
- If golden-yellow fluorescent cells are found, it should be reported as "influenza A viral antigens detected by direct specimen testing."
- If only apple-green fluorescent cells are found, the identification of the virus(es), other than influenza A may be based on the follow-up assay (e.g. individual DFA Reagents in the D^3 Ultra Kit). In such cases, identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according to the respective device's instructions.
- If co-infection of influenza A virus and one of the other respiratory viruses is detected (exhibited by both golden-yellow and apple-green fluorescent cells present) the identification of the virus(es), other than influenza A, may be based on the follow-up assay, such as individual DFA Reagents in the Quidel D^3 Ultra Kit (not provided). In such cases, it should be reported as "Influenza A viral *and* identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device's instructions.

Results from Culture Isolation/Confirmation

- The entire cell spot or monolayer of cells must be examined for virus-infected, golden-yellow or 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 golden-yellow fluorescent cells are found, it should be reported as "influenza A virus isolated in cell culture".
- If only apple-green fluorescing cells are found, the identification of the virus(es), other than influenza A, may be based on the follow-up assay (such as individual DFA Reagents in the D^3 Ultra Kit). In such cases, identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device's instructions.
- If co-infection of influenza A virus and one of the other respiratory viruses is detected (exhibited by both golden-yellow and apple-green fluorescent cells present) the identification of the virus(es), other than influenza A, may be based on the follow-up assay, such as individual DFA Reagents in the D^3 Ultra Kit (not provided). In such cases, it should be reported as "Influenza A viral *and* identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device's instructions.

LIMITATIONS OF THE PROCEDURE

- Inappropriate specimen collection, storage, and transport may lead to false negative culture results.[17]
- Assay performance characteristics for direct staining have not been established for respiratory specimens other than nasal/nasopharyngeal swabs and aspirates. Data supporting the use of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit for direct testing of other respiratory specimens is limited and it is the user's responsibility to establish assay performance parameters.
- 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 reproducibility study indicates a potential for non-detection of low levels of influenza A virus infected cells (golden-yellow fluorescent cells) in the presence of moderate respiratory syncytial virus infected cells (apple-green fluorescent cells). During the clinical studies two influenza A virus/ respiratory syncytial virus co-infections were detected by the D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit. No influenza A virus/ respiratory syncytial virus co-infections were missed by the D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit during the clinical studies. The effects of antiviral therapy on the performance of this kit have not been established.
- The monoclonal antibodies 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 monoclonal antibodies have been prepared using defined virus strains, they may not detect all antigenic variants or new strains of the viruses, should they arise. Monoclonal antibodies may fail to detect strains of viruses which have undergone minor amino acid changes in the target epitope region.
- The viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture. This is particularly true for RSV which is known for its instability and loss of viability.
- A negative *direct* specimen should be inoculated into appropriate cell culture(s) and incubated to isolate and identify any respiratory viruses 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 be assured only when components used in the assay are those supplied by Quidel.
- Prolonged storage of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent under bright light will decrease the staining intensity. Stained slides left under UV light will lose their fluorescence. Remove slide from fluorescence microscope stage as soon as possible.
- Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will bind to the Fc portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, for example, *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots. Therefore, results from cell cultures with bacterial contamination must be interpreted with caution.

EXPECTED VALUES

Prevalence of the respiratory viruses within this population during the 2006/2007 season is noted in Table 2 below.

Table 2. Respiratory Virus Prevalence 2006/2007 Fresh Specimens (n = 1184)

	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
No. of positives	52	99	11	4	1	19	300
Prevalence	4.4%	8.4%	0.9%	0.3%	0.08%	1.6%	25.3%

*Five co-infections were detected (0.4%): 1-influenza A virus/parainfluenza virus type 3, 2-influenza A virus/ respiratory syncytial virus, 1-adenovirus/respiratory syncytial virus, 1-respiratory syncytial virus/ parainfluenza virus type 3.

SPECIFIC PERFORMANCE CHARACTERISTICS

Direct Fresh Specimens

A study was performed prospectively at three sites with 1203 fresh specimens that were received for respiratory virus testing. Each specimen was evaluated by the D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit and a cleared DS-FA (direct specimen fluorescent antibody) device (D^3 Ultra DFA Respiratory Virus Screening and ID Kit) for the presence of influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 in cells derived from clinical specimens.

Study Site 1

Evaluated a total of 575 fresh specimens submitted, February through March, 2007, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in this product insert.

Table 3 shows the age distribution for individuals studied at Site 1.

Table 3. Site 1 – Age Distribution

0 to 1 month	47
> 1 month to 2 years	379
> 2 to 12 years	131
> 12 to 21 years	18
Total	**575**

Study Site 2

Evaluated a total of 300 fresh specimens submitted, January through February, 2007, to the laboratory for respiratory virus testing. Slides were prepared from PBS-washed cells from the fresh specimens and fixed in accordance with the procedure in this product insert. The slides were stored at –70°C until testing was performed. With the fixation in acetone prior to freezing, the cellular morphology is maintained during freezing and on thawing.

Table 4 shows the age distribution for individuals studied at Site 2.

Table 4. Site 2 – Age Distribution

0 to 1 month	7
>1 month to 2 years	130
>2 to 12 years	49
>12 to 21 years	16
22 to 30 years	13
31 to 40 years	13
41 to 50 years	20
51 to 60 years	11
61 to 70 years	8
71 to 80 years	9
81 to 90 years	19
91 to 100 years	4
Unknown age	1
Total	**300**

Study Site 3

Evaluated a total of 328 fresh specimens submitted, January through February, 2007, to the laboratory for respiratory virus testing. Slides were prepared from PBS-washed cells from the fresh specimens and fixed in accordance with the procedure in this product insert. The slides were stored at –70°C until testing was performed.

Upon staining, 4 specimens were found to contain insufficient numbers of cells for interpretation and 5 specimens were found to be un-interpretable due to high background. These 9 specimens along with 10 specimens (9 deviated and 1 duplicate) were removed from analysis total of 309 specimens remained.

Table 5 shows the age distribution for individuals studied at Site 3.

Table 5. Site 3 – Age Distribution

0 to 1 month	37
>1 month to 2 years	200
>2 to 12 years	49
>12 to 21 years	3
22 to 30 years	5
31 to 40 years	3
41 to 50 years	4
51 to 60 years	2
61 to 70 years	2
71 to 80 years	2
81 to 90 years	1
Unknown age	1
Total	**309**

A total of nineteen specimens were excluded from analysis due to site deviations, duplicate specimen, insufficient cell numbers, or high background. These exclusions left 1184 specimen results for analysis.

Table 6 below summarizes the participant age demographics according to test results for a population of 1184 fresh specimens, prospectively collected and evaluated for performance using the Comparator test.

Table 6. Participant Age Demographics

Virus	**Adenovirus**	**Influenza A**	**Influenza B**	**Parainfluenza 1**	**Parainfluenza 2**	**Parainfluenza 3**	**Respiratory Syncytial Virus**	**Negative**
Totals† / **Age***	**52**	**99**	**11**	**4**	**1**	**19**	**300**	**704**
< 1m*	0	1	0	0	0	1	26	67
1m to 2y	35	46	4	4	1	16	237	358
2y to12y	16	36	6	0	0	0	31	147
12y to 21y	1	7	0	0	0	1	0	24

Virus	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus	Negative
Totals† / Age*	52	99	11	4	1	19	300	704
21y to 30y	0	1	0	0	0	0	0	20
31 to 40y	0	1	0	0	0	0	1	17
41 to 50y	0	4	1	0	0	0	0	19
51 to 60y	0	2	0	0	0	0	0	12
61 to 70y	0	0	0	0	0	0	0	10
71 to 80y	0	0	0	0	0	0	2	8
81 to 90y	0	1	0	0	0	1	1	17
91 to 100y	0	0	0	0	0	0	0	4
Age Not reported	0	0	0	0	0	0	2	0

*Age: m = months, and y = years
†Due to 6 co-infections the total adds up to 1190

The following tables detail the summary of the comparison of the D^3 Duet and the cleared DS-FA comparator assay D^3 Duet's phycoerythrin-labeled MAbs identification of influenza A virus positive specimens (Table 7), D^3 Duet's fluorescein-labeled MAbs detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses positive specimens (Table 8), and Virus identification of D^3 Duet FITC Positive Specimens using D^3 Ultra Identification Reagents (Table 9), combined for study Sites 1, 2, and 3.

Table 7. D^3 Duet R-PE Identification of Influenza A Virus Positive Specimens

Direct Specimen (1184 Specimens)		D^3 Ultra Final Identification (influenza A virus)	
		Pos	Neg
D^3 Duet R-PE (influenza A virus)	Pos	99	0
	Neg	1	1084
Positive Percent Agreement (PPA)*		99% (99/100)	–
95% CI- PPA**		94.5, 99.8%	
Negative Percent Agreement (NPA)***		–	100% (1084/1084)
95% CI- NPA			99.7, 100%

*"Positive Percent Agreement", or "PPA", values were calculated according to {[(Total Number of Positive Results in Agreement by both Subject and Predicate Tests) divided by [(Total Number of Positive Results in Agreement by both Subject and Predicate Tests) plus (Number of Results Positive by Predicate but Negative by Subject)]} multiplied by 100%.
**"95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).
***"Negative Percent Agreement", or "NPA", values were calculated according to {[(Total Number of Negative Results in Agreement by both Subject and Predicate Tests) divided by [(Total Number of Negative Results in Agreement by both Subject and Predicate Tests) plus (Number of Results Negative by Predicate but Positive by Subject)]} multiplied by 100%.

Table 8. D^3 Duet FITC Detection of Influenza B Virus, Respiratory Syncytial Virus, Adenovirus, and Parainfluenza Virus Types 1, 2, & 3 Viruses

Direct Specimen (1184 Specimens)		D^3 Ultra Final Identification	
		Pos	Neg
D^3 Duet FITC Screen	Pos	386	0
	Neg	0	798*
Positive Percent Agreement (PPA)		100% (386/386)	–
95% CI- PPA		99.0, 100%	
Negative Percent Agreement (NPA)		–	100% (798/798)
95% CI- NPA			99.5, 100%

*One specimen was screen positive by both devices, but was unable to be identified with the identification reagents.

Table 9. Virus Follow-up Identification of 386 D^3 Duet FITC Positive Specimens for Influenza B Virus, Respiratory Syncytial Virus, Adenovirus, and Parainfluenza Virus Types 1, 2, & 3 Viruses, Using D^3 Ultra Identification Reagents

Virus	**Sensitivity**		**95% CI**	**Specificity**		**95% CI**
	TP/(TP+FN)	**%**	**for Sensitivity**	**TN/(TN+FP)**	**%**	**for Specificity**
Influenza B virus	11/11	100	74.1, 100	1173/1173	100	99.7, 100
Adenovirus	52/52	100	93.1, 100	1132/1132	100	99.7, 100
Parainfluenza type 1	4/4	100	51.0, 100	1180/1180	100	99.7, 100
Parainfluenza type 2	1/1	100	20.1, 100	1183/1183	100	99.7, 100
Parainfluenza type 3	19/19	100	83.2, 100	1165/1165	100	99.7, 100
Respiratory Syncytial Virus	299/299	100	98.7, 100	885/885	100	99.6, 100

The D^3 Duet's ability to identify influenza A virus using phycoerythrin in direct specimens was compared to the D^3 Ultra's ability using fluorescein. The positive percent agreement was 99% (95% CI range of 94.5% to 99.8%). The negative percent agreement was 100% (95% CI range of 99.7% to 100%). When the ability of the D^3 Duet to detect the six other respiratory viruses using fluorescein in direct specimens was compared to the D^3 Ultra's ability using fluorescein, the positive percent agreement was 100% (95% CI range of 99.0-100%). The negative percent agreement was 100% (95% CI range of 99.5% to 100%).

Cultured Specimens

To evaluate the performance of this device using cultured clinical specimens, a fourth study was performed with 298 frozen specimens to compare performance of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Kit with that of the D^3 Ultra DFA Respiratory Virus Screening and ID Kit for the presence of Influenza A, Influenza B, Respiratory Syncytial Virus, Adenovirus, Parainfluenza 1, Parainfluenza 2 and Parainfluenza 3 (Para 3) from cultured clinical specimens. The results of this study are presented below.

Study Site 4

Two hundred and ninety-eight (298) frozen specimens were processed for cell culture testing in accordance with the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices) using R-Mix Too FreshCells™ in 48/24-fill multi-well plates. All specimens at study Site 4 were derived from nasopharyngeal specimens.

Table 10 shows the age distribution for individuals studied at Site 4.

Table 10. Site 4 – Age Distribution

0 to 1 month	5
> 1 month to 2 years	130
> 2 to 12 years	44
> 12 to 21 years	28
22 to 30 years	19
31 to 40 years	20
41 to 50 years	10
51 to 60 years	9
61 to 70 years	8
71 to 80 years	6
81 to 90 years	8
> 90 years	5
Unknown age	6
Total	**298**

The following tables detail the results of the cell culture study's comparison of D^3 Duet's phycoerythrin-labeled MAbs identification of influenza A virus positive specimens (Table 11), and D^3 Duet's fluorescein-labeled MAbs detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 positive specimens (Table 12).

Table 11. Study Site 4 – D^3 Duet R-PE Identification of Influenza A Virus Positive Specimens

Cell Culture (298 Specimens)		D^3 Ultra Final Identification (influenza A virus)	
		Pos	Neg
D^3 Duet R-PE (influenza A virus)	Pos	67	0
	Neg	0	231
Positive Percent Agreement (PPA)		100% (67/67)	–
95% CI- PPA		94.6, 100%	
Negative Percent Agreement (NPA)		–	100% (231/231)
95% CI- NPA			98.4, 100%

Table 12. Study Site 4 – D³ Duet FITC Detection of Influenza B Virus, Respiratory Syncytial Virus, Adenovirus, and Parainfluenza Virus Types 1, 2, & 3 Viruses

Cell Culture (298 Specimens)		D³ Ultra Final Identification	
		Pos	Neg
D³ Duet FITC Screen	Pos	72	0
	Neg	0	226
Positive Percent Agreement (PPA)		100% (72/72)	–
95% CI- PPA		95.0, 100%	
Negative Percent Agreement (NPA)		–	100% (226/226)
95% CI- NPA			98.4, 100%

A variety of viral respiratory pathogens were isolated: influenza A virus [prevalence 22.5% (67/298)], influenza B virus [prevalence 6.7% (20/298)], respiratory syncytial virus [prevalence 11.1% (33/298)], adenovirus [prevalence 3.4% (10/298)], parainfluenza type 1 virus [prevalence 1.7% (5/298)], parainfluenza type 2 virus [prevalence 1.0% (3/298)], and parainfluenza type 3 virus [prevalence 3.0% (9/298)].

There were sixteen co-infections as follows: three (3) influenza A virus + parainfluenza type 3 virus, one (1) influenza A virus + parainfluenza type 1 virus, one (1) influenza A virus + parainfluenza type 2 virus, two (2) influenza A virus + respiratory syncytial virus, one (1) influenza A virus + adenovirus, one (1) influenza B virus + parainfluenza type 2 virus, one (1) influenza B virus + parainfluenza type 3 virus, one (1) influenza B virus + respiratory syncytial virus, one (1) respiratory syncytial virus + parainfluenza type 1 virus, two (2) respiratory syncytial virus + parainfluenza type 3 virus, one (1) adenovirus + parainfluenza type 1 virus and one (1) adenovirus + parainfluenza type 3 virus.

Specimen Type Distribution

Unlike other methodologies for the detection of viral antigens, the immunofluorescent microscopic technique allows the microscopist to visualize the specimen being tested. This allows the microscopist to determine whether the specimen is adequate, i.e., in the case of most respiratory viruses the presence of columnar epithelial cells, contains an adequate number of cells for analysis and the fluorescence pattern is appropriate for the virus(es) being detected. This should be taken into consideration when assessing the impact of the method of specimen collection, i.e. swabs or aspirates, on performance of the device.

Tables 13 and 14 below show the study results by the specimen type. Results from Sites 1, 2, and 3 have been combined.

Table 13. Influenza A Virus by Specimen Type; Study Sites 1, 2, 3 Combined

Specimen type	PPA		95% CI for PPA	NPA		95% CI for NPA
	TP/(TP+FN)	%		TN/(TN+FP)	%	
NPA	61/62	98.4	91.4, 99.7	525/525	100	99.3, 100
NPS	38/38	100	90.8, 100	501/501	100	99.2, 100
Sputum	0/0	–	–	19/19	100	83.2, 100
BAL	0/0	–	–	14/14	100	78.5, 100
Tracheal aspirates	0/0	–	–	13/13	100	78.5, 100
Throat swab	0/0	–	–	7/7	100	64.6, 100
Unknown	0/0	–	–	5/5	100	64.6, 100

Table 14. D^3 Duet FITC Detection of Influenza B Virus, Respiratory Syncytial Virus, Adenovirus, and Parainfluenza Virus Types 1, 2, & 3 Viruses by Specimen Type; Study Sites 1, 2, and 3 Combined

Specimen type	PPA		95%CI for PPA	NPA		95% CI for NPA
	TP/(TP+FN)	%		TN/(TN+FP)	%	
NPA	196/196	100	98.1, 100	391/391	100%	99.0, 100
NPS	173/173	100	97.8, 100	366/366	100%	99.0, 100
Sputum	3/3	100	43.9, 100	16/16	100%	80.6, 100
BAL	7/7	100	64.6, 100	7/7	100%	64.6, 100
Tracheal aspirates	0/0	–	–	13/13	100%	78.5, 100
Throat swab	4/4	100	51.0, 100	3/3	100%	43.9, 100
Unknown	3/3	100	43.9, 100	2/2	100%	34.2, 100

Cross-Reactivity Testing

The D^3 Duet Influenza A/Respiratory Virus Screening Kit was tested for cross-reactivity against a variety of cells and microorganisms. Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent and relatively high titers of microorganisms. The D^3 Duet DFA Influenza A/Respiratory Virus Screening Reagent was prepared at 1.5X the concentration that is provided in the kit. No cross-reactivity was observed for 32 virus strains or for 17 host culture cell types. Twenty-five (25) bacterial strains, one yeast, three *Chlamydia sp.* and one protozoan were evaluated 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*). [See Tables 15, 16 and 17 below for cross-reactivity study results.]

Thirty-two (32) virus strains were tested for cross reactivity. Depending on the particular virus, 71 to 1,400 $TCID_{50}$ were inoculated into shell-vial culture and incubated for 24 to 48 hours, to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFA Reagent according to the procedure as detailed in this product insert. No cross reactivity was observed for the viruses listed below (Table 15).

Table 15. Virus Strains Tested for Cross Reactivity with D³ Duet DFA Influenza A/Respiratory Virus Screening Reagent

Organism	Strain or Type	Inoculum ($TCID_{50}$)	Organism	Strain or Type	Inoculum ($TCID_{50}$)
Parainfluenza 4a	M-25, VR-1378	1,400	CMV	Towne, VR-977	430
Parainfluenza 4b	CH19503, VR-377	1,400		Davis, VR-807	430
Metapneumovirus	Subgroup A1	1,400		AD169, VR-538	430
	Subgroup A2	1,400	Varicella-zoster	Webster, VR-916	430
	Subgroup B1	1,400		Ellen, VR-1367	430
	Subgroup B2	1,400	Rhinovirus 39	209 Picornavirus, VR-340	1,400
Coronavirus	OC43, VR-1558	1,400	Rubeola	–	Commercially available slides stained*
	229E, VR-740	1,400	Mumps		
HSV-1	1F, VR-733	71	Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained*
	MacIntyre, VR-539	71	Coxsackievirus	Types B1, B2, B3, B4, B5, B6	
HSV-2	MS, VR-540	71	*Test material was from commercially available prepared slides. Each positive well contained 10 to 50% reactive cells.		
	Strain G, VR-734	71			

Seventeen (17) host culture cell types were tested for cross reactivity. Cell cultures were prepared in shell-vial format. Confluent monolayers were stained with the 1.5X preparation of the D³ Duet DFA Influenza A/Respiratory Virus Screening Reagent according to the procedure as detailed in this product insert, and then examined for cross reactivity. No cross reactivity was observed for the following (Table 16).

Table 16. Cell lines Tested for Cross Reactivity with D³ Duet DFA Influenza A/Respiratory Virus Screening Reagent

A549	monolayer	pCMK	cell spot
BGMK	monolayer	pRhMK	cell spot
HEp-2	monolayer	RD	monolayer
LLC-MK2	monolayer	RhMK II	cell spot
MDCK	monolayer	pRK	monolayer
MRC-5	monolayer	R-Mix	monolayer
MRHF	monolayer	Vero	cell spot
Mv1Lu	monolayer	WI-38	cell spot
NCI-H292	monolayer		

Thirty (30) microorganisms, including 25 bacterial and one yeast cultures, three *Chlamydia sp.* and one protozoan commercially available slides, were stained with the 1.5X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross reactivity. Except for *Staphylococcus aureus*, which was cross reactive with the D³ Duet DFA Influenza A/Respiratory Virus Screening Reagent, all other microorganisms tested negative. Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*. Concentrations for each bacterial organism cultured by Quidel for cross reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of levels 1.0 and 2.0 (equaling approximately 3.0×10^6 and 6.0×10^6 CFU per mL). Slides were prepared with spots of 0.01 mL of the suspensions to give either 3.0×10^4 or 6.0×10^4 per spot. At the same time, 1 mL of each suspension was plated on an appropriate agar dish for colony

confirmation. According to the confirmation agar cultures, initial concentrations of the bacterial organisms in the study ranged from 6.4 x 10^4 to 2.9 x 10^7 CFU. Microorganisms tested are listed below (Table 17).

Table 17. Microorganisms Tested for Cross Reactivity with D³ Duet DFA Influenza A/Respiratory Virus Screening Reagent

MICROORGANISM	CFU TESTED
Bacteria	
Acholeplasma laidlawii	~6 x 10^7
Acinetobacter calcoaceticus	9.7 x 10^5
Bordetella bronchiseptica	1.7 x 10^5
Bordetella pertussis	4.6 x 10^6
Corynebacterium diphtheriae	2.5 x 10^6
Escherichia coli	2.6 x 10^5
Gardnerella vaginalis	5.0 x 10^5
Haemophilis influenzae type A	9.3 x 10^5
Klebsiella pneumoniae	6.4 x 10^6
Legionella pneumophila	6.5 x 10^4
Moraxella cartarrhalis	6.4 x 10^4
Mycoplasma hominis	~6 x 10^4
Mycoplasma orale	~6 x 10^4
Mycoplasma pneumoniae	~6 x 10^4
Mycoplasma salivarium	~6 x 10^7
Neisseria gonorrhoeae	1.3 x 10^6
Proteus mirabilis	2.1 x 10^6
Pseudomonas aeruginosa	1.0 x 10^7
Salmonella enteriditis	2.5 x 10^6
Salmonella typhimurium	1.8 x 10^6
Staphylococcus aureus*	1.0 x 10^7
Streptococcus agalactiae	9.6 x 10^6
Streptococcus pneumoniae	8.0 x 10^5
Streptococcus pyogenes	2.9 x 10^7
Ureaplasma uralyticum	~6 x 10^4
Chlamydophila pneumoniae	Commercially available slides stained.
Chlamydophila psittaci	Commercially available slides stained.
Chlamydia trachomatis	Commercially available slides stained.
Yeast	
Candida glabrata	8.7 x 10^6
Protozoan	
Trichomonas vaginalis	Commercially available slides stained.

*Reactivity with *Staphylococcus aureus* is more than likely due to binding the Protein A produced by *Staphylococcus aureus*.

Analytical Reactivity (Inclusivity)

Analytical reactivity (inclusivity) of the D³ Duet was evaluated using 10 influenza A virus and 4 influenza B virus strains. Four wells of a 96-well cell culture plate were inoculated with each viral strain (diluted to less than 20-$TCID_{50}$[2] per 0.2 mL inoculum). The plates were centrifuged at 700xg for 60 minutes, and then incubated at 35°C to 37°C for 24 hours. Four wells from each strain were stained with the D³ Duet, and each well was then

[2]50% Tissue Culture Infectious Dose

examined at 200X magnification and the number of fluorescent cells counted. Table 18 below lists the virus identity and strain along with the fluorescent cell count.

Table 18. Analytical Reactivity (inclusivity) of D^3 Duet with Various Influenza A Virus and Influenza B Virus Strains (values are numbers of fluorescent cells per cell monolayer)

	Influenza strain	Fluorescent staining cells/cell monolayer
Influenza A	Wisconsin/56/2005	3, 2, 1, 0
	WS, VR-1520 (H1N1)	6, 6, 6, 4
	Hong Kong, VR-544 (H3N2)	3, 4, 5, 5
	New Jersey, VR-897 (H1N1)	9, 12, 14, 15
	Victoria, VR-822 (H3N2)	3, 3, 3, 5
	PR, VR-95 (H1N1)	3, 9, 9, 6
	Port Chalmers, VR-810 (H3N2)	6, 6, 9, 10
	Aichi, VR-547 (H3N2)	3, 7, 9, 11
	Denver, VR-546 (H1N1)	13, 14, 11, 10
	Mal, VR-98 (H1N1)	8, 3, 6, 4
Influenza B	GL/1739/54, VR-103	7, 6, 7, 7
	Taiwan/2/62, VR-295	3, 1, 2, 5
	Hong Kong/5/72, VR-823	3, 2, 0, 1
	Maryland/1/59, VR-296	5, 6, 6, 8

Based on the data presented above, the D^3 Duet can reliably detect influenza A virus and influenza B virus strains exhibiting both temporal and geographical diversity at viral levels near the limit of detection in cell culture.

Analytical Detection Limit

Analytical detection limit for the seven viruses detected by the subject device D^3 Duet was addressed under conditions similar to those described above, with results reported in numbers of fluorescent cells per cell monolayer. Each master stock virus preparation was diluted in a ten-fold manner. Four wells of a 96-well cell culture plate were inoculated with each dilution. The plates were centrifuged at 700xg for 60 minutes, and then incubated at 35°C to 37°C for 24 hours. Four wells from each dilution were stained with the D^3 Duet. Each well was then examined at 200x magnification and the number of fluorescent cells counted. Table 19 below lists the virus identity and strain along with the fluorescent cell count.

Table 19. Analytical Sensitivity of D³ Duet Compared with that of D³ Ultra MAbs (values are numbers of fluorescent cells per cell monolayer)

Virus strain	Virus Dilutions from master stock	Fluorescent staining cells/well	
		D³ Duet	D³ Ultra
Influenza A virus (PR, VR-95 H1N1)	$1x10^{-5}$	1, 3, 2, 6	1, 3, 0, 5
	$1x10^{-6}$	1, 0, 1, 1	0, 0, 1, 0
	$1x10^{-7}$	0, 0, 0, 0	0, 0, 0, 0
Influenza B virus (Hong Kong, VR-823)	$1x10^{-4}$	4, 1, 6, 2	0, 4, 3, 5
	$1x10^{-5}$	1, 0, 1, 1	0, 0, 2, 2
	$1x10^{-6}$	0, 0, 0, 0	0, 0, 0, 0
Adenovirus (Type 8, VR-8)	$1x10^{-6}$	1, 1, 3, 5	1, 3, 2, 4
	$1x10^{-7}$	0, 0, 0, 0	0, 0, 0, 0
RSV (Washington, VR-1401)	$1x10^{-2}$	1, 0, 3, 4	2, 3, 2, 0
	$1x10^{-3}$	0, 1, 1, 0	2, 1, 0, 0
	$1x10^{-4}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 1 (C-35, VR-94)	$1x10^{-4}$	7, 7, 6, 8	9, 8, 4, 6
	$1x10^{-5}$	2, 2, 3, 0	1, 0, 2, 1
	$1x10^{-6}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 2 (Greer, VR-92)	$1x10^{-4}$	4, 0, 3, 1	4, 3, 1, 2
	$1x10^{-5}$	0, 2, 0, 0	0, 1, 1, 1
	$1x10^{-6}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 3 (C 243, VR-93)	$1x10^{-6}$	3, 3, 0, 6	1, 1, 3, 5
	$1x10^{-7}$	1, 0, 1, 1	1, 1, 1, 0
	$1x10^{-8}$	0, 0, 0, 0	0, 0, 0, 0

Reproducibility

Assay precision, intra-assay variability and inter assay variability were assessed with a panel of proficiency-level antigen control slides. The panel consisted of slides spotted with cell preparations of the following:

- Low level influenza A (Victoria strain)
- Mid-level influenza A (Victoria strain)
- Low level influenza A (Victoria strain) mixed with Mid-level RSV (Washington strain)
- Mid-level influenza A (Victoria strain) mixed with Low level RSV (Washington strain)
- Low level respiratory virus (either influenza virus B {Taiwan strain}, adenovirus type 1, Parainfluenza virus types 1, 2, or 3 (strains C35, Greer, C243 respectively). This panel member will be rotated during the 5 days of testing so that each virus is tested twice.
- Negative – no infected cells present

The low level is estimated to contain between 4 to 10% infected cells per cell spot. The mid-level is estimated to contain between 20 to 25% infected cells per cell spot. Both levels were below the level used in quality control slides. Each panel member was re-coded daily to prevent its identification.

Each panel was stained twice per day for 5 days by three different laboratories. The following results were recorded for both the control slide and the panel slide:

- Presence or absence of Yellow-gold fluorescence.
- Percent of cells exhibiting Yellow-gold fluorescence
- Presence or absence of Green fluorescence
- Percent of cells exhibiting Green fluorescence

The combined data for negative specimens – no infected cells present - from the three sites demonstrates that the R-PE labeled and FITC labeled MAbs do not stain non-infected cells in a reproducible manner. No fluorescent cells were seen in 100% (60/60) of the wells lacking infected cells.

The combined data from the three sites demonstrates that the detection of influenza A virus by the R-PE labeled MAbs occurs in a reproducible manner. The presence of influenza A virus infected cells was reported in 95.3% (143/150) of the wells in which the infected cells were expected (Table 20).

Table 20. Influenza A Virus Detection Summary

Positive Control Slide	Low Level Slide	Mid-Level Slide	Low Level with Mid-Level RSV	Mid-Level with Low Level RSV
100% (30/30)	100% (30/30)	100% (30/30)	83.3% (25/30)	93.3% (28/30)

The combined data demonstrates that the detection of respiratory syncytial virus by the FITC labeled MAbs occurs in a reproducible manner. The presence of respiratory syncytial virus infected cells was reported in 100% (90/90) of the wells in which the infected cells were expected (Table 21).

Table 21. Respiratory Syncytial Virus Detection Summary

Positive Control Slide	Low Level Influenza A with Mid-Level RSV	Mid-Level Influenza A with Low Level RSV
100% (30/30)	100% (30/30)	100% (30/30)

The combined data demonstrates that the presence of R-PE fluorescent cells does not interfere with the detection of respiratory syncytial virus by the FITC labeled MAbs in a reproducible manner. The presence of respiratory syncytial virus infected cells was reported in 100% (53/53) of the wells in which the R-PE stained infected cells were present (Table 22).

Table 22. Respiratory Syncytial Virus Detection in the Presence of R-PE Positive Cells Summary

Low Level R-PE stained cells with Mid-Level RSV	Mid-Level R-PE stained cells with Low Level RSV
100% (25/25)	100% (28/28)

The combined data from all three sites demonstrates that the presence of R-PE in the stain does not interfere with the FITC staining of other viruses in a reproducible manner. The presence of influenza B virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of adenovirus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 1 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 2 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 3 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected.

Table 23. Respiratory virus detection in the presence of R-PE Summary

Adenovirus Control Slide	Low Level Adenovirus	Influenza B Virus Control Slide	Low Level Influenza B Virus	Parainfluenza type 1 Control Slide	Low Level Parainfluenza type 1
100% (30/30)	100% (6/6)	100% (30/30)	100% (6/6)	100% (30/30)	100% (6/6)

Parainfluenza type 2 Control Slide	Low Level Parainfluenza type 2	Parainfluenza type 3 Control Slide	Low Level Parainfluenza type 3
100% (30/30)	100% (6/6)	100% (30/30)	100% (6/6)

The reproducibility study data demonstrates that the presence of R-PE in the stain does not interfere with the detection of the 5 respiratory viruses by their respective FITC labeled MAbs in a reproducible manner.

CUSTOMER AND TECHNICAL SUPPORT

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. Bischofberger, N., Webster, R.G. and Laver, G. (1999). Disarming Flu Viruses. *Scientific American,* January.
4. Wiedbrauk, D.L. and Johnston, S.L.G. (1993). Chapter 17, Influenza Virus. In: Manual of Clinical Virology. New York, Raven Press, 127-140.
5. 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.
6. 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.
7. Fete, T.J., Noyes, B. (1996). Common (but not always considered) viral infections of the lower respiratory tract. *Pediatr. Ann.,* **25**:10), 577-584.
8. 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.
9. 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.
10. Falsey, Ann R. and Walsh, E.E. (2000). Respiratory Syncytial Virus Infection in Adults. Clinical Microbiology Reviews 13(3):371-384.
11. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2007, CDC-NIH manual. [http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm]
12. *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/]

13. *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]
14. 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.
15. 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,
16. Isenberg, Henry D., 2004. *Clinical Microbiology Procedures Handbook,* published by American Society for Microbiology, Washington DC, 10.7.1 – 10.7.10
17. Leland, Diane S. (1996). *Clinical Virology,* published by W.B. Saunders, Philadelphia, PA.

REF 01-200000 – D³ Duet DFA Influenza A/Respiratory Virus Screening 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 **PI1630000EN00 (01/19)**

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
 Consult e-labeling instructions for use	 Do not reuse
IVD For *In Vitro* diagnostic use	CONT NaN_3 Contents/Contains
NaN_3 4% Contents/Contains	