Symbols Lexicon/Glossary

- **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.**

- **SUMMARY AND EXPLANATION OF THE TEST**

With the addition of new antiviral drugs for the treatment of influenza, more rapid and sensitive tests for respiratory virus detection are 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 25-35 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.

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 group 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 3.9

### 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.10 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.11 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.12 RSV is also now recognized as a significant problem in certain adult populations. These include the elderly, persons with cardiopulmonary diseases, and immunocompromised hosts.13

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents 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.14
III. PRINCIPLE OF THE PROCEDURE

The Diagnostic Hybrids, Inc. D³ 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 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 antigens. After incubating at 35º to 37ºC, 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. 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 35º to 37ºC, 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.

IV. REAGENTS

A. KIT COMPONENTS

1. Respiratory Virus DFA 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.

2. 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.

3. 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.

4. 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.

5. 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 5-tonsil 99 strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

6. 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.

7. 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.

8. 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.

9. 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 individually packaged.

10. 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.

11. 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).

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

B. WARNINGS AND PRECAUTIONS

For in vitro diagnostic use.

1. 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.

a) Cell culture isolation may have some potential to be hazardous. Personnel working with cell cultures must be properly trained in safe handling techniques11,12,13 and have experience with cell cultures before attempting this procedure.

b) 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.

2. All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.

a) Biosafety Level 2 or other appropriate biosafety practices should be used when handling these materials.

b) Decontamination of specimens and cultures is most effectively accomplished using a solution of sodium hypochlorite (1:10 final dilution of household bleach).

3. 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.

3. Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.

4. Avoid splashing and the generation of aerosols with clinical samples.

5. Use aseptic technique and sterile equipment and materials for all cell culture procedures.

6. 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.

7. Sodium azide is included in the 40X Wash Solution Concentrate at 4%, and in the other solutions in this kit at 0.1%. A MSDS for sodium azide or for Diagnostic Hybrids, Inc. (DHI) reagents containing sodium azide is available by contacting Diagnostic HYBRIDS Technical Services.

T: Sodium azide at very low levels causes damage to health.

R28 Very toxic if swallowed.

R32 Contact with acids liberates very toxic gas.

S45 In case of accident, or if you feel unwell, seek medical advice immediately.

S60 This material and its container must be disposed of as hazardous waste.

N: Sodium azide may present an immediate or delayed danger to one or more components of the environment.

R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

a) Reagents containing sodium azide should be considered poisons. If products containing sodium azide are swallowed,
seek medical advice immediately and show product container, label, or MSDS to medical personnel. (Refer to NIOSH, National Institute for Occupational Safety and Health; CAS# 2628-22-8; EC# 247-852-1; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.)

b) Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.

c) Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with a large volume of water to prevent azide build-up. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.

8. Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.

9. The DFA Reagents are supplied at working strength. Any dilution of the DFA Reagents will decrease sensitivity.

10. Reagents should be used prior to their expiration date.

11. Each Antigen Control Slide and Reagent should be used only once. Do not re-use a Control Slide.

12. Microbial contamination of DFA Reagents may cause a decrease in sensitivity.

13. Store 1X Wash Solution and PBS (Phosphate Buffered Saline) in clean containers to prevent contamination.

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

15. Do not expose DFA Reagents to bright light during staining or storage.

16. Use of other reagents than those specified with the components of this kit may lead to erroneous results.

C. PREPARATION OF 1X WASH SOLUTION

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

2. Add contents of the fully dissolved 25-mL 40X Wash Solution Concentrate to 975-mL of de-mineralized water.

3. Label the 1X Wash Solution with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

D. STORAGE INSTRUCTIONS

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<td>12. 40X Wash Solution Concentrate</td>
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<tr>
<td>NOTE 1: The Concentrate may crystallize when stored at 2° to 8°C. The crystals will dissolve when the Concentrate is warmed to ambient temperature.</td>
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<tr>
<td>13. 1X Wash Solution</td>
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E. 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.

V. 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

A. SPECIMEN COLLECTION

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.

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

Specimens should be stored at 2° 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.

VI. PROCEDURE

A. MATERIALS PROVIDED

1. Respiratory Virus DFA Screening Reagent

2. Influenza A DFA Reagent

3. Influenza B DFA Reagent

4. RSV DFA Reagent

5. Adenovirus DFA Reagent

6. Parainfluenza 1 DFA Reagent

7. Parainfluenza 2 DFA Reagent

8. Parainfluenza 3 DFA Reagent

9. Normal Mouse Gamma Globulin DFA Reagent

10. Respiratory Virus Antigen Control Slides

11. Mounting Fluid

12. 40X Wash Solution Concentrate

B. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.

2. 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 DHI).

3. Coverslips (22 x 50mm) for Antigen Control Slides and for specimen slides.

4. Universal Transport Medium (available from DHI).

5. R-Mix™ Reagent Medium (for use with R-Mix™ and R-Mix Too™ MixedCells™) or other standard reagent medium (available from DHI).

6. Reagent-grade acetone (>99% pure) chilled at 2° to 8°C for fixation of direct specimen slides, shell-vials and cultured cell preparations.

7. Sterile graduated pipettes: 10-mL, 5-mL, and 1-mL.

8. Sterile Pasteur pipettes or other transfer pipettes.

Caution: One should not use solvents such as acetone with polyethylene transfer pipettes.


10. 200-mL wash bottle.

11. Bent-tip teasing needle (for removal of coverslip from a shell-vial by bending the tip of a syringe needle or similar object (e.g., mycology teasing needle) against a bendtop or with a pair of forceps taking care to avoid injury.

12. Sodium hypo-chlorite solution (1:10 final dilution of household bleach).

13. Humid chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).


15. Acetone-cleaned multi-well glass microscope slides (2-well and 8-well masked slides).

16. Bioters for multi-well glass microscope slides: Two- and 8-well absorbent bioters, used to blot excess liquid from the mask to prevent spread of liquid or stained cells from one well to the other.
17. Sterile, nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell cultures.
18. Incubator, 35° to 37°C (5% CO₂ or non-CO₂, depending on the cell culture format used).
20. De-mineralized water for dilution of 40X Wash Solution Concentrate (see Section IV.C) and for dilution of the reagent-grade acetone for use in polystyrene multi-well plates (See item VI.B.6. Note 2).
21. PBS, sterile, for use in rinsing and suspending cells.
22. Live control viruses for positive culture controls: Known strains of culture format used).
23. 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.
24. Wash Container: Beaker, wash bottle or Coplin jar for washing slides.
25. Fixing Container: Coplin jar, slide dish or polyethylene holder for slides for use in fixing the cells on the slides.
26. 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.

C. PRELIMINARY COMMENTS AND PRECAUTIONS
1. Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
2. 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.
3. 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.
4. Place the closed, humidified chamber for holding slides during 2. 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.
5. Examine the positive and negative controls before examining the slides.
6. 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.

D. SPECIMEN PREPARATION
For specimen processing recommendations, refer to CLSI Approved Viral Culture Guidelines. For use in Cell Culture Testing (See Section VI.D.3. above), add the supernatant that was reserved in Step VI.D.3. above, 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.

E. DIRECT SPECIMEN TESTING
1. Spot 25 μL of the prepared cell suspension onto 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° 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. 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° 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.
G.  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):
   a) 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).
   b) 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. Centrifuge the shell-vials at 700xg for 1-hour at 20° to 25°C.

5. Place stopped shell-vials in an incubator at 35° to 37°C.

6. When a monolayer is ready to be stained, using the DFA Screening Reagent, remove the medium by aspiration and add 1-mL of PBS.

7. Remove the de-mineralized water by aspiration.

8. Wash the shell-vials at 35° to 37°C for 15 to 30 minutes.

9. Centrifuge the shell-vials at 700xg for 1-hour at 20° to 25°C.

10. Add 1-mL of fresh, chilled 100% acetone and allow to stand for 5 to 10 minutes at 20° to 25°C.

Caution: Acetone is volatile and flammable; keep away from open flames.

11. Aspirate the DFA Screening Reagent from the fixed monolayers of patient and control samples, and rock to ensure complete coverage of the monolayer by the Reagent.

12. Place stopped shell-vials in a 35° to 37°C incubator for 15 to 30 minutes.

13. For the Respiratory Virus Antigen Control Slide, add one drop of the DFA Screening Reagent. An Antigen Control Slide should be stained only once, do not re-stain.

14. Add 1-mL of de-mineralized water.

15. Remove the fixative by aspiration.

16. Discard the used wash and repeat the washing step using new 1X Wash Solution.

17. Rinse the de-mineralized water by aspiration.

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. Continue with steps 14 through 21 above.

NOTE: An Antigen Control Slide should be stained only once, do not re-stain.

2. 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.

3. Continue with steps 14 through 21 above.

F. CELL CULTURE TESTING - TUBE CULTURE

1. Calculate the number of wells needed for the staining protocol to be used (this staining protocol requires 3 wells):
   a) 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).
   b) One additional well 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 well.

4. Add 0.2 to 0.4-mL of prepared specimen to each well. Centrifuge the shell-vials at 700xg for 1-hour at 20° to 25°C.

5. Place stopped shell-vials in an incubator at 35° to 37°C.

6. When a monolayer is ready to be stained, using the DFA Screening Reagent, remove the medium by aspiration and add 1-mL of PBS.

7. Remove the de-mineralized water by aspiration.

8. Wash the shell-vials at 35° to 37°C for 15 to 30 minutes.

9. Centrifuge the shell-vials at 700xg for 1-hour at 20° to 25°C.

10. Add 1-mL of fresh, chilled 100% acetone and allow to stand for 5 to 10 minutes at 20° 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 the well to re-stain 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 stopped shell-vials in a 35° 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 fluoresence microscope with magnifications between 200X to 400X. (See Section VI. C. 11-14, 'Immunofluorescence Microscopy').

23. Refer to Section VII., 'Interpretation of Results'.

24. If the result is positive for respiratory virus, process a reserved replicative culture as a cell suspension and spot onto an 8-well specimen slide in order to identify which respiratory virus may be present (See Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:
   a) 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.
   b) For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent. An Antigen Control Slide should be stained only once, do not re-stain.
   c) Continue with VI. F. steps 14 through 21.

H. 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):
   a) 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).
   b) One additional well is required for preparing 8-well slides used to identify the viruses from positive screens.
   c) 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 refeed 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. This allows each plate to be processed on the appropriate day.

5. Centrifuge the multi-well plates at 700xg for 1-hour at 20° to 25°C.

6. Place the covered multi-well plates in a 35° to 37°C incubator with a humidified, 5% CO2 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 the 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° 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 stopped shell-vials in a 35° 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 fluoresence microscope with magnifications between 200X to 400X. (See Section VI. C. 11-14, 'Immunofluorescence Microscopy').

23. Refer to Section VII., 'Interpretation of Results'.

24. If the result is positive for respiratory virus, process a reserved replicative culture as a cell suspension and spot onto an 8-well specimen slide in order to identify which respiratory virus may be present (See Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:
   a) 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.
   b) For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well.
   c) Continue with VI. F. steps 14 through 21.
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° 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 Section VI.C. 11-14, “Immunofluorescence Microscopy”).
23. Refer to Section VII., ‘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 Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:
   a) 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.
   b) 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.
   c) Continue with VI.F, steps 14 through 21.

I. QUALITY CONTROL

1. Reagents
   a) A fresh Respiratory Virus Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
   b) 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.
   c) The negative well will show only negative cells staining a dull red.
   d) 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.
   e) 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.

2. Cell Culture
   a) Positive and negative virus controls should be run with each new batch of cells to confirm their performance in culturing specific viruses.
   b) To ensure viral sensitivity, virus-inoculated control monolayers should be included each time a new lot of cell culture is used.
   c) 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.
   d) If control cultures fail to perform correctly, results are considered invalid.

VII. INTERPRETATION OF RESULTS

A. EXAMINATION OF SAMPLES AND CONTROLS

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

B. ARTIFACTS OF STAINING

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

C. 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 virus infections 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.”

D. RESULTS FROM DIRECT SPECIMEN STAINING

1. Evaluation of sample suitability
   a) 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.
   b) 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.
   c) 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.

2. Reporting Results of Direct Specimen Staining
   a) The entire cell spot must be examined for virus-infected, apple-green fluorescent cells.
   b) 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.
   c) 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, respiratory syncytial virus, adenovirus, respiratory syncytial virus, parainfluenza virus type 1, 2, or 3 (see Section VII.E, ‘Results from Culture Isolation / Confirmation’, below).
   d) If apple-green fluorescent cells are found, the identification of the virus(es) may be based on the individual DFA Reagents (according to Section V.I.E.). 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.

E. RESULTS FROM CULTURE ISOLATION/CONFIRMATION

1. 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.”
2. If apple-green fluorescent cells are found, the identification of the virus(es) may be based on the individual DFA Reagents...
VIII. LIMITATIONS OF PROCEDURE

1. Inappropiate specimen collection, storage, and transport may lead to false negative results.1

2. 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.

3. Incubation times or temperatures other than those cited in the test instructions may give erroneous results.

4. 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.

5. The effects of antiviral therapy on the performance of this kit have not been established.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. A negative result on a direct or cultured specimen does not rule out the presence of virus.

12. Performance of the kit can only be assured when components used in the assay are those supplied by DHI.

13. Prolonged storage of the DFA Reagents under bright light will decrease the staining intensity.

14. 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, 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.

IX. 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 Section X (Specific Performance Characteristics) were comprised of respiratory specimens collected during the winter to early spring months of 2005/2006. Prevalence of the respiratory viruses used in these studies came from nasopharyngeal (NP) aspirates, 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 shows 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 7 shows the overall cell culture results for 474 frozen specimens. The table includes the number of positive results, negative results, and the percentage agreement between the subject and comparator devices. The results are presented for each respiratory virus type, including influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, and parainfluenza type 3.

Table 8 shows the prevalence of the respiratory viruses within the study population based on fresh and frozen specimens. The table includes the percentage of positive results for each respiratory virus type.

Table 9 shows the overall direct specimen results for 409 specimens. The table includes the number of positive results, negative results, and the percentage agreement between the subject and comparator devices. The results are presented for each respiratory virus type, including influenza A, influenza B, parainfluenza type 1, parainfluenza type 2, and parainfluenza type 3.
A. 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 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

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 identified one as Flu B and three as Para 3 infections. All but one of the Para device identified 4 specimens as being negative while the Subject device identified one as Flu B and three as Para 3 infections.

Subject and Comparator devices.

Slides were prepared from the specimens according to instructions detailed in the Comparator device’s product insert. The results of this 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 8 below:

Table 8: Study 2-DS – Direct Specimen Results

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 700g and incubated for 24-hours at 35° 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

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 DHI, 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 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, two (2) Flu A+Para 1, two (2) Flu B+Para 1, one (1) Flu B+Para 2, one (1) Flu A+Adeno, one (1) Flu B+Para 3, one (1) Flu A+Para 3, one (1) Adeno+Para 3, one (1) Flu B+Para 2, and one (1) Flu B+Para 3. Because of the ten (10) co-infections, the Negatives and Positives added 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 48/24-48 cluster plates. The results of this testing are summarized in Table 10 below:

Table 10: Study 3-DS – Direct Specimen Results

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 A+Para 1, one (1) Flu A+Para 2, two (2) Flu A+RSV, one (1) Flu A+Adeno, one (1) Flu A+Para 2, one (1) Flu A+Para 3, one (1) Flu B+BSV, 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.

B. 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

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**Table 6: Age Distribution**

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Adeno</th>
<th>Flu A</th>
<th>Flu B</th>
<th>Para 1</th>
<th>Para 2</th>
<th>Para 3</th>
<th>RSV</th>
<th>No Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1m</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1m to 2y</td>
<td>8</td>
<td>9</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>2y to 12y</td>
<td>8</td>
<td>7</td>
<td>6</td>
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<td>1</td>
<td>42</td>
</tr>
<tr>
<td>&gt;12y</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Not reported</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 7: Study 1-DS – Direct Specimen Results**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Negative</th>
<th>Screen Positive</th>
<th>Adeno</th>
<th>Flu A</th>
<th>Flu B</th>
<th>Para 1</th>
<th>Para 2</th>
<th>Para 3</th>
<th>RSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparator Results:</td>
<td>236</td>
<td>90</td>
<td>18</td>
<td>32</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Subject Results:</td>
<td>232</td>
<td>94</td>
<td>18</td>
<td>32</td>
<td>19</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>18</td>
</tr>
</tbody>
</table>

**Table 8: Study 2-DS – Direct Specimen Results**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Negative</th>
<th>Screen Positive</th>
<th>Adeno</th>
<th>Flu A</th>
<th>Flu B</th>
<th>Para 1</th>
<th>Para 2</th>
<th>Para 3</th>
<th>RSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comparator Results:</td>
<td>142</td>
<td>50</td>
<td>2</td>
<td>26</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Subject Results:</td>
<td>142</td>
<td>50</td>
<td>2</td>
<td>26</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

**Table 9: Study 2-CC – Cell Culture Results**

**Table 10: Study 3-DS – Direct Specimen Results**

**Table 11: Study 3-CC – Cell Culture Results**
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 DHI, 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 Results**

With the exception of one specimen, the DS test results were concordant for both the Screen and the ID reagents.

**C. 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 48/24-fill cluster plates. One specimen was found to be negative for this specimen.

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

**TABLE 15: Cross-Reactivity Study Results**

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

**D. CROSS-REACTIVITY TESTING**

Diagnostic Hybrids, Inc. D² Ultra DFA RESPIRATORY VIRUS SCREENING & ID KIT DFA Reagents were tested for cross-reactivity against a wide variety of cells, viruses and microorganisms. No cross-reactivity was observed for 64 virus stains (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, Section 12.) 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 levels 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  

<table>
<thead>
<tr>
<th>Organisms</th>
<th>DFA Reagent Results</th>
<th>Adeno</th>
<th>Flu A</th>
<th>Flu B</th>
<th>Para 1</th>
<th>Para 2</th>
<th>Para 3</th>
<th>RSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adeno</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flu A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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X. BIBLIOGRAPHY


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