Special 510(k): Device Modification
D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit

DIAGNOSTIC HYBRIDS
Integrating Science and Humanity

K092300

DATE OF PREPARATION OF 510(k) SUMMARY

July 23, 2009

APPLICANT

AUG 2 8 2009

DIAGNOSTIC HYBRIDS, INC.
1055 East State Street
Suite 100
Athens, OHIO 45701

CONTACT INFORMATION

Ronald H. Lollar
Senior Director, Product Realization, Management, and Marketing
E-mail: lollar@dhiusa.com
Telephone: 740-589-3300
Desk Extension: 740-589-3373
FAX: 740-593-8437

DEVICE NAME

Trade name: D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit
Common name: Respiratory Virus DFA Assay
Classification name: Antisera, Cf, Influenza A, B, C
Product Code: GNW
Regulation: 21 CFR § 866.3330, Class I, Influenza virus serological reagents, Panel Microbiology (83)

LEGALLY MARKETED DEVICE

D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit, K061101

DESCRIPTION of DEVICE MODIFICATION

The product insert has been modified. The following has been added (see below):

Table 15 in the product insert has been updated to include reactivity data on influenza A virus Mexico/4108/2009 and California/07/2009 strains. The following language was included with the data:

"Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical

specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D[3] *Ultra* DFA Respiratory Virus Screening & ID Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes."

INTENDED USE

The Diagnostic Hybrids, Inc. D[3] *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[1] is available to receive and culture specimens.[2]

ASSESSMENT OF NON-CLINICAL PERFORMANCE DATA FOR EQUIVALENCE

Not Applicable

ASSESSMENT OF NON-CLINICAL PERFORMANCE DATA FOR EQUIVALENCE

The risk analysis method used to assess the impact of the modification was a Failure Modes and Effects Analysis (FMEA). The modification to device labeling poses no additional risk.

BIOCOMPATABILITY

[1] www.cdc.gov
[2] FDA Guidance Document: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; Issued 4/10/2006

Not applicable

STERILIZATION

Not applicable

D³ *Ultra* DFA
Respiratory Virus Screening & ID
Kit

For *In Vitro* Diagnostic Use

I. INTENDED USE

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- 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[1] is available to receive and culture specimens.[2]

II. SUMMARY AND EXPLANATION OF THE TEST

With the addition of new antiviral drugs for the treatment of Influenza[3], more rapid and sensitive tests for respiratory virus detection[4,5] and the increasing need to be more discriminating in the use of antibiotics[6], early detection and identification of the infecting viral agent has grown substantially in importance. Viral identification is becoming increasingly important in ruling out bacteria as the cause of respiratory infections. Virus identification by either direct antigen detection or cell culture using fluorescent monoclonal antibodies continues to be the standard method in virology laboratories.

Influenza A and B

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.[7] 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™1), A549/MDCK mixtures (R-Mix Too™1), Rhesus MK, MDCK, MRC-5 and A549 cells[8].

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[9].

Adenovirus can be isolated in A549/Mv1Lu mixtures (R-Mix™), A549/MDCK mixtures (R-Mix Too™), HEp2, HEK, A549 and MRC-5 cells.[8]

Parainfluenza Viruses 1, 2 and 3

Parainfluenza viruses (family *Paramyxoviridae*) are enveloped viruses with a single, negative strand RNA genome. The 4 different types, 1 to 4, cause croup and viral pneumonia in children under the age of 5 years and cause upper respiratory illness in adults. Parainfluenza is the number 2 leading cause of lower respiratory illness in children (after RSV). Outbreaks caused by parainfluenza viruses occur during alternate years in the fall (P1 and P2) or throughout the year, with increased activity in the spring (P3) [10].

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 [8].

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[11]. 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

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[1] The use of R-Mix™ and R-Mix Too™ cells is covered by U.S. Patent Number 6,168,915 with additional patents pending.

clinical illnesses involve Subtype A strains which tend to predominate in most outbreaks[12]. 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[13]. RSV is also now recognized as a significant problem in certain adult populations. These include the elderly, persons with cardiopulmonary diseases, and immunocompromised hosts[14].

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents[12] 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[8].

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 1, Parainfluenza 2, and Parainfluenza 3) plus seven separate DFA Reagents, each consisting of MAb blends directed against a single respiratory virus. The kit can be used for direct specimen or cell culture screening and final virus identification.
The cells to be tested, either derived from a clinical specimen or cell culture, are fixed in acetone. The DFA Screening Reagent is added to the cells to determine the presence of viral antigens. After incubating at 35°C to 37°C, the stained cells are rinsed with the diluted Wash Solution. A drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. Virus infected cells will be stained with viral specific apple-green fluorescence when stained with the DFA Screening Reagent while uninfected cells will contain no fluorescence but will be stained red by the Evan's 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 Evan's Blue, it is recommended that the specimen be cultured and stained using the DFA Screening Reagent. If fluorescent cells are seen, the identification of the virus is determined as described above.
Cell preparations are fixed in acetone. The individual DFA reagents are added to the cell preparations. After incubating at 35° to 37°C, the stained cells are rinsed with the diluted Wash Solution. 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 mixture 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 Evan's 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 (strain Texas 1/77, H3N2) infected cells. The buffered, stabilized, aqueous solution contains Evan's 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) infected cells. The buffered, stabilized, aqueous solution contains Evan's 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 Evan's 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 6-tonsil 99 strain) infected cells. The buffered, stabilized, aqueous solution contains Evan's 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 1 (VP-1 strain) infected cells. The buffered, stabilized, aqueous solution contains Evan's 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 2 (Greer strain) infected cells. The buffered, stabilized, aqueous solution contains Evan's 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 Evan's 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 uninfected cells. Each slide is intended to be stained only one time.

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 Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.

11. Wash Solution Concentrate - 25-mL. One bottle containing a 40X concentrate consisting of Tween 20 and 4% sodium azide (after dilution to 1X in water, the concentration of sodium azide in the solution is 0.1%) in Phosphate Buffered Saline.

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

1. For *in vitro* diagnostic use.
2. Cells may have some potential to be hazardous. Personnel working with these cultures must be properly trained in safe handling techniques[15,16,17], and have experience with tissue culture before attempting this procedure.
3. All procedures must be conducted in accordance with the CDC 4th edition Biosafety in Microbiological and Biomedical Laboratories, 1999, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.
4. 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.
5. Sodium azide is included in the 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 a Diagnostic Hybrids' Technical Service Representative.
    a. Reagents containing sodium azide should be considered a poison. If products containing sodium azide are swallowed, seek medical advice immediately and show product container or label. [Refer to NIOSH, National Institute for Occupational Safety and Health; CAS#: 2628-22-8; 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 (sodium azide in water exists is ionic equilibrium with hydrazoic acid, which when mixed with acid may liberate a 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.
6. Evan's Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
7. The DFA Reagents are supplied at working strength. Any dilution of the DFA Reagents will decrease sensitivity.

8. Reagents should be used prior to their expiration date.
9. Each Respiratory Virus Antigen Control Slide should be used only once. Do not re-use a Control Slide.
10. Microbial contamination of DFA Reagents may cause a decrease in sensitivity.
11. Store 1X Wash Solution and PBS in a clean container to prevent contamination.
12. All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel. Decontamination is most effectively accomplished using a 0.05% solution of sodium hypochlorite (1:100 dilution of household bleach).
13. 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.
14. Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
15. Avoid splashing and the generation of aerosols with clinical samples.
16. Use aseptic technique and sterile equipment and materials for all tissue culture procedures.
17. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
18. Do not expose DFA Reagents to bright light during staining or storage.
19. 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 Wash Solution Concentrate may have crystallized. Warm the solution to room temperature to re-dissolve the crystals and mix.
2. Add contents of the fully dissolved 25-mL 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 room temperature (20° to 25°C).

D. Storage Instructions

TABLE 1	
1. Respiratory Virus DFA Screening Reagent	Store at 2° to 8°C in the dark.
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. Mounting Fluid	
10. Normal Mouse Gamma Globulin DFA Reagent	

11. Respiratory Virus Antigen Control Slides	Store at 2° to 8°C.
12. Wash Solution Concentrate NOTE: The Concentrate may crystallize when stored at 2° to 8°C. The crystals will dissolve when the Concentrate is warmed to room temperature.	Store liquid at 2° to 8°C prior to dilution.
13. 1X Wash Solution	Store at room temperature (20° to 25°C).

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.

A. Specimen Collection[18]

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 on wet ice to the laboratory and 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 48 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 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. 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 = 520nm).
2. Cell culture for respiratory virus isolation. Suggested cell lines that are susceptible to respiratory viruses include LLC-MK_2, HEp-2, A549 cells, R-Mix™ and R-Mix Too™ Mixed Cells, and primary Rhesus monkey kidney cells, all available from DHI.
3. Cover slips (22 x 50mm) for Antigen Control Slides and for specimen slides.
4. Universal Transport Medium (available from DHI).
5. R-Mix Refeed medium (for use with R-Mix™ and R-Mix Too™ Mixed Cells) or other standard Refeed medium. Available from DHI.
6. Reagent grade acetone (>99% pure) chilled at 2° to 8°C for fixation of direct specimen slides and shell vials.

   NOTE 1: Keep the reagent grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, nonspecific, background fluorescence.

   NOTE 2: A mixture of 80% acetone/20% de-mineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature.
7. Sterile graduated pipettes: 10-mL, 5-mL, and 1-mL.
8. Sterile Pasteur pipettes or other "transfer"-type pipettes.
9. Fine-tipped forceps.
10. 200-mL wash bottle.
11. 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 (i.e. mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.

12. Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach).
13. Humid chamber (e.g. covered Petri dish with a damp paper towel placed in the bottom).
14. Glass microscope slides.
15. Acetone-cleaned multi-well glass microscope slides (2-well and 8-well masked slides).
16. Blotters for multi-well glass microscope slides: Two- 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.
17. Sterile nylon flock swab or polyester swab, non-inhibitory to respiratory viruses and tissue culture.
18. Incubator, 35° to 37°C (CO_2 or non-CO_2, depending on the cell culture format used).
19. Centrifuge with free swinging bucket rotor.
20. De-mineralized water for dilution of Wash Concentrate Solution and for dilution of the reagent grade acetone for use in polystyrene multi-well plates (see item VI.B.5).
21. PBS (Phosphate Buffered Saline), sterile, for use in rinsing and suspending cells.
22. Control viruses: Known strains of the 7 respiratory viruses for use in monitoring the cell culture and staining procedures. Such control virus strains can be obtained from Diagnostic Hybrids, Inc.
23. Aspirator Set-up: Vacuum aspirator with disinfectant trap containing sufficient household bleach (5%) that the concentration is not decreased by more than 100 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 and CPE. It should have between 100X to 400X 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. The closed, humidified container for holding the slides during incubation should be kept in the incubator so it is at incubator temperature when the slides are placed in

it. By doing this, the cells and antibody solution will come up to temperature more rapidly, yielding more intense stains in shorter periods of time.

REGARDING CELL CULTURE TESTING:

5. Good Laboratory Practice dictates that positive and negative virus controls be run with each new batch of cells to confirm their performance in culturing specific viruses.
6. It is good practice to retain the medium removed from the positive monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer for repeat testing.
7. If using cell cultures in polystyrene multi-well plates, the acetone fixative must be diluted with water to 80% by adding 20 mL of water to 80 mL of acetone.
8. Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased sensitivity.
9. Do not allow the antibody reagents to dry on the monolayers; this can lead to high background.

REGARDING IMMUNOFLUORESCENCE MICROSCOPY:

10. It is good practice to examine the positive and negative controls before examining the test specimens. If one of these fails to perform as expected, review the steps and conditions under which the test was performed to determine the cause(s). Do not report results until controls perform properly.
11. There are three aspects of the fluorescence microscope that must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
    i. The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the DFAs.
    ii. The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
    iii. The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.
12. There are several fluorescent artifacts that may be observed in the cell monolayers being examined:
    i. Cell debris, lint, etc. can nonspecifically adsorb DFAs, resulting in highly intense fluorescence. These can be identified by their morphology, i.e., they don't have the appearance of a complete cell and typically do not appear to be a part of the monolayer like the other cells.
    ii. A low grade, yellow-green fluorescence may sometimes be seen, particularly in areas that have piled cells or are near holes in the cell monolayer. In both cases, the diffusion of the entrapped DFAs is retarded during the wash step, resulting in the nonspecific fluorescence.
    iii. Intense fluorescence around the periphery of slide wells is indicative of drying of the DFA Reagent during incubation, suggesting that it was incubated too long or the humidity was not controlled.
    iv. Inadequate removal of the mucus from direct specimens can lead to nonspecific adsorption of DFAs.

v. Inadequate washing can lead to a general low grade fluorescence due to residual DFAs remaining on the monolayer of cells.
vi. On direct specimens, beware of trapping of fluorescence by leukocytes and monocytes. Also, the presence of RBCs in the specimen may leave a green haze on the sample.

13. Quenching or fading of the fluorescence of the stained cells may occur on exposure to light, particularly light of high intensity. Slides should be protected as much as possible during the assay.

D. Specimen Preparation

1. Vortex the specimen vigorously for 10 to 15 seconds.
2. Centrifuge at 400 to 600xg for 5 to 10 minutes.
3. Collect and set aside the supernatant for viral isolation. (See Step VI.G.9 below.)
4. Add 5 mL of PBS and vortex vigorously for 10 to 15 seconds.
5. Centrifuge at 400 to 600xg for 5 to 10 minutes.
6. Remove the supernatant and the mucus layer above the cell pellet taking care not to disturb the cell pellet.
7. Repeat steps 4 through 6 until the mucus layer has been completely removed.

NOTE: It is important to remove all the mucus since it can cause nonspecific fluorescence.

8. Add 0.5 to 1-mL of PBS.
9. Mix the suspension by pipetting up and down to re-suspend the cell pellet, forming a slightly cloudy suspension. This cell suspension will be used for Direct Specimen Testing (See Section VI.E., below).

NOTE: The quality of the slide preparation is dependent on the concentration of cells in the suspension; too many cells make it difficult to read the result and too few decrease the sensitivity of the procedure. Specimens may also be cytofuged if a monolayer is preferred.

10. For use in Cell Culture Testing (See Section VI. G., F., and H.), 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 suspension on *each well* of a 2-well and an 8-well slide. Repeat this step for each specimen.
2. Air dry the wells completely.
3. Fix the cells to the slides using fresh, chilled acetone for 5 to 10 minutes.
4. Remove the slides from the fixative and allow to air dry.
5. Add one drop of the DFA Screening Reagent to completely cover the dried, fixed cells on one well of each of the 2-well slides.
6. Also, to each of the wells of a fresh Respiratory Virus Antigen Control Slide add one drop of the DFA Screening Reagent. An Antigen Control Slide should be

stained only once, as it contains individual wells of viral infected cells and non-infected cells.

7. Add one drop of the Normal Mouse Gamma Globulin DFA Reagent to completely cover the dried, fixed cells on the other well of each of the 2-well slides.
8. Place the slides in a covered 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. Blot the excess 1X Wash Solution, add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
13. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 100X to 400X. (See Section VI. C. 10-12, 'Regarding Immunofluorescence Microscopy')
14. Refer to Section VII., 'Interpretation of Results'.
15. If the result is positive for respiratory virus, the staining procedure may be repeated using the reserved 8-well specimen slides in order to identify which respiratory virus may be present.
    i. 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.
    ii. For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
    iii. Continue with steps 8 through 14, above.

F. Cell Culture Testing - Tube Culture

1. Examine the monolayers for proper morphology prior to inoculation.
2. Aspirate maintenance medium from the monolayers and add 0.2 to 0.5-mL of each prepared specimen (Step VI.D., above) to each of the cell lines used for respiratory virus culture.
3. Place the tubes at an angle sufficient for the monolayers to be covered by the inoculum and allow virus adsorption to occur for 1 hour at 35° to 37°C.
4. After adsorption, add 2-mL of appropriate refeed medium.
5. Incubate the tubes at 35° to 37°C in a roller drum at 1 to 3 rpm. Examine the monolayers daily for evidence of toxicity or viral CPE or test for hemadsorption.
6. When the monolayers are ready to be stained, remove the medium by aspiration and gently rinse the monolayer two times with 1 to 2-mL PBS.

7. Add 0.5-mL of PBS to the tube and prepare a suspension of the cells by scraping the monolayer using a pipette and breaking the cell aggregates up by pipetting up and down several times.
8. Prepare cell spots using about 25-µL of the suspension on *each well* of a 2-well and an 8-well slide. Repeat this step for each specimen.
9. Air dry the wells completely.
10. Fix the cells to the slides using fresh, chilled acetone. Let stand for 5 to 10 minutes, at 20° to 25°C.
11. Remove the slides from the fixative and allow to air dry.
12. Add one drop of the DFA Screening Reagent to completely cover the dried, fixed cells on one well of each of the 2-well slides.
13. Also, to each of the wells of a fresh Respiratory Virus Antigen Control Slide, add one drop of the DFA Screening Reagent. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
14. Place the slides in a covered chamber at 35°C to 37°C for 15 to 30 minutes.
15. Rinse the stained cells using the 1X Wash Solution. For only a few slides, this can be done using a beaker of the 1X Wash Solution. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with 1X Wash Solution. For effective rinsing, dip the slide(s) up and down a minimum of four times.
16. Discard the used wash and repeat the washing step using new 1X Wash Solution.
17. Rinse the stained cells using de-mineralized water. For only a few slides, this can be done using a beaker of the de-mineralized water. For man slides, a slide carrier that holds 10 to 20 slides can be placed in its container with de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
18. Remove the de-mineralized water by aspiration.
19. Blot the excess liquid, add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
20. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 100X to 400X (See Section VI.C. 10-12, 'Regarding Immunofluorescence Microscopy', page 10).
21. Refer to Section VII., 'Interpretation of Results'.
22. If the result is positive for respiratory virus, the staining procedure may be repeated using the reserved 8-well specimen slides in order to identify which respiratory virus may be present.
    i. 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.
    ii. For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
    iii. Continue with steps 14 through 21 above.

G. Cell Culture Testing - Shell Vial

1. Calculate the number of vials needed based on the staining protocol to be used (this staining protocol requires 3-vials):
    i. One 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 then at 48- to 72-hours, requires 2 vials).
    ii. One additional vial is required for preparing 8-well slides used to identify the viruses from positive screens.
2. Examine the monolayers for proper morphology prior to inoculation.
3. Aspirate maintenance medium from the monolayers and add 1-mL of appropriate refeed medium to each shell vial.
4. Add 0.2 to 0.4-mL of prepared specimen to each shell vial.
5. Centrifuge the shell vials at 700xg for 1-hour at 20° to 25°C.
6. Place stoppered shell vials in an incubator at 35° to 37°C.
7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium and add 1-mL of PBS.
8. Swirl to mix and then aspirate.
9. Repeat this wash with another 1-mL of PBS and then aspirate.
10. Add 1-mL of chilled 100% acetone and allow to stand for 5 to 10 minutes at 18° to 26°C.
11. Remove the fixative by aspiration.
12. Add 0.5-mL of PBS to wet the monolayer.
13. Swirl and then aspirate completely.
14. Add 4 drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples, and rock to ensure complete coverage of the monolayer by the Reagent.
15. Place stoppered shell vials in a 35° 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, and, grasping it with the fine tipped forceps, transfer it, monolayer-side down, to a small drop of Mounting Fluid on a standard microscope slide.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 100X to 400X. (See Section VI. C. 10-12, 'Regarding 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:
    i. 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.

ii. For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.

iii. Continue with VI. F. steps 14 through 15.

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):
   i. 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).
   ii. One additional well is required for preparing 8-well slides used to identify the viruses from positive screens.
   iii. 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 well of a multi-well plate.
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% CO_2 atmosphere.
7. When a monolayer is ready to be stained using the DFA Screening Reagent, remove the medium and add 1-mL of PBS.
8. Swirl to mix and then aspirate.
9. Repeat this wash with another 1-mL of PBS and then aspirate.
10. Add 1-mL of 80% aqueous acetone and let stand 5 to 10 minutes.

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.

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 24-well multi-well plate monolayer; add 3 drops of the DFA Screening Reagent to the fixed monolayers of patient and control samples in each 48-well plate monolayer. 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.

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 100X to 400X. (See Section VI.C. 10-12, 'Regarding 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:
    i. 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.
    ii. For the Respiratory Virus Antigen Control Slide, add one drop of each individual virus DFA Reagent to its corresponding labeled well. An Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
    iii. Continue with VI.F, steps 14 through 21.

I. Quality Control

A fresh Respiratory Virus Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance. The positive wells will show multiple infected cells of bright apple-green fluorescence with negative cells staining a dull red due to the included Evan's 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 have validity. Antigen Control Slides may also aid in the interpretation of patient specimens.

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

VII. INTERPRETATION OF RESULTS

It is recommended that controls be examined first to ensure proper test performance before examination of the specimens. A positive reaction is one in which bright apple-green fluorescence is observed in the infected cells. Uninfected cells will stain dull red due to the Evan's Blue counter-stain included in the DFA Reagent. Technologists should not confuse cell clumps which may fluoresce due to entrapment of antibody with virus-specific staining. Occasionally, dead, rounded cells due to specimen toxicity or improper cell storage may nonspecifically stain a dull olive green due to trapped antibody. Adequate washing between steps will help to eliminate this type of nonspecific staining.

FLUORESCENT STAINING PATTERN OF RESPIRATORY VIRUS INFECTED CELLS

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

Influenza A and B: 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 1, 2, 3: The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.

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

A. Results from Direct Specimen Testing

The quality of the specimen with respect to the number of epithelial cells in the sample can be assessed by examining the 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 minimal sampling of 20 columnar epithelial cells. An inadequate sample 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 of the remaining specimen should be initiated.

A satisfactory specimen with no fluorescent cells found should be reported as "Presumptively negative, no Influenza A, Influenza B, Adenovirus, Respiratory Syncytial Virus, Parainfluenza 1, Parainfluenza 2, or Parainfluenza 3 detected by direct specimen testing". However, such negative results should be confirmed using cell culture.

Specimens negative by direct specimen testing but yielding positive culture results should be reported as "... isolated by cell culture", where '...' is the appropriate virus, e.g. Influenza A, Influenza B, Adenovirus, Respiratory Syncytial Virus, Parainfluenza 1, Parainfluenza 2, or Parainfluenza 3 (see section VII.B, 'Results from Culture Isolation / Confirmation', below).

If fluorescent cells are found, continue with the Testing Procedure, staining with the individual virus DFA Reagents (according to section VI.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 1, Parainfluenza 2, or Parainfluenza 3.

B. Results from Culture Isolation / Confirmation

The entire cell spot or monolayer of cells must be examined for virus-infected, fluorescent cells. If no fluorescent cells are found, the results of testing of the specimen should be reported as, "No Influenza A, Influenza B, Adenovirus, Respiratory Syncytial Virus, Parainfluenza 1, Parainfluenza 2, or Parainfluenza 3 isolated by cell culture."
If fluorescent cells are found, continue with the Testing Procedure, staining with the individual virus DFA Reagents (according to the appropriate sections VI. F., G., and H.). 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 "... isolated by cell culture", where '...' is the appropriate virus, e.g. Influenza A, Influenza B, Respiratory Syncytial Virus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, or Adenovirus.

VIII. LIMITATIONS OF PROCEDURE

1. Inappropriate specimen collection, storage, and transport may lead to false negative culture results[19].
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 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.
7. 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.
8. The monoclonal antibodies 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 Diagnostic Hybrids.
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 nonspecifically 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 5 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 within the population of specimens that were prospectively collected and tested fresh are noted in Table 2 below (also, see Study 1-DS in Section X).

TABLE 2

Expected Values	Adenoviruses	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Fresh Specimens (n = 326)	18	32	19	2	0	5	18
Prevalence	5.5%	9.8%	5.8%	0.6%	0	1.5%	5.5%

X. SPECIFIC PERFORMANCE CHARACTERISTICS

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

803 culture monolayer. All but 30 of the specimens were prospectively collected during the
804 2005-2006 season; those 30 specimens had been archived as Parainfluenza-positive. In
805 addition, a set of 81 clinical isolates were tested by CC methods only. The evaluations
806 were conducted at three laboratory sites: (1) A reference laboratory in northeast United
807 States; (2) A hospital laboratory in northeast United States; and (3) An internal reference
808 laboratory using specimens collected from an external hospital laboratory.

809 Percent Agreement between the Subject and Predicate tests was calculated for
810 prospectively collected specimens. For the DFA Screening Reagent:

811 - By DS method using fresh specimens, positive percent agreement is 95.5% and
812 negative percent agreement is 98.3% (see Table 3). By DS method using frozen
813 specimens, both positive percent agreement and negative percent agreement are
814 100% (see Table 4).

815 - By CC method using frozen specimens, both positive percent agreement and
816 negative percent agreement are 100% (see Table 5).

817 - [See individual study results, in this section, parts A through C, below.]

818

TABLE 3

DS - fresh 326 specimens	Negative	Screen +	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	236	90	18	32	18	2	0	2	18
Subject Results:	232	94	18	32	19	2	0	5	18
Positive Percent Agreement [2] (PPA)		95.5%	100.0%	100.0%	100.0%	100.0%	---	100.0%	100.0%
95% CI [3]- PPA		89.0-98.2%	82.4-100%	89.3-100%	82.4-100%	34.2-100%	---	34.2-100%	82.4-100%
Negative Percent Agreement [4] (NPA)	98.3%		100.0%	100.0%	98.7%	100.0%	100.0%	96.7%	100.0%
95% CI – NPA	95.7-99.3%		95.2-100%	94.2-100%	92.9-99.8%	96.0-100%	96.1-100%	90.8-98.9%	95.2-100%

819

[2] "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%.

[3] "95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).

[4] "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 4

DS - frozen 474 specimens	Negative	Screen +	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	306	168	8	85	19	3	3	9	51
Subject Results:	306	168	8	85	19	3	3	9	51
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		97.8-100%	63.1-100%	95.7-100%	82.3-100%	38.3-100%	38.3-100%	70.1-100%	93.0-100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI– NPA	98.8-100%		97.7-100%	95.6-100%	97.6-100%	97.8-100%	97.8-100%	97.6-100%	96.7-100%

820

TABLE 5

CC - frozen 490 specimens	Negative	Screen +	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	309	181	13	93	23	6	4	9	49
Subject Results:	309	181	13	93	23	6	4	9	49
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		98.0-100%	73.4-100%	95.2-100%	83.1-100%	55.7-100%	45.4-100%	65.5-100%	91.3-100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	98.5 – 100%		97.3-100%	95.0-100%	97.1-100%	97.4-100%	97.4-100%	96.6-100%	96.6-100%

821

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

824

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

TABLE 6

Virus: / Age	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus	Negative
Totals	18	32	18	2	0	2	18	236
<1m	0	0	0	0	0	0	2	1

1m to 2y	8	9	4	1	0	2	8	80
2y to12y	8	7	6	0	0	0	1	42
12y to18y	1	1	5	0	0	0	0	8
18y to 21y	0	0	1	0	0	0	0	2
>21y	0	12	1	0	0	0	1	78
Not reported	1	3	1	1	0	0	6	25

❖ *Age: m = months, and y = years*

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 room temperature and stained in accordance with the procedure in the Predicate product insert (same procedure for both Subject and Predicate devices). Three (3) specimens were found to contain insufficient numbers of cells for interpretation of DS stain results, leaving 326 specimens for evaluation. The results of this testing are summarized in Table 7 below:

TABLE 7 Study 1-DS – Direct Specimen Results

326 specimens	Negative	Screen +	Adeno virus	Influen za A	Influen za B	Parain fluenz a 1	Parain fluenz a 2	Parain fluenz a 3	Respirator y Syncytia Virus
Predicate Results:	236	90	18	32	18	2	0	2	18
Subject Results:	232	94	18	32	19	2	0	5	18
PPA		100%	100%	100%	100%	100%	---	100%	100%
95% CI – PPA		95.1% - 100%	79.3 % - 100%	87.3 % - 100%	79.3 % - 100%	29.0 % - 100%	---	29.0 % - 100%	79.3% - 100%
NPA	98.3%		100%	100%	98.7 %	100%	100%	96.7 %	100%
95% CI – NPA	95.4% - 99.5%		94.2 % - 100%	93.0 % - 100%	92.2 % - >99.9 %	95.2 % - 100%	95.3 % - 100%	90.5 % - 99.3 %	94.2% - 100%

With the exception of 4 specimens, the DS test results were concordant for both the screen and the identification of the individual viruses; the Predicate device

identified 4 specimens as being negative while the Subject device identified one as Flu B and three as Para 3 infections. All but one of the Para 3 specimens were confirmed by culture; the one Para 3, although strongly positive by the Subject assay, could not be cultured to confirm it as a Para 3. The culture method was not performed on the rest of the specimens from this site.

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

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

TABLE 8 Study 2-DS – Direct Specimen Results

192 specimens	Negative	Screen +	Adeno virus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	142	50	2	26	3	1	1	0	17
Subject Results:	142	50	2	26	3	1	1	0	17
PPA		100%	100%	100%	100%	100%	100%	---	100%
95% CI – PPA		91.5% - 100%	29.0% - 100%	84.8% - 100%	38.3% - 100%	16.8% - 100%	16.8% - 100%	---	80.5% - 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	96.8% - 99.5%		92.6% - 100%	96.2% - 100%	96.8% - >99.9%	96.8% - 100%	96.8% - 100	96.8% - 100%	89.4% - 100%

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

Study 2-CC - Cell Culture Method: The same 192 specimens that were evaluated by DS testing were also processed according to the Predicate device's product insert procedure for cell culture (same procedure for both Subject and Predicate devices). Briefly, 200-µL from the specimens were inoculated onto each of 4 monolayers of R-Mix™ Too FreshCells™ contained in shell vials which were centrifuged for 60 minutes at 700xg and incubated for 24-hours at 35° to 37°C. The shell vials were processed according to instructions detailed in the Predicate device's product insert. The results of this testing are summarized in Table 9 below:

TABLE 9 Study 2-CC – Cell Culture Results

192 specimens	Negative	Screen +	Adeno virus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytia Virus
Predicate Results:	142	50	3	26	3	1	1	0	16
Subject Results:	142	50	3	26	3	1	1	0	16
PPA		100%	100%	100%	100%	100%	100%	---	100%
95% CI – PPA		91.5% - 100%	38.3% - 100%	84.8% - 100%	38.3% - 100%	16.8% - 100%	16.8% - 100%	---	77.3% - 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	96.8% - 99.5%		96.8% - 100%	96.2% - 100%	96.8% - >99.9%	96.8% - 100%	96.8% - 100	96.8% - 100%	96.4% - 100%

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

Study 3-DS - Direct Specimen Method: The study consisted of 298 specimens originally received by a hospital laboratory in the eastern US for respiratory virus testing during January through March 2006, with residual specimen material stored at –70°C from 3 to 6 months. The frozen specimens were sent to DHI, where they were thawed and processed between 30 May and 1 June 2006, according to the predicate device's product insert. All specimens used in the studies were tested by both the DS and CC procedures as detailed in the Predicate 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 Mouse Gamma Globulin DFA Reagent), leaving 282 specimens for evaluation.

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

TABLE 10 Study 3-DS – Direct Specimen Results

282 specimens	Negative	Screen +	Adeno virus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytia Virus
Predicate Results:	164	118	6	59	16	2	2	9	34
Subject Results:	164	118	6	59	16	2	2	9	34
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		96.2% - 100%	55.7% - 100%	92.7% - 100%	77.3% - 100%	29.0% - 100%	29.0% - 100%	65.5% - 100%	87.9% - 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	97.3% - 100%		96.0% - 100%	92.7% - 100%	95.6% - >99.9%	96.2% - 100%	96.2% - 100	95.9% - 100%	91.3% - 100%

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

Study 3-CC - Cell Culture Method: The same 298 specimens that were evaluated by DS testing were also processed for CC testing according to the Predicate device's product insert for cell culture using R-Mix™ Too FreshCells™ in 48/24-fill cluster plates. The results of this testing are summarized in Table 11 below:

TABLE 11 Study 3-CC – Cell Culture Results

298 specimens	Negative	Screen +	Adeno virus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	167	131	10	67	20	5	3	9	33
Subject Results:	167	131	10	67	20	5	3	9	33
PPA		100%	100%	100%	100%	100%	100%	100%	100%
95% CI – PPA		96.6% - 100%	67.9 % - 100%	93.5 % - 100%	81.0 % - 100%	51.1 % - 100%	38.3 % - 100%	65.5 % - 100%	87.6% - 100%
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	97.3% - 100%		96.3 % - 100%	93.2 % - 100%	96.0 % - >99.9 %	96.4 % - 100%	96.5 % - 100	96.3 % - 100%	95.5% - 100%

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

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 Predicate Tests by Direct Specimen method (Study 3a-DS; see Table 12, below). The same specimens were

tested by Cell Culture method (see Table 13). Original results reported by the laboratory were unknown to the study investigator. Although the study design has a selection bias, this study offers further analytical information on the assay's ability to detect Parainfluenza viruses.

Study 3a-DS - Direct Specimen Method: The study consisted of 30 specimens originally received by a hospital laboratory in Italy for respiratory virus testing during the period from October 2005 through April 2006, with residual specimen material stored at -70°C from 2 to 6 months. The frozen specimens were sent to 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 Predicate 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 Predicate and Subject devices are summarized in Table 12 below:

TABLE 12 Study 3a-DS – Direct Specimen Results

26 specimens	Negative	Screen +	Adeno virus	Influen za A	Influen za B	Parain fluenz a 1	Parain fluenz a 2	Parain fluenz a 3	Respirat ory Syncytia Virus
Predicate Results:	9	17	0	0	0	1	5	11	0
Subject Results:	8	18	0	0	0	1	5	12	0
PPA		100%	---	---	---	100%	100%	100%	---
95% CI – PPA		78.4% - 100%	---	---	---	16.8 % - 100%	51.1 % - 100%	70.0 % - 100%	---
NPA	88.9%		100%	100%	100%	100%	100%	85.7 %	100%
95% CI – NPA	54.3% - >99.9%		79.3 % - 100%	79.3 % - 100%	79.3 % - 100%	78.4 % - 100%	73.4 % - 100	46.7 % - 99.5 %	79.3 % - 100%

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

Study 3a-CC - Cell Culture Method: The same 30 frozen specimens that were evaluated by DS testing were also processed for CC testing according to the Predicate device's product insert for cell culture using R-Mix™ FreshCells™ in 48/24-fill cluster plates. One specimen was found to be unsuitable for CC testing because it was toxic to the monolayer of cells. The results of this testing are summarized in Table 13 below:

TABLE 13 Study 3a-CC – Cell Culture Results

29 specimens	Negative	Screen +	Adeno virus	Influen za A	Influen za B	Parain fluenz a 1	Parain fluenz a 2	Parain fluenz a 3	Respirator y Syncytial Virus
Predicate Results:	8	21	0	0	0	3	5	13	0
Subject Results:	8	21	0	0	0	3	5	13	0
PPA		100%	---	---	---	100%	100%	100%	---
95% CI – PPA		81.8% - 100%	---	---	---	38.3 % - 100%	51.1 % - 100%	73.4 % - 100%	---
NPA	100%		100%	100%	100%	100%	100%	100%	100%
95% CI – NPA	62.8% - 100%		81.8 % - 100%	81.8 % - 100%	81.8 % - 100%	79.3 % - 100%	77.3 % - 100	62.8 % - 100%	81.8% - 100%

The CC 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 Test, 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 Test.

Study 3b-CC- Cell Culture Method: A total of 81 clinical isolates from a frozen archival repository were processed according to the Predicate device's product insert for cell culture using R-Mix™ FreshCells™ cultures in shell vials. The results of this testing are summarized in Table 14 below:

TABLE 14 Study 3b-CC – Cell Culture Results

81 specimens	Negative	Screen +	Aden ovirus	Influen za A	Influen za B	Parain fluenz a 1	Parain fluenz a 2	Parain fluenz a 3	Respirator y Syncytial Virus
Predicate Results:	0	81	11	18	17	4	1	26	5
Subject Results:	0	81	11	18	17	4	1	26	5
PPA		100%	100 %	100%	100%	100%	100%	100%	100%
95% CI – PPA		94.6% - 100%	70.0 % - 100 %	79.3 % - 100%	78.4 % - 100%	45.4 % - 100%	16.8 % - 100%	84.8 % - 100%	51.1% - 100%
NPA		100%	100 %	100%	100%	100%	100%	100%	100%
95% CI – NPA		97.3% - 100%	93.8 % - 100 %	93.1 % - 100%	93.2 % - 100%	94.3 % - 100%	94.5 % - 100	92.2 % - 100%	94.2% - 100%

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

D. Cross-reactivity Testing

Diagnostic Hybrids, Inc. D^3 *Ultra* DFA RESPIRATORY VIRUS SCREENING & ID KIT DFA Reagents were tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 64 virus strains (cultured and processed for staining) or for 18 host culture cell types. Eighteen (18) 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 DFAs and high titers of microorganisms. The DFAs (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 DFAs 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 DFAs according to the procedure in this product insert. Cell cultures were stained as confluent monolayers.

TABLE 15		*DFA Reagent (Results are Positive (+) or Negative (-) for Reactivity)*						
Organism	Strain	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Adenovirus	Type 1	+	-	-	-	-	-	-
	Type 3	+	-	-	-	-	-	-
	Type 5	+	-	-	-	-	-	-
	Type 6	+	-	-	-	-	-	-
	Type 7	+	-	-	-	-	-	-
	Type 10	+	-	-	-	-	-	-
	Type 13	+	-	-	-	-	-	-
	Type 14	+	-	-	-	-	-	-
	Type 18	+	-	-	-	-	-	-
	Type 31	+	-	-	-	-	-	-
	Type 40	+	-	-	-	-	-	-
	Type 41	+	-	-	-	-	-	-
Influenza A	Mexico/4108/2009 (H1N1) from CDC*	-	+	-	-	-	-	-
	California/07/2009 (H1N1) from CDC*	-	+	-	-	-	-	-
	Aichi (H3N2)	-	+	-	-	-	-	-
	Mal (H1N1)	-	+	-	-	-	-	-
	Hong Kong (H3N2)	-	+	-	-	-	-	-
	Denver (H1N1)	-	+	-	-	-	-	-
	Port Chalmers (H3N2)	-	+	-	-	-	-	-
	Victoria (H3N2)	-	+	-	-	-	-	-

TABLE 15		*DFA Reagent (Results are Positive (+) or Negative (-) for Reactivity)*						
Organism	Strain	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
	New Jersey ($H_{sw}N1$)	-	+	-	-	-	-	-
	WS (H1N1)	-	+	-	-	-	-	-
	PR (H1N1)	-	+	-	-	-	-	-
Influenza B	Hong Kong	-	-	+	-	-	-	-
	Maryland	-	-	+	-	-	-	-
	Mass	-	-	+	-	-	-	-
	Taiwan	-	-	+	-	-	-	-
	GL	-	-	+	-	-	-	-
	Russia	-	-	+	-	-	-	-
RSV	Long	-	-	-	-	-	-	+
	Wash	-	-	-	-	-	-	+
	9320	-	-	-	-	-	-	+
Rhinovirus 39	209 Picornavirus	-	-	-	-	-	-	-
Parainfluenza 1	C-35	-	-	-	+	-	-	-
Parainfluenza 2	Greer	-	-	-	-	+	-	-
Parainfluenza 3	C 243	-	-	-	-	-	+	-
Parainfluenza 4a	M-25	-	-	-	-	-	-	-
Parainfluenza 4b	CH19503	-	-	-	-	-	-	-
Metapneumovirus	A1	-	-	-	-	-	-	-
	A2	-	-	-	-	-	-	-
	B3	-	-	-	-	-	-	-
	B4	-	-	-	-	-	-	-
Coronavirus	OC43	-	-	-	-	-	-	-
	229E	-	-	-	-	-	-	-
Herpes simplex virus Type 1	1F	-	-	-	-	-	-	-
	MacIntyre	-	-	-	-	-	-	-
Herpes simplex virus Type 2	MS	-	-	-	-	-	-	-
	Strain G	-	-	-	-	-	-	-
Cytomegalovirus	Towne	-	-	-	-	-	-	-
	Davis	-	-	-	-	-	-	-
	AD169	-	-	-	-	-	-	-
Varicella-zoster	Webster	-	-	-	-	-	-	-
	Ellen	-	-	-	-	-	-	-
Echovirus	9	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	-
	30	-	-	-	-	-	-	-
	34	-	-	-	-	-	-	-
Coxsackievirus	B1	-	-	-	-	-	-	-
	B2	-	-	-	-	-	-	-
	B3	-	-	-	-	-	-	-
	B4	-	-	-	-	-	-	-
	B5	-	-	-	-	-	-	-
	B6	-	-	-	-	-	-	-
Mumps		-	-	-	-	-	-	-
Rubeola		-	-	-	-	-	-	-
Rhinovirus	209 Picornavirus	-	-	-	-	-	-	-
Acholeplasma laidlawii		-	-	-	-	-	-	-
Bordetella bronchiseptica		-	-	-	-	-	-	-
Bordetella pertussis		-	-	-	-	-	-	-
Chlamydia pneumoniae		-	-	-	-	-	-	-
Clostridium diphtheriae		-	-	-	-	-	-	-
Haemophillus influenzae type A		-	-	-	-	-	-	-
Klebsiella pneumoniae		-	-	-	-	-	-	-
Listeria pneumophila		-	-	-	-	-	-	-
Moraxella cartarrhalis		-	-	-	-	-	-	-
Mycobacterium avium		-	-	-	-	-	-	-
Mycobacterium intracellulare		-	-	-	-	-	-	-
Mycoplasma hominis type 1		-	-	-	-	-	-	-
Mycoplasma orale		-	-	-	-	-	-	-

TABLE 15		DFA Reagent (Results are Positive (+) or Negative (-) for Reactivity)						
Organism	Strain	Adeno	Flu A	Flu B	Para 1	Para 2	Para 3	RSV
Mycoplasma pneumoniae		-	-	-	-	-	-	-
Mycoplasma salivarium		-	-	-	-	-	-	-
Pseudomonas aeruginosa		-	-	-	-	-	-	-
Streptococcus pneumoniae		-	-	-	-	-	-	-
Streptococcus pyogenes		-	-	-	-	-	-	-
Ureaplasma urealyticum		-	-	-	-	-	-	-
Cell cultures:								
A549		-	-	-	-	-	-	-
BGMK		-	-	-	-	-	-	-
HEp-2		-	-	-	-	-	-	-
LLC-MK2		-	-	-	-	-	-	-
MDCK		-	-	-	-	-	-	-
MRC-5		-	-	-	-	-	-	-
MRHF		-	-	-	-	-	-	-
Mv1Lu		-	-	-	-	-	-	-
NCI-H292		-	-	-	-	-	-	-
pCMK		-	-	-	-	-	-	-
pRhMK		-	-	-	-	-	-	-
pRK		-	-	-	-	-	-	-
RD		-	-	-	-	-	-	-
RhMK II		-	-	-	-	-	-	-
R-Mix™		-	-	-	-	-	-	-
R-Mix™ Too		-	-	-	-	-	-	-
Vero		-	-	-	-	-	-	-
WI-38		-	-	-	-	-	-	-

* Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

XI. BIBLIOGRAPHY

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Special 510(k): Device Modification
D[3] *Ultra* DFA Respiratory Virus Screening & ID Kit

DIAGNOSTIC HYBRIDS
Integrating Science and Humanity

DATE OF PREPARATION OF 510(k) SUMMARY

July 23, 2009

APPLICANT

DIAGNOSTIC HYBRIDS, INC.
1055 East State Street
Suite 100
Athens, OHIO 45701

CONTACT INFORMATION

Ronald H. Lollar
Senior Director, Product Realization, Management, and Marketing
E-mail: lollar@dhiusa.com
Telephone: 740-589-3300
Desk Extension: 740-589-3373
FAX: 740-593-8437

DEVICE NAME

Trade name: D[3] *Ultra* DFA Respiratory Virus Screening & ID Kit
Common name: Respiratory Virus DFA Assay
Classification name: Antisera, Cf, Influenza A, B, C
Product Code: GNW
Regulation: 21 CFR § 866.3330, Class I, Influenza virus serological reagents, Panel Microbiology (83)

LEGALLY MARKETED DEVICE

D[3] *Ultra* DFA Respiratory Virus Screening & ID Kit, K061101

DESCRIPTION of DEVICE MODIFICATION

The product insert has been modified. The following has been added (see below):

Table 15 in the product insert has been updated to include reactivity data on influenza A virus Mexico/4108/2009 and California/07/2009 strains. The following language was included with the data:

"Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical

specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D[3] *Ultra* DFA Respiratory Virus Screening & ID Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes."

INTENDED USE

The Diagnostic Hybrids, Inc. D[3] *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[1] is available to receive and culture specimens.[2]

ASSESSMENT OF NON-CLINICAL PERFORMANCE DATA FOR EQUIVALENCE

Not Applicable

ASSESSMENT OF NON-CLINICAL PERFORMANCE DATA FOR EQUIVALENCE

The risk analysis method used to assess the impact of the modification was a Failure Modes and Effects Analysis (FMEA). The modification to device labeling poses no additional risk.

BIOCOMPATABILITY

[1] www.cdc.gov
[2] FDA Guidance Document: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; Issued 4/10/2006

Not applicable

STERILIZATION

Not applicable

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
10903 New Hampshire Avenue
Document Mail Center - WO66-G609
Silver Spring, MD 20993-0002

AUG 2 8 2009

Ron Lollar
Senior Director, Product Realization, Management, and Marketing
DIAGNOSTIC HYBRIDS, INC.
1055 East State Street, Suite 100
Athens, Ohio 45701

Re: K092300
Trade/Device Name: D3 Ultra DFA Respiratory Virus Screening & ID Kit
Regulation Number: 21 CFR 866.3330
Regulation Name: Influenza virus serological reagents
Regulatory Class: Class I
Product Code: GNW
Dated: July 23, 2009
Received: July 29, 2009

Dear Mr. Lollar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket

notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, Ph.D.
Director, Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): k092300

Device Name: D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit

Indication For Use:

The Diagnostic Hybrids, Inc. D^3 *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[1] is available to receive and culture specimens.[2]

Prescription Use _X____ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Division Sign-Off

Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) 092300

1 www.cdc.gov

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