D³® Ultra 8™ DFA
Respiratory Virus Screening & Identification Kit
REF: I-01-110000
For in vitro Diagnostic Use

I. INTENDED USE

The Diagnostic Hybrids, Inc. D³® Ultra 8™ DFA Respiratory Virus Screening & Identification Kit is intended for the qualitative detection and identification of influenza A, influenza B, respiratory syncytial virus, metapneumovirus, adenovirus, and parainfluenza virus types 1, 2, and 3 in respiratory specimens, by either direct detection or cell culture method, by immunofluorescence using fluorescein-conjugated 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.

II. SUMMARY AND EXPLANATION OF THE TEST

With the addition of new antiviral drugs for the treatment of influenza,2 more rapid and sensitive tests for respiratory virus detection14, and the increasing need to be more discriminating in the use of antibiotics3, 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.
Adenoviruses can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCx 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 four different types cause croup and lower respiratory illness in children. Parainfluenza type 3 is the most common cause of lower respiratory illness in adults. Parainfluenza is the second leading cause of respiratory illness in adults. Outbreaks caused by parainfluenza viruses usually occur in the fall during alternate years (P1 and P2) or throughout the year, with increased activity in the spring (P3).8

Parainfluenza viruses can be isolated in cell cultures of A549/Mv1Lu mixtures (R-Mix), A549/MDCx 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.

III. PRINCIPLE OF THE PROCEDURE
The Diagnostic Hybrids, Inc. D³ Ultra δ DFA (direct fluorescent antibody) Respiratory Virus Screening & Identification 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 MAbs directed against eight respiratory viruses (influenza A, influenza B, RSV, MPV, adenovirus, parainfluenza virus types 1, 2, and 3) plus eight 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° to 37°C, the staining cells are rinsed with the diluted Wash Solution (1X). A drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. Virus-infected cells will be stained with viral specific apple-green fluorescence when stained with the DFA Screening Reagent while non-infected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain. The positive well contains infected cells. The negative well contains non-infected cells. Each slide is intended to be stained only one time.

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.

2. Cell culture material may have some potential to be hazardous. Personnel working with cell cultures must be properly trained in safe handling practices.9,16,10 and have experience with cell cultures before attempting this procedure.

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

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%. It is intended to be stained only one time.

8. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

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

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 non-reactive with any of the listed respiratory viruses. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

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

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

13. Influenza A DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by influenza A virus infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

14. 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.

15. RSV DFA Reagent, 2-mL. One dropper bottle containing fluorescein-labeled murine monoclonal antibodies directed against antigens produced by respiratory syncytial virus (L strain) infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
RS1/53 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 gases.

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. Reagents should be used prior to their expiration date.

11. Each Antigen Control Slide 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° 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 demineralized water.

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

D. STORAGE INSTRUCTIONS

<table>
<thead>
<tr>
<th>Table 1: Reagent Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Respiratory Virus DFA Screening Reagent</td>
</tr>
<tr>
<td>2. Influenza A DFA Reagent</td>
</tr>
<tr>
<td>3. Influenza B DFA Reagent</td>
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<td>4. RSV DFA Reagent</td>
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<tr>
<td>5. Metapneumovirus DFA Reagent</td>
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<td>6. Adenovirus DFA Reagent</td>
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<tr>
<td>7. Parainfluenza 1 DFA Reagent</td>
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<tr>
<td>8. Parainfluenza 2 DFA Reagent</td>
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<tr>
<td>9. Parainfluenza 3 DFA Reagent</td>
</tr>
<tr>
<td>10. Mounting Fluid</td>
</tr>
<tr>
<td>11. Normal Mouse Gamma Globulin DFA Reagent</td>
</tr>
<tr>
<td>12. Respiratory Virus Antigen Control Slides</td>
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<td>13. hMPV Antigen Control Slides</td>
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<td>14. 40X Wash Solution Concentrate</td>
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<tr>
<td>NOTE: 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>15. 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.®

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.21 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 2-7 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. Metapneumovirus DFA Reagent
6. Adenovirus DFA Reagent
7. Parainfluenza 1 DFA Reagent
8. Parainfluenza 2 DFA Reagent
9. Parainfluenza 3 DFA Reagent
10. Normal Mouse Gamma Globulin DFA Reagent
11. Respiratory Virus Antigen Control Slides
12. hMPV Antigen Control Slides
13. Mounting Fluid
14. 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 MixCells™, and primary Rhesus monkey kidney cells. All are available from DHI.
3. Live control viruses for positive culture controls: Known strains of the 8 respiratory viruses for use in monitoring the cell culture and staining procedures. Such control virus strains can be obtained from DHI.
4. Coverslips (22 x 50mm) for Antigen Control Slides and for specimen slides.
5. Universal Transport Medium. Available from DHI.
6. R-Mix Reused Medium (for use with R-Mix and R-Mix Too MixCells) or other standard refed medium. Available from DHI.
7. Reagent-grade acetone (>99%) pure) chilled at 2° to 8°C for fixation of direct specimen slides, shell-vials and cultured cell preparations.

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

NOTE 2: A mixture of 80% acetone / 20% demineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature (20° to 25°C).
8. Sterile graduated pipettes: 10-mL, 5-mL, and 1-mL.
9. Sterile Pasteur pipettes or other transfer pipettes.
10. Caution: One should not use solvents such as acetone with polyethylene transfer pipettes.

12. Sterile 0.45-µm syringe filter.
13. Sterile 3-mL syringe.
14. 200-mL wash bottle.
15. 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) to a point (20° to 25°C).
16. Sodium hypochlorite solution (1:10 final dilution of household bleach).
17. Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).
18. Glass microscope slides.
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-second 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 staining into the incubator for equilibration to 35° to 37°C prior to staining. By doing this, the test slides and reagents will come to temperature quickly, yielding more rapid, intense staining.
5. Before staining with DFA Reagents, bring the suspension to ambient temperature (20° to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2° to 8°C.

D. SPECIMEN PREPARATION
For specimen processing recommendations, refer to CLSI Approved Viral Culture Guidelines.
1. Vortex the specimen vigorously for 10- to 15-seconds.
2. Centrifuge at 400 to 600 X g for 5- to 10-minutes.
3. Collect and set aside the supernatant for viral isolation. (Step VI.D.10 below.)
4. Add 5-μL of PBS and vortex vigorously for 10- to 15-seconds.
5. Centrifuge at 400 to 600 X g 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.
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 suspension will be used for Direct Specimen Testing (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. Some suspensions may also be centrifuged if a monolayer is preferred.
10. For use in Cell Culture Testing (Section VI. F., G., 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 prepared cell 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 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 1-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 and hMPV Antigen Control Slide add 1-drop of the DFA Screening Reagent. The Antigen Control Slides should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.
7. Add 1-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 deionized water. For only a few slides, this can be done using a beaker of the deionized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with deionized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
12. Gently blot the excess deionized water.
13. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
14. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X. (Section VI. C. 11-14, ‘Immunofluorescence Microscopy’).
15. Refer to Section VII. ‘Interpretation of Results’.
16. 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.
a) Add 1-drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Add the Metapneumovirus DFA Reagent to the well labeled 'Neg'.

b) For the Respiratory Virus Antigen Control Slide, add 1-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) For the MPHV Antigen Control Slide add 1-drop of the Metapneumovirus DFA Reagent to each well. **NOTE:** An Antigen Control Slide should be stained only once, do not re-stain.

d) Continue with steps 8 through 15, 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 0.4-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°C to 37°C.

4. After adsorption, add 2-mL of appropriate refined medium.

5. Incubate the tubes at 35°C to 37°C in a roller drum at 1 to 3 rpm.

6. When the monolayers are ready to be stained, remove the medium by aspiration and gently rinse the monolayer two times with 1- to 2-mL PBS.

7. Add 0.5-mL of PBS to the tube and prepare a suspension of the cells by scraping the monolayer using a pipette and breaking the cell aggregates up by pipetting the PBS up and down several times.

8. Prepare cell spots using about 25-μL of the suspension on each well of a 2-well and an 8-well slide. Repeat this step for each specimen.

9. Air dry the wells completely.

10. Fix the cells to the slides using fresh, chilled 100% acetone. Let stand for 5- to 10-minutes, at 20° to 25°C. **Caution:** Acetone is volatile and flammable; keep away from open flames.

11. Remove the slides from the fixative and allow to dry.

12. Add 1-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 1-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 demineralized water. 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 demineralized water. For only a few slides, this can be done using a beaker of the demineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with demineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.

18. Remove the demineralized water by aspiration.

19. Gently blot the excess liquid.

20. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.

21. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X. (Section VI.C. 11-14, 'Immunofluorescence Microscopy'.) 

22. Refer to Section VII 'Interpretation of Results'.

23. 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 (refer to Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:

   a) Add 1-drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Add the Metapneumovirus DFA Reagent to the well labeled 'Neg'.

   b) For the Respiratory Virus Antigen Control Slide, add 1-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) For the MPHV Antigen Control Slide add 1-drop of the Metapneumovirus DFA Reagent to each well. **NOTE:** An Antigen Control Slide should be stained only once, do not re-stain.

   d) 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 each 8-well specimen slide.

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

6. Place stopped 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 by aspiration and add 1-mL of PBS.

8. Swirl to mix and then aspirate.

9. Repeat this wash with another 1-mL of PBS and then aspirate.

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

20. Remove the demineralized water by aspiration.

21. Examine the fixed monolayer using a bent-tip needle on a syringe barrel. Grabbing it with the fine tipped forceps, transfer it, monolayer-side down, to a small drop of Mounting Fluid on a standard microscope slide.

22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (Section VI.C. 11-14, Immunofluorescence Microscopy.)

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

24. If the result is positive for respiratory virus culture, 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 (refer to Section VI.F. steps 6 through 11, for procedure to prepare a specimen slide), then:

   a) Add 1-drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Add the Metapneumovirus DFA Reagent to the well labeled 'Neg'.

   b) For the Respiratory Virus Antigen Control Slide, add 1-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) For the MPHV Antigen Control Slide add 1-drop of the Metapneumovirus DFA Reagent to each well. **NOTE:** An Antigen Control Slide should be stained only once, do not re-stain.

   d) Continue with VI.F., steps 14 through 21.
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 by aspiration, repeat the wash step, and again remove by aspiration.

18. Remove the 1X Wash Solution by aspiration, repeat the wash step, and again remove by aspiration.

19. Add 1 mL of demineralized water.

20. Add 2 -to -3 -drops of Mounting Fluid to each monolayer, then cover the plate.

21. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (Section VI. C. 11-14, ‘Immunofluorescence Microscopy’).

22. Refer to Section VII. ‘Interpretation of Results’.

23. 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 (Refer to Section VII. F., steps 6 through 11, for procedure to prepare a specimen slide), then:
   a) Add 1-drop of each individual virus DFA Reagent to its corresponding well on the 8-well specimen slide. Add the Metapneumovirus DFA Reagent to the well labeled ‘Neg’.
   b) For the Respiratory Virus Antigen Control Slide, add 1-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) For the hMPV Antigen Control Slide add 1-drop of the Metapneumovirus DFA Reagent to each well.
      NOTE: An Antigen Control Slide should be stained only once, do not re-stain.
   d) Continue with VII. F., steps 14 through 21.

I. QUALITY CONTROL

1. Reagents
   a) A fresh Respiratory Virus and hMPV 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 fluoresce 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.

Metapneumovirus: The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.

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 fluorescent cells 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, metapneumovirus, 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, metapneumovirus, adenovirus, respiratory syncytial virus, parainfluenza type 1, 2, or 3 (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 VII.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, metapneumovirus, 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, metapneumovirus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3 isolated in cell culture.”

2. If apple-green fluorescing cells are found, the identification of the virus(es) may be based on the appropriate Sections VI. F., G., and H.). In such cases, identification of the viral antigen(s) should be reported as “… isolated in cell culture”, where “… is the appropriate virus, e.g., influenza A virus, influenza B virus, respiratory syncytial virus, metapneumovirus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, or parainfluenza virus type 3.

VIII. LIMITATIONS OF PROCEDURE

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

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 supplied 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 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 2007/2008. Prevalence of the respiratory viruses within the population of specimens that was prospectively collected and the cells directly tested is noted in Table 2 below.

<table>
<thead>
<tr>
<th>Expected Values</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>MPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Specimens (n = 516)</td>
<td>23</td>
<td>71</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>74</td>
<td>12</td>
</tr>
<tr>
<td>Prevalence</td>
<td>4.5%</td>
<td>13.8%</td>
<td>1.0%</td>
<td>0.8%</td>
<td>0.2%</td>
<td>0.8%</td>
<td>14.3%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

X. SPECIFIC PERFORMANCE CHARACTERISTICS

A. CLINICAL PERFORMANCE CHARACTERISTICS

1. Study Site 1

The study consisted of a total of 300 fresh specimens submitted, January through February 2007, to the laboratory for respiratory virus testing. Slides were prepared from PBS-washed cells from the fresh specimens and fixed in accordance with the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices). The slides were stored at -70°C until testing was performed. The preparation of specimen slides, fixing in acetone, and freezing is common laboratory practice in order to batch test at a later time. With the fixation in acetone, prior to freezing, the cellular morphology is maintained during freezing and on thawing.

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

<table>
<thead>
<tr>
<th>TABLE 3: Study Site 1 - Age Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 month</td>
</tr>
<tr>
<td>&gt;1 month - 2 years</td>
</tr>
<tr>
<td>&gt;2 - 12 years</td>
</tr>
<tr>
<td>&gt;12 - 21 years</td>
</tr>
<tr>
<td>22 - 30 years</td>
</tr>
<tr>
<td>31 - 40 years</td>
</tr>
<tr>
<td>41 - 50 years</td>
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<tr>
<td>51 - 60 years</td>
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<tr>
<td>71 - 80 years</td>
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<tr>
<td>81 - 90 years</td>
</tr>
<tr>
<td>91 - 100 years</td>
</tr>
<tr>
<td>Unknown age</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>61 - 70 years</td>
</tr>
<tr>
<td>81 - 90 years</td>
</tr>
<tr>
<td>51 - 60 years</td>
</tr>
</tbody>
</table>

The following tables detail the results from Study Site 1:

Table 4 compares the results of the D³ Ultra 8 DFA Respiratory Virus Screening Reagent with those of the D³ Ultra 8 DFA Respiratory Virus Screening Reagent for detecting the seven respiratory viruses identified by the D³ Ultra Screening Reagent in cells derived from a specimen.

Table 5 compares the D³ Ultra 8 Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumoviruses in cells derived from a specimen.

Table 6 compares the results of the D³ Ultra 8 DFA Respiratory Virus Screening Reagent with those of the D³ Metapneumovirus DFA Reagent.

Study Site 1 - CONCLUSION

A variety of viral respiratory pathogens were detected: respiratory syncytial virus [prevalence 15% (45/300)], influenza A virus [prevalence 9% (27/300)], influenza B virus [prevalence 0.3% (1/300)], metapneumovirus [prevalence 1.7% (5/300)], adenovirus [prevalence 7% (21/300)], parainfluenza type 1 virus [prevalence 1% (3/300)] and parainfluenza type 3 virus [prevalence 1.3% (4/300)]. No co-infections were detected.

The D³ Ultra 8’s ability to identify the seven viruses in direct specimens was compared to the D³ Ultra’s ability. The positive percent agreement was 100% (95% CI range of 96.3% to 100%). The negative percent agreement was 100% (95% CI range of 98.1% to 100%). The D³ Ultra 8’s ability to identify metapneumovirus in direct specimens was compared to the D³ Metapneumovirus DFA Reagent’s ability. The positive percent agreement was 100% (95% CI range of 96.6% to 100%). The negative percent agreement was 100% (95% CI range of 97.1% to 100%).

For Study Site 1, the performance results of the D³ Ultra 8, when compared to those of the Comparator devices, D³ Ultra and D³ Metapneumovirus DFA Reagent, demonstrate that the device detect respiratory virus antigens in similar manners, and that the addition of the metapneumovirus MAbs do not adversely impact the other MAbs for the detection of the other respiratory viruses found (i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus and parainfluenza virus types 1 and 3). No increase in background was seen with the D³ Ultra 8.

TABLE 2: Prevalence of the Respiratory Viruses within the Study Population

<table>
<thead>
<tr>
<th>Expected Values</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>MPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Specimens (n = 516)</td>
<td>23</td>
<td>71</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>74</td>
<td>12</td>
</tr>
<tr>
<td>Prevalence</td>
<td>4.5%</td>
<td>13.8%</td>
<td>1.0%</td>
<td>0.8%</td>
<td>0.2%</td>
<td>0.8%</td>
<td>14.3%</td>
<td>2.3%</td>
</tr>
</tbody>
</table>

TABLE 3: Study Site 1 - Age Distribution

<table>
<thead>
<tr>
<th>0 - 1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1 month - 2 years</td>
</tr>
<tr>
<td>&gt;2 - 12 years</td>
</tr>
<tr>
<td>&gt;12 - 21 years</td>
</tr>
<tr>
<td>22 - 30 years</td>
</tr>
<tr>
<td>31 - 40 years</td>
</tr>
<tr>
<td>41 - 50 years</td>
</tr>
<tr>
<td>51 - 60 years</td>
</tr>
<tr>
<td>71 - 80 years</td>
</tr>
<tr>
<td>81 - 90 years</td>
</tr>
<tr>
<td>91 - 100 years</td>
</tr>
<tr>
<td>Unknown age</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

TABLE 4: Study Site 1 – Comparison of D³ Ultra 8 and the D³ Ultra for Detecting all Seven Respiratory Viruses

<table>
<thead>
<tr>
<th>Direct Specimen (300 Specimens)</th>
<th>D³ Metapneumovirus DFA Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>101</td>
</tr>
<tr>
<td>Neg</td>
<td>0</td>
</tr>
<tr>
<td>Positive Percent Agreement (PPA)</td>
<td>100% (101/101)</td>
</tr>
<tr>
<td>Negative Percent Agreement (NPA)</td>
<td>95% CI- NPA §</td>
</tr>
</tbody>
</table>

TABLE 5: Study Site 1 – D³ Ultra 8 Identification of Metapneumovirus Positive Specimens

<table>
<thead>
<tr>
<th>Direct Specimen (300 Specimens)</th>
<th>D³ Metapneumovirus DFA Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>5</td>
</tr>
<tr>
<td>Neg</td>
<td>0</td>
</tr>
<tr>
<td>Positive Percent Agreement (PPA)</td>
<td>100% (5/5)</td>
</tr>
<tr>
<td>Negative Percent Agreement (NPA)</td>
<td>95% CI- NPA §</td>
</tr>
</tbody>
</table>

TABLE 6: Study Site 1 – Comparison of D³ Ultra 8 Screening Reagent with D³ Metapneumovirus DFA Reagent

| Positive Percent Agreement (PPA) | 100% (295/295) |
| Negative Percent Agreement (NPA) | 97.1, 100% |

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

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

* “Negative Percent Agreement”, or “NPA”, values were calculated according to [{(Total Number of Negative Results in Agreement by both Subject Device and Comparator Devices) plus (Number of Results Negative by Comparator Device but Positive by Subject Device)] multiplied by 100%.}.
2. Study Site 2

Two hundred and sixty-eight (268) specimens were processed for direct specimen testing in accordance with the procedure in the D³ Ultra product insert (same procedure for both Subject and Comparator devices). The first forty-eight specimens were not included in analysis due to a processing issue; the cells were not rinsed sufficiently and did not remain fixed on the slide. An additional rinse step was added to the procedure for the remaining 220 specimens; the cells remained fixed to the slides. Of these 220 specimens, 4 had insufficient cells (<20) present to be interpreted. A total of 216 specimens remained for final analysis.

Two hundred and sixty-eight (268) specimens were processed for cell culture testing in accordance with the procedure in the D³ Ultra product insert (same procedure for both Subject and Comparator devices). The specimens were processed for cell culture using R-Mix Too™ FreshCells™ in 48/24/48 multi-well plates. Thirteen (13) specimens were toxic and 1 was contaminated in cell culture. A total of 252 specimens remained for final analysis.

Table 6 shows the age distribution for individuals studied at Study Site 2:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1 month</td>
<td>78</td>
</tr>
<tr>
<td>&gt;1 month - 2 years</td>
<td>35</td>
</tr>
<tr>
<td>&gt;2 - 12 years</td>
<td>35</td>
</tr>
<tr>
<td>&gt;12 - 21 years</td>
<td>35</td>
</tr>
<tr>
<td>22 - 30 years</td>
<td>39</td>
</tr>
<tr>
<td>31 - 40 years</td>
<td>19</td>
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<td>41 - 50 years</td>
<td>19</td>
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<td>51 - 60 years</td>
<td>11</td>
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<td>61 - 70 years</td>
<td>12</td>
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<tr>
<td>71 - 80 years</td>
<td>7</td>
</tr>
<tr>
<td>81 - 90 years</td>
<td>5</td>
</tr>
<tr>
<td>&gt;90 years</td>
<td>1</td>
</tr>
<tr>
<td>Unknown age</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
</tr>
</tbody>
</table>

The following tables detail the direct specimen testing results for Study Site 2:

Table 7 compares the results of the D³ Ultra Screening Reagent with those of the D³ Ultra Screening Reagent in cells derived from a specimen.

Table 8 compares the D³ Ultra 8 Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a specimen.

The following tables detail the cell culture testing results for Study Site 2:

Table 9 compares the results of the D³ Ultra Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a culture.

Table 10 compares the D³ Ultra 8 Screening Reagent with those of the D³ Metapneumovirus DFA Reagent for detecting metapneumovirus in cells derived from a culture.
cultures were negative. [Table 11, Cross-Reactivity Study Results. The table indicates which organisms were reactive with which DFA Reagent.]

Stringent conditions for cross-reactivity testing were achieved by using high concentration of the DFA Reagents and high titers of microorganisms. The DFA Reagents (i.e., directly fluorocesinated 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-hour intervals, 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 11: Cross-Reactivity Study Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
<th>D³ Ultra 8 Respiratory Screening Reagent at 1.5X Concentration</th>
<th>TCID₅₀/Source or CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AS49</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>BGMK</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>CV-1</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>HEp-2</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>Hs27 (HFF)</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>LLC-MK2</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>McCoy</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>MRC-5</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>MRhF</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>MV1Lu</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>NCl-H292</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>pAGMK</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>pCMK</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>pReMK</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>pReMK II</strong></td>
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<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>pRK</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>RD</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>R-Mix</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
</tr>
<tr>
<td><strong>R-Mix Too</strong></td>
<td></td>
<td>*</td>
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</tr>
<tr>
<td><strong>Vero</strong></td>
<td></td>
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<tr>
<td><strong>WI-38</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
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<tr>
<td><strong>Type 1</strong></td>
<td></td>
<td>*</td>
<td>714 TCID₅₀</td>
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<tr>
<td><strong>Type 3</strong></td>
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<td><strong>Type 5</strong></td>
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<td><strong>Type 6</strong></td>
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<td><strong>Type 13</strong></td>
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<td><strong>Type 14</strong></td>
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<td><strong>Type 18</strong></td>
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<td><strong>Type 31</strong></td>
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<td><strong>Type 40</strong></td>
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<td>*</td>
<td>714 TCID₅₀</td>
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<td><strong>Type 41</strong></td>
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<td><strong>WS (H1N1)</strong></td>
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<td><strong>PR (H1N1)</strong></td>
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<td>*</td>
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</table>

### Notes:

1. Staining of *S. aureus* appeared as small points of fluorescence while all other cultures were negative. This will be noted in labeling in the section "Limitations of the Assay": The Protein A produced by the bacterium, *Staphylococcus aureus*, will bind the Fc portion of some of the fluorescein-labeled monoclonal antibodies used in this kit. 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.
TABLE 11: Cross-Reactivity Study Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
<th>D³ Ultra 8 Respiratory Screening Reagent at 1:5X Concentration</th>
<th>TCID₅₀/Source or CFU</th>
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</thead>
<tbody>
<tr>
<td>Chlamydia phila psittaci</td>
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<td>Control slide</td>
<td></td>
</tr>
<tr>
<td>Chlamydia trachomatis</td>
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<td>Control slide</td>
<td></td>
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<tr>
<td>Yeast</td>
<td>Candida glabrata</td>
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<td>8.7 X 10⁶ CFU</td>
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<tr>
<td>Protozoa</td>
<td>Trichomonas vaginalis</td>
<td>-</td>
<td>Control slide</td>
</tr>
</tbody>
</table>

*Although this test has been shown to detect the 2009 H1N1 influenza virus in two cultured isolates, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D³ Ultra 8 DFA Respiratory Screening & Identification Kit can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.

XI. BIBLIOGRAPHY


WARRANTY STATEMENT

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