D³ DFA
Varicella-zoster Virus Identification Kit

REF: 01-020000
For in vitro Diagnostic Use

I. INTENDED USE
The Diagnostic Hybrids, Inc. D³ DFA Varicella-zoster Virus Identification Kit is intended for use in the qualitative detection of Varicella-zoster virus (VZV) in cell cultures by immunofluorescence using fluorescent monoclonal antibodies (MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decision.

Performance testing has not been done on direct patient specimen testing.

II. SUMMARY AND EXPLANATION OF THE TEST
VZV is a latent DNA virus in the family Herpessviridae. The virus is associated with two disease states: chickenpox (primary infections) and shingles (reactivation of latent virus). When an appropriately sensitive cell line is infected with VZV, a characteristic deterioration of cells, termed cytopathic effect (CPE), can be observed. Tube culture, a classic format for virus amplification, can take several days before CPE is evident. In the case of those specimens with low titers of virus, 7-14 days of tube culture may be required before CPE can be observed.

Even so, CPE may be difficult to interpret due to, for instance, deterioration of cells, which can result from toxic components present in the clinical specimen making microscopic examination of the infected cells problematic. Because of this, immunofluorescence confirmation of cell culture is necessary for confirmation of a VZV positive result.

III. PRINCIPLE OF THE PROCEDURE
The Diagnostic Hybrids, Inc. D³ DFA Varicella-zoster Virus Identification Kit uses a blend of VZV antigen-specific murine MAbs that are directly labeled with fluorescein for the rapid identification of VZV in cell culture.

The infected cells are fixed in acetone on a slide prepared from a tube culture or cell monolayer from either a shell-vial or multi-well plate. The VZV DFA Reagent is added to the cells to detect the presence of VZV specific viral antigens. After incubating for 15-30 minutes at 36º to 37ºC, the stained cells are washed with the diluted Phosphate Buffered Saline (1X PBS) and, using the supplied Mounting Fluid, prepared for examination. The slides or wells are examined using a fluorescence microscope equipped with the correct filter combination for fluorescein isothiocyanate (FITC) at a magnification of 200X-400X. By staining with the VZV DFA Reagent, which contains Evans Blue as a counter-stain, virus infected cells will show bright apple-green fluorescence that will be distinguished from the counter-stained non-infected cells exhibiting dull red fluorescence.

IV. REAGENTS
A. Kit Components
1. VZV DFA Reagent, 5-ml. One dropper bottle containing a blend of two fluorescein labeled murine monoclonal antibodies directed against a recombinant glycoprotein E (gE) from the Ellen strain of VZV. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
2. Mounting Fluid, 7-ml. One dropper bottle containing an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.
3. VZV Antigen Control Slides, 5-slides. Five (5) individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each VZV positive well is identified. The negative well contains non-infected cells. Each slide is intended to be stained only one time.
4. 40X PBS Concentrate, 25-ml. One bottle of 40X PBS concentrate consisting of 4% sodium azide (0.1% sodium azide after dilution to 1X using demineralized water).

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 should be handled in accordance with the OSHA Standard on Bloodborne Pathogens.¹

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

b. All procedures must be conducted in accordance with the CDC 5th Edition Biosafety in Microbiological and Biomedical Laboratories, 2007, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.

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

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

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

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

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

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

6. Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.

7. Sodium azide is included in the 40X PBS 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 DHI Technical Services.

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

R28 Very toxic if swallowed.
R32 Contact with acids liberates very toxic gas.
S28 After contact with skin, wash immediately with plenty of water.
S45 In case of accident, or if you feel unwell, seek medical advice immediately.
S60 This material and its container must be disposed of as hazardous waste.
N: Sodium azide may present an immediate or delayed danger to one or more components of the environment.

R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.
a. Reagents containing sodium azide should be considered poisons. If products containing sodium azide are swallowed, seek medical advice immediately and show product container, label, or MSDS to medical personnel. [Refer to NIOSH, National Institute for Occupational Safety and Health; CAS# 7446-50-0; EC# 203-014-0; and also to OSHA, 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 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 buildup. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.

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

9. The VZV DFA Reagent is supplied at working strength. Any dilution to the DFA Reagent will decrease sensitivity.

10. Reagent should be used prior to its expiration date.

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

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

13. Store 1X PBS in a clean container to prevent contamination.

14. Microbial contamination of the VZV DFA Reagent may cause a decrease in sensitivity. Specimen collection, specimen processing, and cell culture strains can be obtained from DHI.

15. Do not expose the VZV DFA Reagent 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 PBS

1. After storage at 2° to 8°C, some salts in the 40X PBS 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 PBS Concentrate to 975-mL of demineralized water.

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

4. Store liquid at 2° to 8°C prior to dilution.

5. Store at ambient temperature (20° to 25°C).

VI. PROCEDURE

A. Materials Provided

1. VZV DFA Reagent
2. Mounting Fluid
3. 40X PBS Concentrate
4. VZV Antigen Control Slides

B. Materials Required But Not Provided

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

2. Cell culture for VZV isolation. Suggested cell lines include H&V-Mix™ MixedCells™, human newborn foreskin, MRC-5, CV-1 and AS49. All are available from DHI.

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

4. Universal Transport Medium. Available from DHI.

5. Tissue culture reseeded medium (Eagle’s Minimum Essential Medium with 2% fetal bovine serum, 25mM HEPES and antibiotics). Available from DHI.

6. Reagent-grade acetone (+99% pure) chilled at 2° to 8°C for fixation of shell-vials.

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).

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

7. Sterile Pasteur pipettes or other transfer pipettes

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


10. Stainless steel, 0.45-µm syringe filter.

11. Sterile 3-mL syringe.

12. 200-mL wash bottle.

13. Bent-tip teasing needle (for removal of coverslip from a shell-vial): fashion the mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.

14. Sodium hypochlorite (1:10 final dilution of household bleach).

15. Mechanical pipetter (e.g., covered Petri dish with a bump paper towel placed in the bottom).


17. Sterile nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell culture.

18. Incubator, 35° to 37°C (5% CO2 or non-CO2, depending on the cell culture format used).


20. Denitrogenized water for dilution of 40X PBS Concentrate (Section IV.C.) and for dilution of the reagent-grade acetone for use in polystyrene multi-well plates (Item VI.B.6. Note 2).

21. Live control viruses for positive culture controls: Known strains of VZV for use in monitoring the cell culture and staining procedures. Such control virus strains can be obtained from DHI.

22. Aspirator Set-up: Vacuum aspirator with disinfectant trap containing sufficient household bleach (5%) such that the concentration is not decreased by more than 10-fold as it is diluted with discarded fluids.

23. Wash Container: Beaker, wash bowl or Coplin jar for washing slides.

24. Fixing Container: Coplin jar, slide dish or polyethylene holder for slides in use for fixing the cells on the slides.

25. Inverted Light Microscope: Used for examining monolayers of cells prior to inoculation and examination for toxicity, confluency and for CPE. It should have between 40X to 100X magnification capability.

C. Preliminary Comments and Precautions

1. Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.

2. For specimen swabs received in transport medium with glass beads, vortex vigorously for about 15-seconds to dissociate adhered cells. For swabs not received in transport medium, transfer them to a tube of transfer medium containing glass beads and vortex vigorously for about 15-seconds to dissociate adhered cells.
3. When staining with fluorescent reagents and examining cells microscopically for fluorescence, it is very important to include controls, both positive and negative, to monitor the procedure and performance of the reagents. It is recommended that such controls be run with each batch of patient specimens.

4. Place the closed, humidified chamber for holding slides during 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. Bring the DFA Reagent to ambient temperature (20° to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2° to 8°C.

6. 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.

7. It is good practice to retain the medium removed from the monolayers until after staining results have been obtained. If there are any questions concerning the specimen results, the medium can be passed to another monolayer and incubated for the appropriate time period for repeat testing.

8. When using cell cultures in polystyrene multi-well plates, dilute the acetone fixative to 80% by adding 20-mL of demineralized water to 80-mL of acetone (Section VI.B.6 Note 2).

9. Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased sensitivity.

10. Do not allow the DFA Reagent to dry on the monolayers; this can lead to high background.

IMMUNOFLUORESCENCE MICROSCOPY:

11. Examine the positive and negative controls before examining the test specimens. If one of these fails to perform as expected, review the steps and conditions under which the test was performed to determine the root cause(s).

12. Do not report results for patient samples until controls perform as expected.

13. Three aspects of the fluorescence microscope must be functioning properly and optimally in order to achieve maximum brightness of fluorescence.

14. a. The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the DFA Reagent.

15. b. The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.

16. c. The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.

17. Fluorescent artifacts may be observed in the cell monolayers:

18. a. Cell debris, lint, etc. can non-specifically absorb the DFA Reagent, resulting in highly intense fluorescence. These can be identified by their morphology, i.e., they don’t have the appearance of a complete cell and typically are not seen on the same plane of the monolayer as the other cells would be.

19. b. A low grade, yellow-green fluorescence may sometimes be seen, particularly in areas that have pitted cells or are near holes in the cell monolayer. In both cases, the diffusion of the entrapped DFA Reagent is retarded during the wash step, resulting in the non-specific fluorescence.

20. Intense fluorescence around the periphery of slide wells is indicative of drying of the DFA Reagent during incubation, suggesting that it was incubated too long or the humidity was not well controlled.

21. Inadequate washing can lead to general low grade fluorescence due to residual DFA Reagent remaining on the monolayer of cells.

22. Protect stained slides and monolayers from light as much as possible during testing:

23. a. Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.

24. b. This bleaching can occur when a stained cell is viewed in a fluorescence microscope for an extended period of time.

D. Specimen Preparation

1. Swabs containing specimen material should be handled with sterile forceps. The swab should be rotated in viral transport medium and then pressed against the inside of the tube to allow excess fluid to drain back into the transport medium. Discard the swab into an appropriate disinfectant such as a sodium hypochlorite solution (1:10 dilution of household bleach).

2. Decontaminate the forceps after specimen disposal.

3. Swirl to mix and then aspirate.

4. Add 4 drops of the VZV DFA Reagent to each of the wells of a fresh VZV Antigen Control Slide. Each Antigen Control Slide can be stained only once.

5. Place the slides in a covered humidified chamber at 35° to 37°C for 15- to 30-minutes.

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

7. 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.

8. Blot the excess liquid.

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

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

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

F. Cell Culture Testing - Shell-vial

1. The laboratory that conducted studies for clearance of this assay using shell-vial culture [presented in Section X (‘Specific Performance Characteristics’)], used one shell-vial per specimen per test, and terminated cultures at a minimum of 72-hours.

NOTE: Shell-vial cultures are typically terminated after 1- to 7-days, depending on the particular agent. Frequently two time points are utilized, e.g., day two and days four to five for VZV. Selection of time points for termination of cultures and immunostaining of monolayers for the detection of viral antigens is best based on the laboratory’s experience and needs.

2. Examine the monolayers for proper morphology prior to inoculation.

3. Aspirate maintenance medium from the monolayers and add 1-mL of appropriate refed 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 stopped shell-vials in an incubator at 35° to 37°C.

7. When a monolayer is ready to be stained using the VZV DFA Reagent, remove the medium and add 1-mL of 1X PBS.

8. Swirl to mix and then aspirate.

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

10. Add 1-mL of 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. Discard the acetone into a biohazard container.

12. Add 0.5-mL of 1X PBS to wet the monolayer.

13. Swirl and then aspirate.

14. Add 4 drops of the VZV DFA Reagent to the fixed monolayers of patient and control samples, and rock to ensure complete coverage of the monolayer by the Reagent.

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

16. Aspirate the VZV DFA Reagent from the monolayers.

17. Add 1-mL of the 1X PBS.

18. Remove the 1X PBS by aspiration; repeat the wash step, and again remove by aspiration.

19. Add 0.5- to 1.0-mL of demineralized water.

20. Remove the demineralized water by aspiration.
G. Cell Culture Testing – Multi-well Plate

1. The laboratories that conduct studies for clearance of this assay using multi-well plates (presented in Section X ‘Specific performance characteristics’) used one well per specimen, and terminated cultures at a minimum of 72-hours.

NOTE: Multi-well plate cultures are typically terminated after 1- to 4-days, depending on the particular agent. Frequently two time points are utilized, e.g., day two and days four to five for VZV. Selection of time points for termination of cultures and immunostaining of monolayers for the detection of viral antigens is best based on the laboratory’s experience and needs.2

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% CO2 atmosphere.

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

8. Swirl to mix and then aspirate.

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

10. Add 1-mL of 80% acetone and let stand 5- to 10-minutes at 20° to 25°C.

NOTE: Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10-minutes since it may craze and cloud the plastic, making it difficult to examine the monolayers.

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

11. Remove the fixative by aspiration.

12. Add 0.5-mL of the 1X PBS to wet the monolayer.

13. Swirl and then aspirate.

14. To each well of a 24-well plate, add 4 drops of the VZV DFA Reagent to the fixed monolayers of patient and control samples; to each well of a 48-well plate, add 3 drops of the VZV DFA Reagent to the fixed monolayers of patient and control samples. 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 VZV DFA Reagent from the monolayers.

17. Add 1-mL of the 1X PBS.

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

19. Add 0.5- to 1.0-mL of demineralized water.

20. Remove the demineralized water by aspiration.

21. Add 3 drops of Mounting Fluid to each monolayer, and cover the plate.

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

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

H. Quality Control

1. Reagents
   a. A fresh VZV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
   b. The positive well will show infected areas of bright apple-green fluorescence where negative cells will fluoresce 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.

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, a VZV-inoculated control monolayer should be included each time a new lot of cell culture is used.
   c. Also, a non-inoculated monolayer from each lot should be kept and re-fed every 3- to 7-days and observed for normal cell growth; it may be used as a negative cell control when examining for CPE. All cell cultures should be stored at 35° to 37°C. Adverse storage conditions or handling procedures may also be noted in the negative control.
   d. If control cultures fail to perform correctly, results are considered invalid.

III. LIMITATIONS OF PROCEDURE

1. Intradermal inoculation of cultures or monolayers may yield a false positive result due to non-specific staining.

2. The fluorescence of the monolayers may be enhanced by minute amounts of specimen contamination with proteins or other materials.

3. Non-infected cells will stain dull red due to the Evans Blue counter-stain included in the DFA Reagents.

4. Examine the entire cell spot or monolayer of cells before reporting final negative 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 to eliminate non-specific staining.

C. Results from Culture Isolation/Confirmation

1. The bright apple-green fluorescence staining pattern is cytoplasmic.

2. Examine the entire cell spot or monolayer of cells for VZV-specific fluorescent cells. If no fluorescent cells are found, report: ‘No Varicella-zoster virus isolated by cell culture.’

3. If fluorescent cells are found, report results as, ‘Varicella-zoster virus isolated by cell culture’.

VIII. EXPECTED VALUES

The clinical studies described in Section X (‘Specific Performance Characteristics’) used only specimens collected and cultured for the presence of VZV. Most of the specimen types used in the clinical studies were swabs taken from skin lesions (with two taken as respiratory specimens (NP) and one CSF). Specimens were taken from the following body sites (and presented as # positive/# specimens) are described in Table 2 below.

<table>
<thead>
<tr>
<th>Site</th>
<th>Unknown</th>
<th>Facial</th>
<th>Genital</th>
<th>Perianal</th>
<th>Vaginal</th>
<th>Rectal</th>
<th>Eye</th>
<th>Face</th>
<th>Mouth</th>
<th>Other</th>
<th>CSF Brain</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>99</td>
<td>0/8</td>
<td>0/1</td>
<td>0/0</td>
<td>0/0</td>
<td>0/1</td>
<td>0/11</td>
<td>0/1</td>
<td>14/4</td>
<td>0/2</td>
<td>1/7</td>
<td>1/61</td>
</tr>
<tr>
<td>Site 2</td>
<td>35</td>
<td>0/0</td>
<td>0/0</td>
<td>1/2</td>
<td>0/0</td>
<td>0/0</td>
<td>1/3</td>
<td>0/0</td>
<td>0/2</td>
<td>0/0</td>
<td>9/2</td>
<td>0/7</td>
</tr>
<tr>
<td>Site 3</td>
<td>120</td>
<td>2/51</td>
<td>0/6</td>
<td>0/1</td>
<td>0/9</td>
<td>0/1</td>
<td>0/0</td>
<td>0/3</td>
<td>0/0</td>
<td>1/9</td>
<td>0/3</td>
<td>4/3</td>
</tr>
</tbody>
</table>

*mouth: mouth, lip, tongue, gum, throat

**perineum: groin, buttock, gluteal, coccyx, sacral, pubic, perianal

*skin: skin lesion, skin, finger, wrist, chest, axilla, abdomen, thigh, blister

Demographics by age and gender for the specimens that were tested at the three Study Sites are tabulated below.

Of the specimens evaluated in these studies (which had been submitted to the laboratories as swabs taken from lesions for both HSV and V2Z testing), a large
proportion were from patients between the ages of 18 and 40. Prevalence of VZV within the population tested was quite low (due in part to varicella vaccination programs). The patient demographics are listed below (Table 3).

### Table 3: Demographics by Age and Gender

<table>
<thead>
<tr>
<th>Source</th>
<th>Site 1 Values are # pos / Total</th>
<th>Site 2 Values are # pos / Total</th>
<th>Site 3 Values are # pos / Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>F M</td>
<td>F M</td>
<td>F M</td>
</tr>
<tr>
<td>&lt;2y</td>
<td>0/1 0/4</td>
<td>0/1 0/1</td>
<td>0/0 0/0</td>
</tr>
<tr>
<td>2y to 10y</td>
<td>0/1 0/1</td>
<td>0/1 0/1</td>
<td>0/0 0/0</td>
</tr>
<tr>
<td>10y to 18y</td>
<td>1/6 1/3</td>
<td>1/1 1/1</td>
<td>0/4 0/3</td>
</tr>
<tr>
<td>18y to 40y</td>
<td>0/18 0/18</td>
<td>0/1 0/1</td>
<td>0/3/6 0/13</td>
</tr>
<tr>
<td>&gt;40y</td>
<td>11/38 7/24</td>
<td>3/6 4/5</td>
<td>2/33 5/21</td>
</tr>
<tr>
<td>Age not reported</td>
<td>0/0 1/1</td>
<td>0/1 0/1</td>
<td>0/12 1/1</td>
</tr>
</tbody>
</table>

#### X. SPECIFIC PERFORMANCE CHARACTERISTICS

This study included two hundred and fifty-four (254) prospectively collected specimens submitted for VZV culture. Each specimen was evaluated by the D³ DFA Varicella-zoster Virus Identification Kit (D³ DFA VZV Kit) and compared to a currently-marketed VZV identification kit. A combination of fresh (61) and frozen (193) specimens were tested. Three fresh specimens from Site 2 were toxic to cell culture and were not evaluated by either test. One specimen from Site 3 was negative in the multi-well plate culture, but was positive in the tube culture 10-days post inoculation. These evaluations were conducted at two external laboratory sites and one in-house laboratory: (1) A reference laboratory in the Southeastern United States; (2) A reference laboratory in the Southwestern United States; and (3) Diagnostic Hybrids, Inc. in-house virology laboratory. The numbers of fresh and frozen specimens tested are summarized below (Table 4).

#### Table 4: Number of Fresh vs. Frozen Specimens

<table>
<thead>
<tr>
<th>Site</th>
<th>Culture</th>
<th>Site Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Frozen</td>
</tr>
<tr>
<td>1</td>
<td>57</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>120</td>
</tr>
</tbody>
</table>

Percent Agreement between the D³ DFA VZV Kit and comparator tests was calculated and tabulated in Table 5 for all the tested specimens is presented below (Table 4).

#### Table 5: Percent Agreement of All Tests

<table>
<thead>
<tr>
<th>Comparison Device</th>
<th>Positive Percent Agreement (^a) (PPA)</th>
<th>Negative Percent Agreement (^a) (NPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D³ DFA VZV Kit</td>
<td>100%</td>
<td>99.5%</td>
</tr>
<tr>
<td></td>
<td>95% CI – PPA</td>
<td>97.3% to 99.9%</td>
</tr>
<tr>
<td></td>
<td>Positive (\times)</td>
<td>Negative (\times)</td>
</tr>
<tr>
<td></td>
<td>42 1</td>
<td>0 208</td>
</tr>
</tbody>
</table>

#### A. Study Site 1: A total of 99 specimens were cultured for VZV using multi-well plates. Briefly, two hundred microliters (200-µL) of each specimen were inoculated using one well per specimen. The inoculated cells were centrifuged at 700xg for 60-minutes, incubated at 35° to 37°C for up to 72-hours then stained in accordance with each respective product insert (DHI and Comparison device). All calculations for confidence intervals were done according to the Exact Method\(^b\). The results of this testing are summarized below (Table 6).

#### Table 6: Study Site 1 – D³ DFA VZV Kit and Comparison Device in Multi-Well Plates

<table>
<thead>
<tr>
<th>Comparison Device</th>
<th>Positive (\times)</th>
<th>Negative (\times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D³ DFA VZV Kit</td>
<td>+ 22</td>
<td>– 0</td>
</tr>
<tr>
<td>PPA = Agreed</td>
<td>100%</td>
<td>95.1% to 100%</td>
</tr>
<tr>
<td>NPA = Agreed</td>
<td>100%</td>
<td>95.2% to 100%</td>
</tr>
</tbody>
</table>

#### B. Study Site 2: A total of 35 specimens were cultured for VZV. Three fresh specimens from this Site were toxic to cell culture and were not evaluated. Briefly, 200-µL from the specimens was inoculated into duplicate shell-vials. The inoculated cells were incubated at 35° to 37°C for 72-hours then stained in accordance with the respective product insert (DHI and Comparison devices). All calculations for confidence intervals were done according to the Exact Method\(^d\). The results of this testing are summarized below (Table 7).

#### Table 7: Study Site 2 – D³ DFA VZV Kit and Comparison Device in Shell-vials

<table>
<thead>
<tr>
<th>Comparison Device</th>
<th>Positive (\times)</th>
<th>Negative (\times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D³ DFA VZV Kit</td>
<td>+ 11</td>
<td>– 0</td>
</tr>
<tr>
<td>PPA = Agreed</td>
<td>100%</td>
<td>95.1% to 100%</td>
</tr>
<tr>
<td>NPA = Agreed</td>
<td>100%</td>
<td>95.1% to 100%</td>
</tr>
</tbody>
</table>

#### C. Study Site 3: A total of 120 specimens were cultured for VZV. Briefly, two hundred microliters (200-µL) of each specimen was inoculated into one well per specimen of a multi-well plate and a single cell culture tube. The inoculated plates were centrifuged at 700xg for 60-minutes, incubated at 35° to 37°C for 72-hours then stained in accordance with each respective product insert (DHI and Comparison devices). The results of this testing are summarized in Table 8a. The inoculated tubes were read for CPE daily for 14-days. Tubes exhibiting CPE were scraped and cell spots made on multi-well slides according to the Comparison device’s product insert procedure (the same procedure was used for both the DHI and the Comparison devices). Tubes exhibiting no CPE at 14-days were also scraped and cell spots made to confirm the absence of VZV. The cell spots were fixed with acetone in accordance with each respective product insert (DHI and Comparison device). All calculations for confidence intervals were done according to the Exact Method\(^d\). The results of this testing are summarized below (Table 8b).

#### Table 8a: Study Site 3 – D³ DFA VZV Kit and Comparison Device in Multi-Well Plates

<table>
<thead>
<tr>
<th>Comparison Device</th>
<th>Positive (\times)</th>
<th>Negative (\times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D³ DFA VZV Kit</td>
<td>+ 8</td>
<td>– 0</td>
</tr>
<tr>
<td>PPA = Agreed</td>
<td>100%</td>
<td>63.1% to 100%</td>
</tr>
<tr>
<td>NPA = Agreed</td>
<td>100%</td>
<td>96.8% to 100%</td>
</tr>
</tbody>
</table>

#### Table 8b: Study Site 3 – D³ DFA VZV Kit and Comparison Device in Tube Cultures

<table>
<thead>
<tr>
<th>Comparison Device</th>
<th>Positive (\times)</th>
<th>Negative (\times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D³ DFA VZV Kit</td>
<td>+ 9</td>
<td>– 0</td>
</tr>
<tr>
<td>PPA = Agreed</td>
<td>100%</td>
<td>66.4% to 100%</td>
</tr>
<tr>
<td>NPA = Agreed</td>
<td>100%</td>
<td>96.7% to 100%</td>
</tr>
</tbody>
</table>
D. Cross-Reactivity Testing

The Diagnostic Hybrids, Inc. D³ DFA Varicella-zoster Virus Identification Kit VZV DFA Reagent was tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 55 virus strains (cultured and processed for staining) or for 20 host culture cell types. Twenty-seven (27) bacterial cultures and one (1) yeast culture 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 (Limitations of Procedure, Section VIII,) while all other bacterial cultures were negative. [See Tables below for cross-reactivity study results.]

Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the VZV DFA Reagent and relatively high titers of microorganisms. The DFA Reagent was prepared at 1.5X the concentration that is provided in the kit.

- Fifty-five (55) virus strains were tested for cross-reactivity. Depending on the particular virus, 150 to 2100 TCID50 viruses were inoculated into a shell-vial culture and incubated for 24 to 48-hours, to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFA Reagent according to the procedure detailed in the product insert. No cross-reactivity was observed for the viruses listed below (Table 10).

- Twenty (20) host culture cell types were tested for cross-reactivity. Cell cultures were prepared in shell-vial format. Confluent monolayers were stained with the DFA Reagent and incubated for 24- to 48-hours, to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFA Reagent according to the procedure detailed in the product insert. No cross-reactivity was observed for the cells listed below (Table 11).

- Twenty-eight (28) microorganisms, including one (1) yeast culture and twenty-seven (27) bacterial cultures, were stained with the 1.5X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. No cross-reactivity was observed for the following strains presented as listed below (Table 11).

### TABLE 10: Virus Strains Tested for Cross-Reactivity with VZV DFA Reagent

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
<th>Inoculum Concentration (TCID50)</th>
<th>Organism</th>
<th>Strain or Type</th>
<th>Inoculum Concentration (TCID50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus Type 1</td>
<td>350</td>
<td>Adenovirus Type 5</td>
<td>350</td>
<td>Parainfluenza 1</td>
<td>C-35</td>
</tr>
<tr>
<td>Adenovirus Type 6</td>
<td>350</td>
<td>Adenovirus Type 8</td>
<td>350</td>
<td>Parainfluenza 2</td>
<td>Greer</td>
</tr>
<tr>
<td>Adenovirus Type 10</td>
<td>350</td>
<td>Adenovirus Type 14</td>
<td>350</td>
<td>Parainfluenza 5</td>
<td>1F</td>
</tr>
<tr>
<td>Adenovirus Type 18</td>
<td>350</td>
<td>Adenovirus Type 31</td>
<td>350</td>
<td>HSV-1</td>
<td>1F</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Achille</td>
<td>2.100</td>
<td>Influenza A</td>
<td>Maculhydrate</td>
<td>150</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Mai</td>
<td>2.100</td>
<td>Influenza A</td>
<td>H1N1</td>
<td>1.000</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Hong Kong</td>
<td>2.100</td>
<td>Influenza A</td>
<td>Strain G</td>
<td>150</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Denver</td>
<td>2.100</td>
<td>Influenza A</td>
<td>CMV</td>
<td>700</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Port Chalmers</td>
<td>2.100</td>
<td>Influenza A</td>
<td>HSV-2</td>
<td>56</td>
</tr>
<tr>
<td>Influenza A</td>
<td>Victoria</td>
<td>2.100</td>
<td>Influenza A</td>
<td>Echovirus</td>
<td>4</td>
</tr>
<tr>
<td>Influenza A</td>
<td>PR</td>
<td>2.100</td>
<td>Influenza B</td>
<td>Echovirus</td>
<td>6</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Hong Kong</td>
<td>3.100</td>
<td>Influenza B</td>
<td>Echovirus</td>
<td>9</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Bombay</td>
<td>3.100</td>
<td>Influenza B</td>
<td>Echovirus</td>
<td>11</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Mass</td>
<td>3.500</td>
<td>Influenza B</td>
<td>Echovirus</td>
<td>30</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Taiwan</td>
<td>3.500</td>
<td>Influenza B</td>
<td>CMV</td>
<td>34</td>
</tr>
<tr>
<td>Influenza B</td>
<td>GL</td>
<td>3.500</td>
<td>Influenza B</td>
<td>Coxsackievirus</td>
<td>B1</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Russia</td>
<td>3.500</td>
<td>Influenza B</td>
<td>Coxsackievirus</td>
<td>B2</td>
</tr>
<tr>
<td>Poliovirus Type 1</td>
<td>Coxsackievirus</td>
<td>B2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poliovirus Type 2</td>
<td>Coxsackievirus</td>
<td>B3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poliovirus Type 3</td>
<td>Coxsackievirus</td>
<td>B4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epstein-Barr</td>
<td>Commercially available slides stained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mumps</td>
<td>Commercially available slides stained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 11: Cell Lines Tested for Cross-Reactivity with VZV DFA Reagent

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain or Type</th>
<th>Inoculum Concentration (TCID50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>MV1Lu</td>
<td>RD</td>
</tr>
<tr>
<td>BGMK</td>
<td>HFF</td>
<td>RhMK II</td>
</tr>
<tr>
<td>HEP-2</td>
<td>McCoy</td>
<td>R-Mix</td>
</tr>
<tr>
<td>LLC-MK2</td>
<td>NCI-H292</td>
<td>Vero</td>
</tr>
<tr>
<td>MDCK</td>
<td>pCMK</td>
<td>WI-38</td>
</tr>
<tr>
<td>MRC-5</td>
<td>pRhMK</td>
<td>Vero 76</td>
</tr>
<tr>
<td>MRHF</td>
<td>pRK</td>
<td></td>
</tr>
</tbody>
</table>

XI. BIBLIOGRAPHY


[WARRANTY STATEMENT]

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