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D3®

DFA Varicella-zoster Virus
IDENTIFICATION KIT

For the qualitative detection of Varicella-zoster virus (VZV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

INTENDED USE

The Diagnostic Hybrids, Inc. D^3 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 fluoresceinated 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.

SUMMARY AND EXPLANATION OF THE TEST

VZV is a latent DNA virus in the family *Herpesviridae.* 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 to 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.

PRINCIPLE OF THE PROCEDURE

The D^3 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 to 30 minutes at 35°C to 37°C, 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.

REAGENTS AND MATERIALS PROVIDED

The D³ DFA Varicella-zoster Virus Identification Kit contains the following:

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.

Mounting Fluid **7 mL**

One dropper bottle containing an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

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.

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).

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.
- Cell culture for VZV isolation. Suggested cell lines include H&V-Mix™ MixedCells™, human newborn foreskin, MRC-5, CV-1 and A549.[7] All are available from Quidel.
- Coverslips (22 x 50 mm) for Antigen Control Slides and for specimen slides.
- Universal Transport Medium. Available from Quidel.
- Tissue culture refeed medium (Eagle's Minimum Essential Medium with 2% fetal bovine serum, 25 mM HEPES and antibiotics). Available from Quidel.
- Reagent-grade acetone (> 99% pure) chilled at 2°C to 8°C for fixation of shell-vials
  NOTES:
  - Keep the reagent-grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, non-specific, background fluorescence.
  - A mixture of 80% acetone/20% demineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature (20°C to 25°C).
- Sterile graduated pipettes: 10 mL, 5 mL, and 1 mL
- Sterile Pasteur pipettes or other transfer pipettes
  CAUTION: One should not use solvents such as acetone with polyethylene transfer pipettes.
- Fine-tipped forceps
- Sterile 0.45 µm syringe filter
- Sterile 3 mL syringe
- Wash bottle, 200 mL
- Bent-tip teasing needle (for removal of coverslip from a shell-vial): fashion the teasing needle by bending the tip of a syringe needle or similar object (i.e., mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.
- Sodium hypochlorite solution (1:10 final dilution of household bleach)
- Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom)
- Glass microscope slides
- Sterile nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell culture
- Incubator, 35°C to 37°C (5% CO_2 or non-CO_2, depending on the cell culture format used)

- Centrifuge with free-swinging bucket rotor
- Demineralized water for dilution of 40X PBS Concentrate (see *REAGENT PREPARATION*) and for dilution of the reagent-grade acetone for use in polystyrene multi-well plates (see *MATERIALS REQUIRED BUT NOT PROVIDED*).
- 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 Quidel.
- 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.
- Wash Container: Beaker, wash bottle or Coplin jar for washing slides.
- Fixing Container: Coplin jar, slide dish or polyethylene holder for slides for use in fixing the cells on the slides.
- Inverted Light Microscope: 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.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens are handled in accordance with the OSHA Standard on Bloodborne Pathogens.[1]
  - Cell culture isolation may have some potential to be hazardous. Personnel working with cell cultures must be properly trained in safe handling techniques[2] and have experience with cell culture before attempting this procedure.
  - All procedures must be conducted in accordance with the CDC 5th Edition Biosafety in Microbiological and Biomedical Laboratories, 2009 and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.
- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.
  - Biosafety Level 2 or other appropriate biosafety practices should be used when handling these materials.
  - Decontamination of specimens and cultures is most effectively accomplished using a solution of sodium hypochlorite (1:10 final dilution of household bleach).
  - Although Antigen Control Slides have been shown to be non-infectious, the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
- Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
- Avoid splashing and the generation of aerosols with clinical samples.
- Use aseptic technique and sterile equipment and materials for all tissue culture procedures.
- Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.
- Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
- The DFA Reagent is supplied at working strength. Any dilution of the DFA reagent will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Each Respiratory Virus Antigen Control Slide should be used only once. Do not re-use a control slide.
- Microbial contamination of the DFA Reagent may cause a decrease in sensitivity.
- Store 1X PBS in a clean container to prevent contamination.
- Sodium azide is included in the 40X PBS Concentrate at a concentration of 4% (w/v), and in the other solutions in this kit at 0.1% concentration.

- Sodium azide is considered poisonous. If the 40X PBS Concentrate is swallowed, seek medical advice immediately.
- 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. Avoid disposal of these solutions down sanitary or industrial plumbing systems.
- Avoid release to the environment.

- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Do not expose the DFA Reagent to bright light during staining or storage.
- Use of reagents other than those specified with the components of this kit may lead to erroneous results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

Preparation of 1X PBS Solution

- After storage at 2°C to 8°C, some salts in the 40X PBS Concentrate may have crystallized.
- Warm the solution to ambient temperature (20°C to 25°C) to re-dissolve the crystals, then mix. Add contents of the fully dissolved 25 mL 40X PBS Concentrate to 975 mL of demineralized water. Label the 1X PBS with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

Storage

Table 1. Reagent Storage Conditions

VZV DFA Reagent	Store at 2°C to 8°C in the dark.
Mounting Fluid	
VZV Antigen Control Slides	Store at 2°C to 8°C.
40X PBS Concentrate NOTE: The Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when the Concentrate is warmed to room temperature.	Store liquid at 2°C to 8°C prior to dilution.
1X PBS	Store at ambient temperature (20°C to 25°C).

Stability

Reagent 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 VZV DFA Reagent should be kept to a minimum.

Discard 1X PBS if it becomes cloudy.

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful VZV 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.

Specimen Collection[3,4]

The possibility of virus isolation is increased when specimens are collected from the suspected site of infection as soon as possible after onset of the disease state. When possible, the specimen of choice is vesicular fluid removed from a fresh lesion by aspiration with a 26 or 27-gauge needle attached to a tuberculin syringe. For ulcerated lesions, use a sterile nylon flocked[5,6], rayon or polyester fiber-tipped swab to remove and discard pus without disrupting the lesion base, and then use a fresh sterile collection swab dipped in sterile physiological saline to vigorously swab the lesion base to obtain cells. Crusted lesions should have the crust removed and discarded by lifting the crust from the lesion with a sterile needle. A sterile nylon flocked, rayon or polyester fiber-tipped swab moistened in sterile physiological saline is then used to vigorously swab the base of the lesion. All specimens should be immediately placed into viral transport medium to stabilize virus and inhibit microbial growth. Several factors of specimen collection may affect the successful isolation of VZV. When swabs are used for specimen collection, sterile nylon flocked, rayon or polyester fiber-tipped swabs should be used. **Do not use calcium alginate and cotton swabs because they have been shown to inhibit virus replication.**

Specimen Transport and Storage

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the US Code of Federal Regulations, or other regulatory requirements as may be applicable.

Specimens should be transported to the laboratory at 2°C to 8°C. This temperature can be attained by using cold packs, wet ice, foam refrigerant, or other coolants.**Error! Bookmark not defined.** The specimen should be processed and tested as soon as possible and then stored at 2°C to 8°C.

Specimens should be stored at 2°C to 8°C for no longer than 2 days before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

Freezing and thawing of specimens should be avoided since this will result in a loss of viability of viruses, leading to decreased sensitivity of the test.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
- 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.
- 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.
- Place the closed, humidified chamber for holding slides during staining into the incubator for equilibration to 35°C to 37°C prior to staining. By doing this, the test slides and reagent will come to temperature quickly, yielding more rapid, intense staining.
- Bring the DFA Reagent to ambient temperature (20°C to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2°C to 8°C.

Regarding Cell Culture Testing

- 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.
- It is good practice to retain the medium removed from the monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer and incubated for the appropriate time period for repeat testing.
- 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 (see *MATERIALS REQUIRED BUT NOT PROVIDED*).
- Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased sensitivity.
- Do not allow the DFA Reagent to dry on the monolayers; this can lead to high background.

Regarding Immunofluorescence Microscopy

- Examine the positive and negative controls before examining the test specimens. If 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). Do not report results for patient samples until controls perform as expected.
- Three aspects of the fluorescence microscope that must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
  - The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the DFA Reagent.
  - The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
  - The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.
- Fluorescent artifacts may be observed in the cell monolayers:
  - Cell debris, lint, etc. can non-specifically adsorb 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.
  - A low grade, yellow-green fluorescence may sometimes be seen, particularly in areas that have piled cells or are near holes in the cell monolayer. In both cases, the diffusion of the entrapped DFA Reagent is retarded during the wash step, resulting in the non-specific fluorescence.
  - 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.
  - Inadequate washing can lead to general low-grade fluorescence due to residual DFA Reagent remaining on the monolayer of cells.
- Protect stained slides and monolayers from light as much as possible during testing.
  - Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
  - This bleaching can occur when a stained cell in viewed in a fluorescence microscope for an extended period of time.

Specimen Preparation

- 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). Decontaminate the forceps after specimen disposal.
- Disrupt cellular material in the transport medium by vortexing with sterile glass beads for 30 to 60 seconds, sonication at 10kc/sec for 30 to 60 seconds or by other methods determined by the laboratory to

be effective in disrupting cellular debris. This will enhance the release of cell-associated virus into the medium.

- To remove bacterial, fungal, and cellular debris, centrifuge the transport medium at 700xg for 10 minutes. Supernatant is then used as the inoculum. Heavily contaminated specimens, noted by a cloudy yellow coloration, may be further clarified by filtration through a sterile 0.45-micron membrane filter. The filtrate is then used as the inoculum. Since such procedures may reduce the number of viruses in a specimen, each laboratory should establish the efficacy of its specimen preparation procedure.

Cell Culture Testing – Tube Culture

1. The laboratory that conducted studies for clearance of this assay using tube culture [presented in *SPECIFIC PERFORMANCE CHARACTERISTICS*] used one tube per specimen and terminated cultures within 14 days.
   - It is generally recommended that specimens be inoculated into a minimum of two vessels (tubes, shell-vials, or wells; or a combination of these) containing the same or different cell types that are permissive for the suspected or requested virus(es).[8]
2. Examine the monolayers for proper morphology prior to inoculation.
3. Using a sterile pipette, remove medium from the cell culture container and re-feed with at least 2 mL of fresh pre-warmed (25°C to 37°C) refeed medium. Aseptic technique is essential at all times during inoculation and cell culture handling.
4. Using a sterile 1 mL graduated pipette, inoculate 0.2 to 0.4 mL of the clinical specimen into each tube. It is recommended that all clinical specimens be inoculated in duplicate for backup.
5. Incubate the tubes at 35°C to 37°C. Examine the monolayers daily for evidence of toxicity or viral CPE.
6. Examine the monolayers daily for at least 5 to 7 days and every other day thereafter for 14 days. Assess cultures for evidence of viral replication (CPE) and also identification of problems such as toxicity, microbial contamination, cell death, pH extremes and non-specific cellular degeneration.[9]
7. Rinse the cells 2 to 3 times with 1 mL volumes of 1X PBS.
8. Discard each rinse into a biohazard container.
9. Add 0.5 to 1.0 mL of 1X PBS to each tube.
10. Scrape cells from the tube surface and re-suspend in the 1X PBS using a sterile pipette.
11. Prepare cell spots using about 25 µL of the suspension onto an acetone cleaned slide. Repeat this step for each specimen.
12. Air-dry the wells completely.
13. Fix the cells to the slides using fresh, chilled 100% acetone. Let stand for 5 to 10 minutes, at 20°C to 25°C. **Caution: Acetone is volatile and flammable. Keep away from open flames.**
14. Remove the slides from the fixative and allow to air dry.
15. Add one drop of the VZV DFA Reagent to completely cover the dried, fixed cells on each of the slides.
16. Also, add one drop 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.
17. Place the slides in a covered humidified chamber at 35°C to 37°C for 15 to 30 minutes.
18. Rinse the stained cells using the 1X PBS. For only a few slides, this can be done using a beaker of the 1X PBS. 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.
19. Discard the used 1X PBS and repeat the washing step using fresh 1X PBS.
20. 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.
21. Blot the excess liquid.
22. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.

23. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X (See *Regarding Immunofluorescence Microscopy*).
24. Refer to *INTERPRETATION OF RESULTS*.

Cell Culture Testing – Shell-vial

1. The laboratory that conducted studies for clearance of this assay using shell-vial culture [presented in *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.[Error! Bookmark not defined.]
2. Examine the monolayers for proper morphology prior to inoculation.
3. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each shell-vial.
4. Add 0.2 to 0.4 mL of prepared specimen to each shell-vial.
5. Centrifuge the shell-vials at 700xg for 1 hour at 20°C to 25°C.
6. Place stoppered shell-vials in an incubator at 35°C 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°C 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 stoppered shell-vials in a 35°C 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.
21. Lift the coverslip from the bottom of the shell-vial using a bent-tip needle on a syringe barrel. Grasping it with the fine-tipped forceps, transfer it, monolayer-side down, to a small drop of Mounting Fluid on a standard microscope slide.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*).
23. Refer to *INTERPRETATION OF RESULTS*.

Cell Culture Testing – Multi-well Plate

1. The laboratories that conducted studies for clearance of this assay using multi-well plates (presented in *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.[Error! Bookmark not defined.]

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°C to 25°C.
6. Place the covered multi-well plates in a 35°C to 37°C incubator with a humidified, 5% CO_2 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°C to 25°C.
    NOTE: Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10 minutes since it may craze and cloud the plastic, making it difficult to examine the monolayers.
    CAUTION: Acetone is volatile and flammable; keep away from open flames.
11. Remove the fixative by aspiration.
12. Add 0.5 mL of the 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°C 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 *Regarding Immunofluorescence Microscopy.*)
23. Refer to *INTERPRETATION OF RESULTS*.

Quality Control

Reagents

- A fresh VZV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
- 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.
- The negative well will show only negative cells staining a dull red.
- Positive and negative controls must demonstrate appropriate fluorescence for specimen results to be considered valid.

Cell Culture

- Positive and negative virus controls should be run with each new batch of cells to confirm their performance in culturing specific viruses.
- To ensure viral sensitivity, a VZV-inoculated control monolayer should be included each time a new lot of cell culture is used.

- 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°C to 37°C. Adverse storage conditions or handling procedures will also be reflected in the negative control.
- If control cultures fail to perform correctly, results are considered invalid.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

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

Artifacts of Staining

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

Results from Culture Isolation/Confirmation

- The bright apple-green fluorescence staining pattern is *cytoplasmic*.
- 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.”
- If fluorescent cells are found, report results as, “Varicella-zoster virus isolated by cell culture”.

LIMITATIONS OF THE PROCEDURE

- Inappropriate specimen collection, storage, and transport may lead to false negative culture results.[10]
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of viruses will vary greatly depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of virus infection. Results of the test should be interpreted in conjunction with information available from epidemiological studies, clinical evaluation of the patient and other diagnostic procedures.
- The effects of antiviral therapy on the performance of this kit have not been established.
- 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.
- The VZV DFA Reagent cannot differentiate between the various VZV genotypes or the wild type or vaccine strain(s).
- Performance of the kit can only be assured when components used in the assay are those supplied by Quidel.
- Prolonged storage of the VZV DFA Reagent under bright light will decrease the staining intensity.
- Light background staining may occur from specimens contaminated with certain *Staphylococcus aureus* (e.g. Cowan) 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.

EXPECTED VALUES

The clinical studies described in *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 2. Specimen Sources

Source	Total specimens	Unknown	Genital	Penis	Vaginal	Cervical	Rectal	perineum**	Eyelid	Face	Mouth*	Skin‡	NP	CSF/Brain
Site 1	99	0/8	0/1	0/0	0/0	0/0	0/1	0/11	0/1	4/14	0/2	17/61	0/0	0/0
Site 2	35	0/0	0/0	1/2	0/0	0/0	0/0	1/3	0/0	0/2	0/0	9/27	0/1	0/0
Site 3	120	2/51	0/6	0/1	0/9	0/1	0/0	0/3	0/0	1/9	0/5	4/33	1/1	0/1

* 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 VZV 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).[11] The patient demographics are listed below (Table 3).

Table 3. Demographics by Age and Gender

Source	Site 1 Values are # pos/Total		Site 2 Values are # pos/Total			Site 3 Values are # pos/Total	
Gender / Age	F	M	F	M	Gender not reported	F	M
TOTALS	63	36	10	10	12	80	40
< 2 years	0/1	0/4	0	0/2	0	0	0/1
2 years to 10 years	0	0/1	0/1	0/1	0	1/3	0/2
10 years to 18 years	1/6	1/3	1/1	1/1	0	0/4	0/3
18 years to 40 years	0/18	1/3	0/1	0/1	0	0/39	0/13
> 40 years	11/38	7/24	3/6	4/5	0	2/33	5/21
Age not reported	0/0	1/1	0/1	0	1/12	1/1	0

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 D^3 DFA Varicella-zoster Virus Identification Kit (D^3 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

Site	Culture		Site Total
	Fresh	Frozen	
1	57	42	99
2	1	31	32
3	0	120	120

Percent Agreement between the D^3 DFA VZV Kit and comparator tests was calculated and tabulated in Table 5 for all the tested specimens is presented below.

Table 5. Percent Agreement of All Tests

		Comparison Device +	Comparison Device –
D^3 DFA VZV Kit	+	**42**	1
	–	0	**208**

Positive Percent Agreement† (PPA): 100%
95% CI‡ – PPA: 91.6% to 100%
Negative Percent Agreement‡‡ (NPA): 99.5%
95% CI – NPA: 97.3% to 99.9%

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

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°C to 37°C for up to 72 hours then stained in accordance with each respective

product insert (Quidel and Comparison device). All calculations for confidence intervals were done according to the Exact Method‡. 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

		Comparison Device	
		+	–
D³ DFA VZV Kit	+	**22**	0
	–	0	**77**

	Agreed	95% CI
PPA =	100%	84.6% to 100%
NPA =	100%	95.2% to 100%

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°C to 37°C for 72 hours then stained in accordance with the respective product insert (Quidel and Comparison devices). All calculations for confidence intervals were done according to the Exact Method‡. 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.

		Comparison Device	
		+	–
D³ DFA VZV Kit	+	**11**	1
	–	0	**20**

	Agreed	95% CI
PPA =	100%	71.5% to 100%
NPA =	95.1%	76.2% to 99.9%

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°C to 37°C for 72 hours then stained in accordance with each respective product insert (Quidel 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 Quidel 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 (Quidel and Comparison device). All calculations for confidence intervals were done according to the Exact Method‡. 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

		Comparison Device +	Comparison Device –
D³ DFA VZV Kit	+	**8**	0
	–	0	**112**

	Agreed	95% CI
PPA =	100%	63.1% to 100%
NPA =	100%	96.8% to 100%

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

		Comparison Device +	Comparison Device –
D³ DFA VZV Kit	+	**9**	0
	–	0	**111**

	Agreed	95% CI
PPA =	100%	66.4% to 100%
NPA =	100%	96.7% to 100%

Cross-Reactivity Testing

The 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 $TCID_{50}$ 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).

Table 10. Virus Strains Tested for Cross-Reactivity with VZV DFA Reagent

Organism	Strain or Type	Inoculum Concentration ($TCID_{50}$)
Adenovirus	Type 1	350
	Type 5	350
	Type 6	350
	Type 7	350
	Type 8	350
	Type 10	350
	Type 14	350
	Type 18	350
	Type 31	350
Influenza A	Aichi	2,100
	Mal	2,100
	Hong Kong	2,100
	Denver	2,100
	Port Chalmers	2,100
	Victoria	2,100
	PR	2,100
Influenza B	Hong Kong	350
	Maryland	350
	Mass	350
	Taiwan	350
	GL	350
	Russia	350
Poliovirus	Type 1	Commercially available slides stained*
	Type 2	
	Type 3	
Epstein-Barr	Commercially available slides stained*	
Rubeola		
Mumps		

Organism	Strain or Type	Inoculum Concentration ($TCID_{50}$)
RSV	Long	350
	Wash	350
	9320	350
Parainfluenza 1	C-35	Commercially available slides stained*
Parainfluenza 2	Greer	
Parainfluenza 3	C 243	
HSV-1	1F	150
	CWOH 0026	150
	CWOH 0015	150
	MacIntyre	150
HSV-2	MS	150
	Strain G	150
CMV	Towne	700
	Davis	700
	AD169	700
Echovirus	4	Commercially available slides stained*
	6	
	9	
	11	
	30	
	34	
Coxsackievirus	B1	Commercially available slides stained*
	B2	
	B3	
	B4	
	B5	
	B6	

* Test material is from commercially available prepared slides. Each positive well contains 10 to 50% reactive cells.

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 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 cell lines as presented below (Table 11).

Table 11. Cell Lines Tested for Cross-Reactivity with VZV DFA Reagent

A549	Mv1Lu	RD
BGMK	HFF	RhMK II
HEp-2	McCoy	R-Mix
LLC-MK2	NCI-H292	Vero
MDCK	pCMK	WI-38
MRC-5	pRhMK	Vero 76
MRHF	pRK	

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. Except for *Staphylococcus aureus*, which was cross-reactive with the VZV DFA Reagent (see above), all microorganisms tested negative. Concentrations for each bacterial organism cultured by Quidel for cross-reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of 1.0 and 2.0 (equaling approximately 3.0×10^6 and 6.0×10^6 CFU per mL). Slides were prepared with spots of 0.01 mL of the suspensions to give either 3.0×10^4 or 6.0×10^4 per spot. At the same time, 1 mL of each suspension was plated on an appropriate agar dish for colony confirmation. According to the confirmation agar cultures, initial concentrations of the bacterial organisms in the study ranged from 6.4×10^4 to 2.9×10^7 CFU. Results of testing are listed below (Table 12).

Table 12. Bacteria and Yeast Tested for Cross-Reactivity with VZV DFA Reagent

BACTERIA	**CFU TESTED**
Acinetobacter calcoaceticus	9.7×10^5
Bordetella bronchiseptica	1.7×10^5
Bordetella pertussis	4.6×10^6
Corynebacterium diphtheriae	2.5×10^6
Escherichia coli	2.6×10^5
Gardnerella vaginalis	5.0×10^5
Haemophilis influenzae type A	9.3×10^5
Klebsiella pneumoniae	6.4×10^6
Legionella pneumophila	6.5×10^4
Moraxella cartarrhalis	6.4×10^4
Neisseria gonorrhoeae	1.3×10^6
Proteus mirabilis	2.1×10^6
Pseudomonas aeruginosa	1.0×10^7
Salmonella enteriditis	2.5×10^6
Salmonella typhimurium	1.7×10^6
Staphylococcus aureus	1.0×10^7
Streptococcus agalactiae	9.6×10^6
Streptococcus pneumoniae	8.0×10^5
Streptococcus pyogenes	2.9×10^7
Acholeplasma laidlawi	$\sim 6 \times 10^7$
Mycoplasma hominis	$\sim 6 \times 10^4$
Mycoplasma orale	$\sim 6 \times 10^4$
Mycoplasma pneumoniae	$\sim 6 \times 10^4$
Mycoplasma salivarium	$\sim 6 \times 10^7$
Ureaplasma urealyticum	$\sim 6 \times 10^4$
These were procured as prepared slides:	Proportion of cells reactive
Chlamydophila pneumoniae	10 to 50%
Chlamydia trachomatis	10 to 50%
YEAST	
Candida glabrata	8.7×10^6

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

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REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	 Do not reuse
 Biological risks	IVD For *In Vitro* diagnostic use
CONT NaN_3 Contents/Contains	NaN_3 4% Contents/Contains