D³ DFA Herpes Simplex Virus Identification and Typing Kit

REF: 01-090000

For *in vitro* Diagnostic Use

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US Patent Nos. 6,168,915; 6,280,928; 6,306,582; 6,376,172; 6,406,842; 6,495,316; and patents pending

Symbols Lexicon/Glossary

IVD	In Vitro Diagnostic Medical Device	LOT	Batch code/lot number
	Temperature limit	REF	Catalog number
	Consult instructions for use		Use by YYYY-MON-DD
	Do not reuse		Manufacturer
iP	Patent Numbers		
CONT NaN_3	Contains sodium azide	EC REP	Authorized representative in the European Community
NaN_3 4%	Contains 4% sodium azide when undiluted	CE	CE mark of conformity (Conformité Européen)

I. INTENDED USE

The Diagnostic Hybrids, Inc. D³ DFA Herpes Simplex Virus Identification and Typing Kit is intended for use in the qualitative detection and typing of human herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) 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 decisions.

Performance using direct specimen testing has not been evaluated.

II. SUMMARY AND EXPLANATION OF THE TEST

HSV infections in humans can cause lesions at a variety of sites, e.g., oral-facial, genital, eye and cutaneous sites.

When an appropriately sensitive cell line is infected with HSV, 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-days of culture may be required by the standard tube culture method before CPE can be observed[1,2,3,4,5].

The rate of isolation may be enhanced and the time required for HSV identification may be decreased by centrifugation of specimens in shell-vials or multi-well plates containing appropriately sensitive cell lines (centrifuge enhanced technique) [3,5,6].

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. Therefore, determination of results should not depend on CPE alone. Confirmation of cell culture using fluoresceinated monoclonal antibodies is considered the gold standard for confirmation and typing of a HSV type 1 or HSV type 2 positive viral culture.

III. PRINCIPLE OF THE PROCEDURE

The Diagnostic Hybrids, Inc. D³ DFA Herpes Simplex Virus Identification and Typing Kit uses a blend of HSV antigen-specific and type-specific murine MAbs that are directly labeled with fluorescein for the rapid detection and typing of HSV-1 and HSV-2 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 HSV-1 and HSV-2 DFA Reagents are added to two separate slide wells containing the fixed cells or to two separate fixed cell monolayers in shell-vials or multi-well plates to detect the presence of HSV type-specific viral antigens. After incubating for 15- to 30-minutes at 35º 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 200-400X. Upon staining with the HSV-1 and HSV-2 DFA Reagents, which contain Evans Blue as a counter-stain, HSV-1 infected cells will be stained by the HSV-1 DFA Reagent with bright apple-green fluorescence that will be distinguished from the counter-stained non-infected cells exhibiting a dull red fluorescence[7]. Likewise, HSV-2 infected cells will be stained with bright apple-green fluorescence by the HSV-2 DFA Reagent.

IV. REAGENTS

A. Kit Components

1. **HSV-1 DFA Reagent**, 5-mL. One dropper bottle containing a blend of two fluorescein-labeled murine monoclonal antibodies directed against HSV-1 specific glycoproteins. The HSV-1 MAbs were developed using HSV-1(f) cell lysate as immunogen – one MAb has been determined to be directed against HSV-1 glycoprotein C1 but, the antigen to the other is undetermined. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
2. **HSV-2 DFA Reagent**. 5-mL. One dropper bottle containing a blend of two fluorescein-labeled murine monoclonal antibodies directed against HSV-2 specific glycoproteins. The HSV-2 MAbs were developed using a HSV-2 recombinant glycoprotein G immunogen. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
3. **HSV-1/HSV-2 Antigen Control Slides**, 5-slides. Five (5) individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each slide consists of four wells containing acetone fixed cells; two wells of non-infected cells and one well each of HSV-1 infected cells and HSV-2 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 de-mineralized water).
5. **Mounting Fluid**, 7-mL. One dropper bottle of an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

B. Warnings and Precautions

For *in vitro* diagnostic use.

1. No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens should be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[8]
   a. Cell culture isolation may have some potential to be hazardous. Personnel working with these cultures must be properly trained in safe handling techniques[9] 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).
   c. Although Antigen Control Slides have been shown to be non-infectious, the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
3. Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
4. Avoid splashing and the generation of aerosols with clinical samples.
5. Use aseptic technique and sterile equipment and materials for all cell culture procedures.
6. Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.
7. Sodium azide is included in the 40X 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# 2628-22-8; EC# 247-852-1; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.]
b. Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.
c. Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with a large volume of water to prevent azide build-up. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous waste.

8. Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
9. The HSV-1 and HSV-2 DFA Reagents are supplied at working strength. Any dilution to the DFA Reagents will decrease sensitivity.
10. Reagents should be used prior to their expiration date.
11. Each HSV-1/HSV-2 Antigen Control Slide should be used only once. Do not re-use a Control Slide.
12. Microbial contamination of the HSV-1 and HSV-2 DFA Reagents may cause a decrease in sensitivity.
13. Store 1X PBS in a clean container to prevent contamination.
14. Reusable glassware must be cleaned and thoroughly rinsed free of all detergents.
15. Do not expose the HSV-1 and HSV-2 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 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 de-mineralized water.
3. Label the 1X PBS with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

D. Storage Instructions

TABLE 1 – Reagent Storage Conditions	
1. HSV-1 DFA Reagent	Store at 2° to 8°C in the dark.
2. HSV-2 DFA Reagent	
3. Mounting Fluid	
4. HSV-1/HSV-2 Antigen Control Slides	Store at 2° to 8°C.
5. 40X PBS Concentrate NOTE: The Concentrate may crystallize when stored at 2° to 8°C. The crystals will dissolve when the Concentrate is warmed to ambient temperature.	Store liquid at 2° to 8°C prior to dilution.
6. 1X PBS	Store at ambient temperature (20° to 25°C).

E. Stability

Reagents and components will retain their full potency through the expiration date shown on the label of each bottle when stored at recommended temperatures. Light exposure of the HSV-1 and HSV-2 DFA Reagents should be kept to a minimum.

Discard 1X PBS if it becomes cloudy.

V. SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful HSV detection. Specimen collection, specimen processing, and cell culture of viruses should be attempted only by personnel that have been trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

A. Specimen Collection[10,11]

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[12,13] 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[14]. Several factors of specimen collection may affect the successful isolation of HSV-1 and HSV-2. 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.**

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.[15] The specimen should be processed and tested as soon as possible and then stored at 2° to 8°C.

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

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

VI. PROCEDURE

A. Materials Provided

1. HSV-1 DFA Reagent
2. HSV-2 DFA Reagent
3. HSV-1/HSV-2 Antigen Control Slides
4. 40X PBS Concentrate
5. Mounting Fluid

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 HSV isolation. Suggested cell lines include H&V-Mix™ MixedCells™, human newborn foreskin, MRC-5, Vero, and A549[16]. All available from DHI. Examples of HSV isolation methods include:
   a. Tube cultures containing monolayers of either a commercially prepared or user propagated cell line.
   b. Shell-vials, with glass coverslips, containing monolayers of either a commercially prepared or user propagated cell line.
   c. Multi-well plates (either 24-, or 48-well size), containing monolayers of either a commercially prepared or user propagated cell line.
3. Live control viruses for positive culture controls: Known strains of HSV concentration 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. Tissue culture refeed medium (Eagle's Minimum Essential Medium with 2% fetal bovine serum, 25mM HEPES, and antibiotics). 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% de-mineralized 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.
   Caution: One should not use solvents such as acetone with polyethylene transfer pipettes.
10. Fine-tipped forceps.
11. 200-mL wash bottle.
12. 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.
13. Sodium hypochlorite solution (1:10 final dilution of household bleach).
14. Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).
15. Glass microscope slides.
16. Acetone-cleaned multi-well glass microscope slides.

17. Blotters for multi-well glass microscope slides used to blot excess liquid from the mask to prevent spread of liquid or stained cells from one well to the other.
18. Sterile nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell culture.
19. Incubator, 35° to 37°C (5% CO_2 or non-CO_2, depending on the cell culture format used).
20. Centrifuge with free-swinging bucket rotor.
21. De-mineralized 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 (See Section VI.B.7.Note 2).
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 bottle or Coplin jar for washing slides.
24. Fixing Container: Coplin jar, slide dish or polyethylene holder for slides for use in 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 transport 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 37ºC prior to staining. By doing this, the test slides and reagents will come to temperature quickly, yielding more rapid, intense staining.
5. Bring the HSV-1 and HSV-2 DFA Reagents to ambient temperature (20° to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2° to 8°C.

CELL CULTURE TESTING:

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 positive monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer 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 de-mineralized water to 80 mL of acetone (See Section VI.B.7.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 Reagents 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). Do not report results for patient samples until controls perform as expected.
12. Three aspects of the fluorescence microscope that must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
    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.
    b. The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
    c. The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.
13. Fluorescent artifacts may be observed in the cell monolayers being examined:
    a. 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.
    b. 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.
    c. 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.
    d. Inadequate washing can lead to general low grade fluorescence due to residual DFA Reagent remaining on the monolayer of cells.
14. Protect stained slides and monolayers from light as much as possible during testing.
    a. Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
    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 sodium hypochlorite solution (1:10 final dilution of household bleach). Decontaminate the forceps after specimen disposal.
2. 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.
3. 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.

E. Cell Culture Testing – Tube Culture

1. One of the laboratories that conducted studies for clearance of this assay using tubes [presented in Section X ('Specific Performance Characteristics')], used one tube per specimen and the other used two; both laboratories terminated the cultures within 7-days.
    a. 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). However – providing the specimen is appropriately collected and taken during the early infectious process (indicated by a viable lesion) – a single vessel may be sufficient for HSV cultures from genital specimens, which often contain high viral titers[17].
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 (25º to 37ºC) 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 35º to 37ºC in a roller drum at 1 to 3 rpm.
6. Examine the monolayers daily for evidence of viral CPE (including toxicity, microbial contamination, cell death, pH extremes and non-specific cellular degeneration)[15], for at least 5-to 7-days and every other day thereafter for 14-days.
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-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 duplicate cell spots using 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 20º to 25ºC.
    Caution: Acetone is volatile and flammable; keep away from open flames.
14. Remove the slides from the fixative and allow to air dry.
15. Stain the specimen slides and a HSV-1/HSV-2 Antigen Control Slide in the following manner:
    a. Add one drop of HSV-1 DFA Reagent to completely cover one of the dried, fixed cell spots on each of the slides (labeled as "HSV-1").
    b. Add one drop of HSV-2 DFA Reagent to completely cover the duplicate dried, fixed cell spots on each of the slides (labeled as "HSV-2").
    c. For a HSV-1/HSV-2 Antigen Control Slide: Add one drop of the HSV-1 DFA Reagent to the well labeled "HSV-1" of a fresh HSV-1/HSV-2 Antigen Control Slide, and one drop of the HSV-2 DFA Reagent to the well labeled "HSV-2" of the same HSV-1/HSV-2 Antigen Control Slide. An Antigen Control Slide should be stained only once, as it contains individual wells of viral-infected cells and non-infected cells.
16. Place the slides in a covered chamber at 35º to 37ºC for 15- to 30-minutes.
17. 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.
18. Discard the used 1X PBS and repeat the washing step using fresh 1X PBS.
19. Rinse the stained cells using de-mineralized water. For only a few slides, this can be done using a beaker of the de-mineralized water. For many slides, a slide carrier that holds 10 to 20 slides can be placed in its container with de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
20. Gently blot the excess liquid.
21. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
22. Examine the stained, mounted cells using a fluorescence microscope with magnifications between 200X to 400X (See Section VI.C. 11-14, 'Immunofluorescence Microscopy').
23. Refer to Section VII. 'Interpretation of Results'.

F. Cell Culture Testing – Shell-vial

1. The laboratory that conducted studies for clearance of this assay using shell-vials [presented in Section X ('Specific Performance Characteristics')] used one shell-vial per HSV type for each specimen and terminated cultures within 24-hours of incubation.
   a. It is generally recommended that specimens be inoculated into a minimum of two vessels (tubes, shell-vials, or wells per specimen; or a combination of these) containing the same or different cell types that are permissive for the suspected or requested virus(es).
   b. Shell-vial cultures are typically terminated after 1- to 4-days, depending on the particular agent. Overnight incubation may be adequate for rapidly growing viruses such as HSV[18].
2. Examine the monolayers for proper morphology prior to inoculation.
3. Aspirate medium from the monolayers and add 1-mL of appropriate refeed medium to each shell-vial.
4. Add 0.2 to 0.4-mL of prepared specimen to each shell-vial.
5. Centrifuge the shell-vials at 700xg for 1 hour at 20° to 25°C.
6. Place stoppered shell-vials in an incubator at 35° to 37°C.
7. When a monolayer is ready to be stained, 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 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 1X PBS to wet the monolayer.
13. Swirl and then aspirate completely.
14. Stain the cell monolayers (cultured specimens and cultured control viruses) and a HSV-1/HSV-2 Antigen Control Slide in the following manner:
    a. For the shell-vial cultures: Add 4 drops of the HSV-1 DFA Reagent or HSV-2 DFA Reagent to respective fixed monolayers of patient and respective control samples. Rock to ensure complete coverage of the monolayer by the Reagent.
    b. For a HSV-1/HSV-2 Antigen Control Slide, add one drop of the HSV-1 DFA Reagent to the well labeled "HSV-1" of a fresh HSV-1/HSV-2 Antigen Control Slide, and one drop of the HSV-2 DFA Reagent to the well labeled "HSV-2", of the same HSV-1/HSV-2 Antigen Control Slide. An Antigen Control Slide should be stained only once, as it contains individual wells of viral-infected cells and non-infected cells. (For complete Antigen Control Slide staining procedure, see Section VI.E. 16-23.)
15. Place stoppered shell-vials in a 35° to 37°C incubator for 15- to 30-minutes.
16. Aspirate the HSV-1 and HSV-2 DFA Reagents 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 1-mL of de-mineralized water.
20. Remove the de-mineralized water by aspiration.
21. Lift the coverslip from the bottom of the shell-vial, grasping it with the fine tipped forceps; then transfer it, monolayer-side down, to a small drop of Mounting Fluid on a labeled microscope slide.
22. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See Section VI.C.11-14, 'Immunofluorescence Microscopy')
23. Refer to Section VII. 'Interpretation of Results'.

G. Cell Culture Testing – Multi-well Plate

1. The laboratory that conducted studies for clearance of this assay using multi-well plates [presented in Section X ('Specific Performance Characteristics')] used one well per HSV type for each specimen and terminated cultures within 24-hours of incubation.
   a. 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).
   b. Multi-well plate cultures are typically terminated after 1- to 4-days, depending on the particular agent. Overnight incubation may be adequate for rapidly growing viruses such as HSV[18].
2. Determine the number of wells needed based on the staining protocol to be used (this staining protocol requires 2 wells: 1 each for HSV-1 and HSV-2 staining).
3. Examine the monolayers for proper morphology prior to inoculation.
4. Aspirate 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.
5. Add 0.2 to 0.4-mL of prepared specimen to the appropriate wells of a multi-well plate.
6. Centrifuge the multi-well plates at 700xg for 1 hour at 20° to 25°C.
7. Place the covered multi-well plates in a 35° to 37°C incubator with a humidified, 5% CO_2 atmosphere.
8. When a monolayer is ready to be stained, remove the medium by aspiration and add 1-mL of 1X PBS.
9. Swirl to mix and then aspirate.
10. Repeat this wash with another 1-mL of 1X PBS and then aspirate.
11. Add 1-mL of 80% aqueous 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.
12. Remove the fixative by aspiration.
13. Add 0.5-mL of the 1X PBS to wet the monolayer.
14. Swirl and then aspirate completely.
15. Stain the cell monolayers (cultured specimens and cultured control viruses) and a HSV-1/HSV-2 Antigen Control Slide in the following manner:
    a. For 24-well plate monolayers, add 4 drops of the HSV-1 DFA Reagent or HSV-2 DFA Reagent to respective fixed monolayers of patient and respective control samples. Rock to ensure complete coverage of the monolayer by the Reagent.
    b. For 48-well plate monolayers, add 3 drops of the HSV-1 DFA Reagent or HSV-2 DFA Reagent to respective fixed monolayers of patient and respective control samples. Rock to ensure complete coverage of the monolayer by the Reagent.
    c. For a HSV-1/HSV-2 Antigen Control Slide, add one drop of the HSV-1 DFA Reagent to the well labeled "HSV-1" of a fresh HSV-1/HSV-2 Antigen Control Slide, and one drop of the HSV-2 DFA Reagent to the well labeled "HSV-2", of the same HSV-1/HSV-2 Antigen Control Slide. An Antigen Control Slide should be stained only once, as it contains individual wells of viral-infected cells and non-infected cells. (For complete Antigen Control Slide staining procedure, see Section VI.E. 16-23.)
16. Place the covered multi-well plate in a 35° to 37°C, humidified incubator for 15- to 30-minutes.
17. Aspirate the HSV-1 and HSV-2 DFA Reagents from the monolayers.
18. Add 1-mL of the 1X PBS and mix to wash.
19. Remove the 1X PBS by aspiration, repeat the wash step, and again remove by aspiration.
20. Add 1-mL of de-mineralized water.
21. Remove the de-mineralized water by aspiration.
22. Add 3 drops of Mounting Fluid to each monolayer, and cover the plate.
23. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See Section VI.C.11-14, 'Immunofluorescence Microscopy')
24. Refer to Section VII. 'Interpretation of Results'.

H. Quality Control

1. Reagents
   a. A fresh HSV-1/HSV-2 Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
   b. The positive well will show multiple infected areas of bright apple-green fluorescence with negative cells fluorescing 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.

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, an HSV-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 monitored 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 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 for HSV 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 to eliminate non-specific staining.

C. Results from Culture Isolation/Confirmation

1. The bright apple-green fluorescence staining pattern is finely granular in appearance, and associated with the cytoplasm and/or nucleus.
2. Examine the entire cell spot or monolayer of cells for herpes simplex virus-specific fluorescent cells. If no fluorescent cells are found, report: "No Herpes simplex virus isolated by cell culture."
3. If fluorescent cells are found in the HSV-1 stained monolayer showing an expected staining pattern, report the result as "herpes simplex virus type 1 isolated by cell culture".

4. If fluorescent cells are found in the HSV-2 stained monolayer showing an expected staining pattern, report the result "herpes simplex virus type 2 isolated by cell culture."
5. If fluorescent cells are found in both the HSV-1 and HSV-2 stained wells, report the results as "herpes simplex virus types 1 and 2 isolated by cell culture".

VIII. LIMITATIONS OF PROCEDURE

1. Inappropriate specimen collection, storage, and transport may lead to false negative culture results[19].
2. Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
3. Since the MAbs used in this kit have been prepared using defined HSV strains, they may not detect all antigenic variants or new strains of the virus should they arise. MAbs may fail to detect strains of viruses which have undergone minor amino acid changes in the target epitope region. The following HSV strains were shown to be reactive with these MAbs (following 24-hr incubation with approximately150 $TCID_{50}$ inoculum):
   a. MAbs in the HSV-1 DFA Reagent have been shown to react with cells infected with the HSV strains HSV-1(f) and HSV-1 (MacIntyre).
   b. MAbs in the HSV-2 DFA Reagent have been shown to react with cells infected with the HSV strains HSV-2(G) and HSV-2(MS).
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. Performance of this kit can only be assured when components used in the assay are those supplied by DHI.
7. Prolonged storage of the HSV-1 DFA Reagent or HSV-2 DFA Reagent under bright light will decrease the staining intensity.
8. Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will bind 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

The clinical studies described in Section X ('Specific Performance Characteristics') used only specimens that were collected and cultured for the presence of *Herpes simplex* types 1 and 2. Most of the specimen types (n=370) evaluated in the clinical studies were swabs taken from surface lesions, e.g., skin or genital. The rest of the specimens were respiratory specimens (n=8) and CSF specimens (n=1), or were from unknown body sites (n=19). The specimen sources and positivity with the Comparator device are described below (Table 2).

TABLE 2: Specimen Sources

Study Site	Total specimens	Unknown +/Total	Genital +/Total	Penis +/Total	Vaginal +/Total	Labia +/Total	Cervical +/Total	Rectal +/Total	perineum** +/Total	Eye +/Total	Urethra +/Total	Eyelid +/Total	Face +/Total	Mouth* +/Total	Skin‡ +/Total	Bronchial/BAL Sputum/NP+/Total	CSF/Brain +/Total
1	107		18/37	1/1	12/14	6/8	1/1	0/1	4/9	0/0	1/1	1/1	2/4	9/14	3/10	5/6	0/0
2	150	7/19	1/3	2/8	16/42	3/6	1/2		3/4	0/3	0/1		2/3	3/12	9/44	1/2	0/1
3	141		40/141														

*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 two of the three Study Sites are tabulated below (specimen information from Study Site 3 was not available).

Of the specimens evaluated in these studies (which had been submitted to the laboratories for HSV testing and typing), 141 of 257 were taken from patients between 18 and 40. Of those in that age range, an average of 21.3% tested positive for HSV-1 and 24.8% tested positive for HSV-2. Of the 34 patients younger (less than 18 years), 29.4% tested positive for HSV-1 and only 8.8% for HSV-2. The prevalence of HSV in the population of specimens that were evaluated in these studies ranged from 32% to 58%, with an average of 45%. The patient demographics are listed below (Table 3).

TABLE 3: Demographics by Age and Gender

Study Site	HSV-1					HSV-2				
	Study Site 1 Values are # pos / Total		Study Site 2 Values are # pos / Total			Study Site 1 Values are # pos / Total		Study Site 2 Values are # pos / Total		
Age / Gender	F	M	F	M	Gender not reported	F	M	F	M	Gender not reported
TOTALS	84	23	111	36	3	84	23	111	36	3
<2y	0/3	1/2	1/2	1/2	0	0/3	0/2	0/2	0/2	0
2y to 10y	4/5	1/2	0/3	0/1	1/1	0/5	0/2	0/3	0/1	0/1
10y to18y	1/4	0	0/6	0/3	0	2/4	0	1/6	0/3	0
18y to 40y	14/47	5/11	9/66	2/16	0/1	16/47	2/11	15/66	2/16	0/1
>40y	7/25	2/8	4/32	3/14	0	5/25	2/8	9/32	0/14	0
Age not reported	0	0	0/2	0	0/1	0	0	0/2	0	0/1

X. SPECIFIC PERFORMANCE CHARACTERISTICS

This study included four hundred and one (401) prospectively collected specimens submitted for herpes simplex culture. Each specimen was evaluated by the Diagnostic Hybrids, Inc. D^3 DFA Herpes Simplex Virus Identification and Typing Kit ("D^3 DFA HSV ID & Typing Kit") and compared to a currently-marketed HSV identification kit ("Comparator device"). A combination of fresh (154) and frozen (247) specimens were tested. Three specimens from Study Site 3 were not evaluated due to bacterial contamination of the monolayers, leaving 398 for analysis. These studies were conducted at two external laboratory sites located in the mid-west United States and one in-house virology laboratory. The number/type of specimens tested at each study site is summarized below (Table 4).

TABLE 4: Numbers of Specimens Evaluated per Study Site

Study Site	Culture Confirmation		Study Site Total
	Fresh	Frozen	
1	4	103	107
2	150	0	150
3	0	141	141

Percent Agreement between the D^3 DFA HSV ID & Typing Kit and the Comparator device was calculated and tabulated for all the tested specimens, and is presented below (Table 5).

TABLE 5: Specimen Total: D^3 DFA HSV ID & Typing Kit and Comparator Device using Various Culture Methodologies

Table 5a – HSV-1

D^3 DFA HSV ID & Typing Kit \ Comparator Device	+	–
+	**73**	0
–	1	**324**

	95% CI [a]
Positive Percent Agreement [b] (PPA) =	98.6% (92.7% to 100%)
Negative Percent Agreement [c] (NPA) =	100% (98.9% to 100%)

Table 5b – HSV-2

D^3 DFA HSV ID & Typing Kit \ Comparator Device	+	–
+	**76**	1
–	0	**321**

	95% CI
Positive Percent Agreement [b] (PPA) =	100% (95.3% to 100%)
Negative Percent Agreement [c] (NPA) =	99.7% (98.3% to100%)

A. Study Site 1: A total of 107 specimens (4 fresh and 103 frozen) were tested using the D^3 DFA HSV ID & Typing Kit. Briefly, an aliquot from each specimen was inoculated into MRC-5 and A549 tubes, one tube for each HSV type. The inoculated

[a] "95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).
[b] "Positive Percent Agreement", or "PPA", values were calculated according to {[Total Number of Positive Results in Agreement by both Subject and Comparator Device) divided by [(Total Number of Positive Results in Agreement by both Subject and Comparator Device) plus (Number of Results Positive by Comparator Device but Negative by Subject)]} multiplied by 100%.
[c] "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 Device) plus (Number of Results Negative by Comparator Device but Positive by Subject)]} multiplied by 100%.

cells were incubated at 35° to 37°C and examined daily for CPE for 7-days. Tube cultures exhibiting CPE were scraped and cell spots made on multi-well slides. Tube cultures exhibiting no CPE at 7-days were also scraped and cell spots made on multi-well slides to confirm the absence of HSV. The cell spots were fixed with acetone in accordance with the respective product insert (Subject and Comparator devices). All calculations for confidence intervals were done according to the Exact Method. The results of this study site are summarized below (Table 6).

TABLE 6: Study Site 1 Results: D³ DFA HSV ID & Typing Kit and Comparator Device using Tube Culture

D³ DFA HSV ID & Typing Kit	Table 6a – HSV-1 Comparator Device +	Table 6a – HSV-1 Comparator Device –	Table 6b – HSV-2 Comparator Device +	Table 6b – HSV-2 Comparator Device –
+	**35**	0	**28**	0
–	0	**72**	0	**79**

	Table 6a – HSV-1	95% CI	Table 6b – HSV-2	95% CI
PPA =	100%	90.0% to 100%	100%	87.7% to 100%
NPA =	100%	95.0% to 100%	100%	95.4% to 100%

B. Study Site 2: A total of 150 fresh specimens were cultured for HSV. Briefly, an aliquot from each specimen was inoculated into MRC-5 tubes, one tube for each HSV type. The inoculated cultures were incubated at 35° to 37°C and examined daily for CPE for 7-days. Tube cultures exhibiting CPE were scraped and cell spots made on multi-well slides according to the D³ DFA HSV ID & Typing Kit and Comparator device product insert procedures. Tube cultures exhibiting no CPE at 7-days were also scraped and cell spots made to confirm the absence of HSV. All 150 specimens were tested for the presence of HSV. All calculations for confidence intervals were done according to the Exact Method.[a] The results of this study site are summarized below (Table 7).

TABLE 7: Study Site 2 Results: D³ DFA HSV ID & Typing Kit and Comparator Device using Tube Culture

D³ DFA HSV ID & Typing Kit	Table 7a – HSV-1 Comparator Device +	Table 7a – HSV-1 Comparator Device –	Table 7b – HSV-2 Comparator Device +	Table 7b – HSV-2 Comparator Device –
+	**21**	0	**25**	1
–	1	**128**	0	**124**

	Table 7a – HSV-1	95% CI	Table 7b – HSV-2	95% CI
PPA =	95.5%	77.2% to 99.9%	100%	86.3% to 100%
NPA =	100%	97.2% to 100%	99.2%	95.6% to 100%

C. Study Site 3: The study was conducted at a Diagnostic Hybrids, Inc. in-house virology laboratory. A total of 141 frozen specimens provided by an independent laboratory were processed. Briefly, an aliquot from each specimen was inoculated into duplicate wells of DHI H&V-Mix™ multi-well plates. The inoculated cells were centrifuged at 700xg for 60-minutes, incubated at 35° to 37°C for up to 24-hours then stained in accordance with the respective product insert. All calculations for confidence intervals were done according to the Exact Method. The results of this study site are summarized below (Table 8).

TABLE 8: Study Site 3 Results: D³ DFA HSV ID & Typing Kit and Comparator Device using Multi-well Plate Culture

D³ DFA HSV ID & Typing Kit	Table 8a – HSV-1 Comparator Device +	Table 8a – HSV-1 Comparator Device –	Table 8b – HSV-2 Comparator Device +	Table 8b – HSV-2 Comparator Device –
+	**17**	0	**23**	0
–	0	**124**	0	**118**

	Table 8a – HSV-1	95% CI	Table 8b – HSV-2	95% CI
PPA =	100%	80.5% to 100%	100%	85.2% to 100%
NPA =	100%	97.1% to 100%	100%	96.9% to 100%

D. Cross-reactivity Testing

The Diagnostic Hybrids, Inc. D³ DFA Herpes Simplex Virus Identification and Typing Kit's HSV-1 DFA Reagent and HSV-2 DFA Reagent were tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 59 virus strains or for 17 host culture cell types. Twenty-seven (27) bacterial cultures, one yeast and one protozoan 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 (See Limitations of Procedure, Section VIII.). [See Tables below for cross-reactivity study results.]

Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the HSV-1 DFA Reagent or the HSV-2 DFA Reagent and relatively high titers of microorganisms. The DFA Reagents were prepared at 1.5X the concentrations that are provided in the kit.

- Fifty-nine (59) virus strains were tested for cross-reactivity. Depending on the particular virus, 500 to 715 $TCID_{50}$ 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 HSV-1 or HSV-2 DFA Reagent at 1.5X concentration according to the procedure as detailed in this product insert. No cross-reactivity was observed for the viruses listed below (Table 9).

TABLE 9: Virus Strains Tested for Cross-Reactivity with HSV-1 DFA Reagent and HSV-2 DFA Reagent

Organism	Strain or Type	Inoculum ($TCID_{50}$)
Adenovirus	Type 1	715
	Type 3	715
	Type 5	715
	Type 6	715
	Type 7	715
	Type 8	715
	Type 10	715
	Type 13	715
	Type 14	715
	Type 18	715
	Type 31	715
	Type 40	715
	Type 41	715
Influenza A	Aichi	715
	Mal	715
	Hong Kong	715
	Denver	715
	Port Chalmers	715
	Victoria	715
	New Jersey	715
	PR	715
	WS	715
Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained.[d]
Coxsackievirus	Types B1, B2, B3, B4, B5, B6	Commercially available slides stained.[d]
Influenza B	Hong Kong	715
	Maryland	715
	Mass	715
	Taiwan	715
	GL	715
	JH-001 isolate	715
	Russia	715
RSV	Long	715
	Wash	715
	9320	715
Parainfluenza 1	C-35	715
Parainfluenza 2	Greer	715
Parainfluenza 3	C 243	715
Parainfluenza 4a	M-25	715
Parainfluenza 4b	CH19503	715
CMV	Towne	700
	Davis	700
	AD169	700
VZV	Webster	500
	Ellen	500
Epstein-Barr	Commercially available slides stained.[d]	
Rubeola		
Mumps		
HPV	Types 6, 11	Commercially available slides stained.[d]

- Seventeen (17) host culture cells types were tested for cross-reactivity. Cell cultures were prepared in shell-vial format. Confluent monolayers were stained with the HSV-1 or HSV-2 DFA Reagent at 1.5X concentration according to the procedure as detailed in the product insert, then examined for cross-reactivity. No cross-reactivity was observed for cell culture types listed below (Table 10).

TABLE 10: Cell lines Tested for Cross-Reactivity with HSV-1 DFA Reagent and HSV-2 DFA Reagent

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

- Twenty-nine (29) microorganisms, including 27 bacterial cultures, one yeast and one protozoan, were stained with the HSV-1 or HSV-2 DFA Reagent at 1.5X concentration according to the procedure as detailed in the product insert, then examined for cross-reactivity. Except for *Staphylococcus aureus*, which was cross-reactive with the DFA Reagents (see above), all microorganisms tested negative. Concentrations for each bacterial organism cultured by DHI for cross-reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of levels 1.0 and 2.0 (equaling approximately 3×10^6 and 6×10^6 CFU per mL). Slides were prepared with spots of 0.01-mL of the suspensions to give either 3×10^4 or 6×10^4 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. No cross-reactivity was observed for the microorganisms

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

listed below (Table 11) (with the exception of *Staphylococcus aureus*, marked with an asterisk [*]).

TABLE 11: Microorganisms Tested for Cross-Reactivity with HSV-1 DFA Reagent and HSV-2 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	2.1×10^5
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	~6×10^7
Mycoplasma hominis	~6×10^4
Mycoplasma orale	~6×10^4
Mycoplasma pneumoniae	~6×10^4
Mycoplasma salivarium	~6×10^7
Ureaplasma uralyticum	~6×10^4
Chlamydophila pneumoniae	Commercially available slides stained.[d]
Chlamydia trachomatis	
YEAST	
Candida glabrata	8.7×10^6
PROTOZOAN	
Trichomonas vaginalis	[Commercially available slides]

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