QUIDEL

ELVIS® HSV

ID and D³ TYPING TEST SYSTEM

A Test System for the Culture, Identification and Typing of Herpes simplex virus using the Enzyme Linked Virus Inducible System®

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

INTENDED USE

The ELVIS HSV ID and D³ Typing Test System provides Cells, Replacement Medium and Test Reagents for the culture, qualitative identification and typing of herpes simplex virus (HSV) from cutaneous or mucocutaneous specimens as an aid in the diagnosis of HSV type 1 (HSV-1) and HSV type 2 (HSV-2) infections. The performance characteristics of this assay have not been established for antiviral therapy, prenatal monitoring or use with cerebral spinal fluid specimens.

SUMMARY AND EXPLANATION OF THE TEST

Herpes simplex virus (HSV) infections in humans can cause lesions at a variety of sites, e.g., oral-facial, genital, visceral, eye, cutaneous and the central and peripheral nervous system. These lesions can be a result of the primary infection by the virus or they can result from a reactivation of the latent virus, causing recurrent episodes of the disease. There are two genetically- and antigenically-distinct forms of HSV, termed HSV type 1 (HSV-1) and HSV type 2 (HSV-2). HSV-2 is most commonly the cause of genital infections, due to venereal transmission; HSV-1 is commonly associated with other disease locations although both serotypes have been shown to cause disease in all locations of the body.

Studies have shown an increasing prevalence of genital HSV infections with a concomitant increase of the disease in neonates. The consequences of HSV infection can range from inconsequential (cold sores in otherwise healthy patients) to highly morbid and fatal (neonates). There is an effective antiviral chemotherapeutic agent (acyclovir) available to treat HSV infections.

Cell culture is widely recognized and used as a sensitive method for the detection of HSV in cutaneous or mucocutaneous lesion samples. When an appropriately sensitive cell type is infected with HSV, a characteristic deterioration of cells, termed cytopathic effect (CPE), can be observed. CPE appears as enlargement and swelling of infected cells at the early stage of infection; radial spread of virus to adjacent cells produces a focal plaque on the cell monolayer during later stages of infection, or at an earlier stage when specimens contain high titers of virus. 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.[1,2,3,4,5,6,7,8]

Deterioration of cells can also result from toxic components present in the clinical specimen making microscopic examination of the infected cells for CPE difficult to interpret. In addition, other viruses that may be present in the specimen can cause CPE. Therefore, confirmation that the cellular changes are due specifically to HSV infection is critical to the identification of HSV in clinical specimens.

ELVIS HSV ID and D³ Typing Test System (ELVIS) combines the cell culture amplification with identification of HSV. The ELVIS test eliminates the need for detecting viruses in culture by CPE and has a turn-around time of < 1 day. The System is offered in two formats: (1) shell-vials with and without coverslips and (2) multi-well plates. Both formats are based on transgenic reporter technology and share the same reagents for detection of HSV in clinical specimens.

PRINCIPLE OF THE PROCEDURE

The ELVIS HSV ID and D³ Typing Test System is comprised of Cells, Replacement Medium and Test Reagents for the culture, qualitative identification and typing of Herpes simplex virus (HSV) isolated from patient specimens.

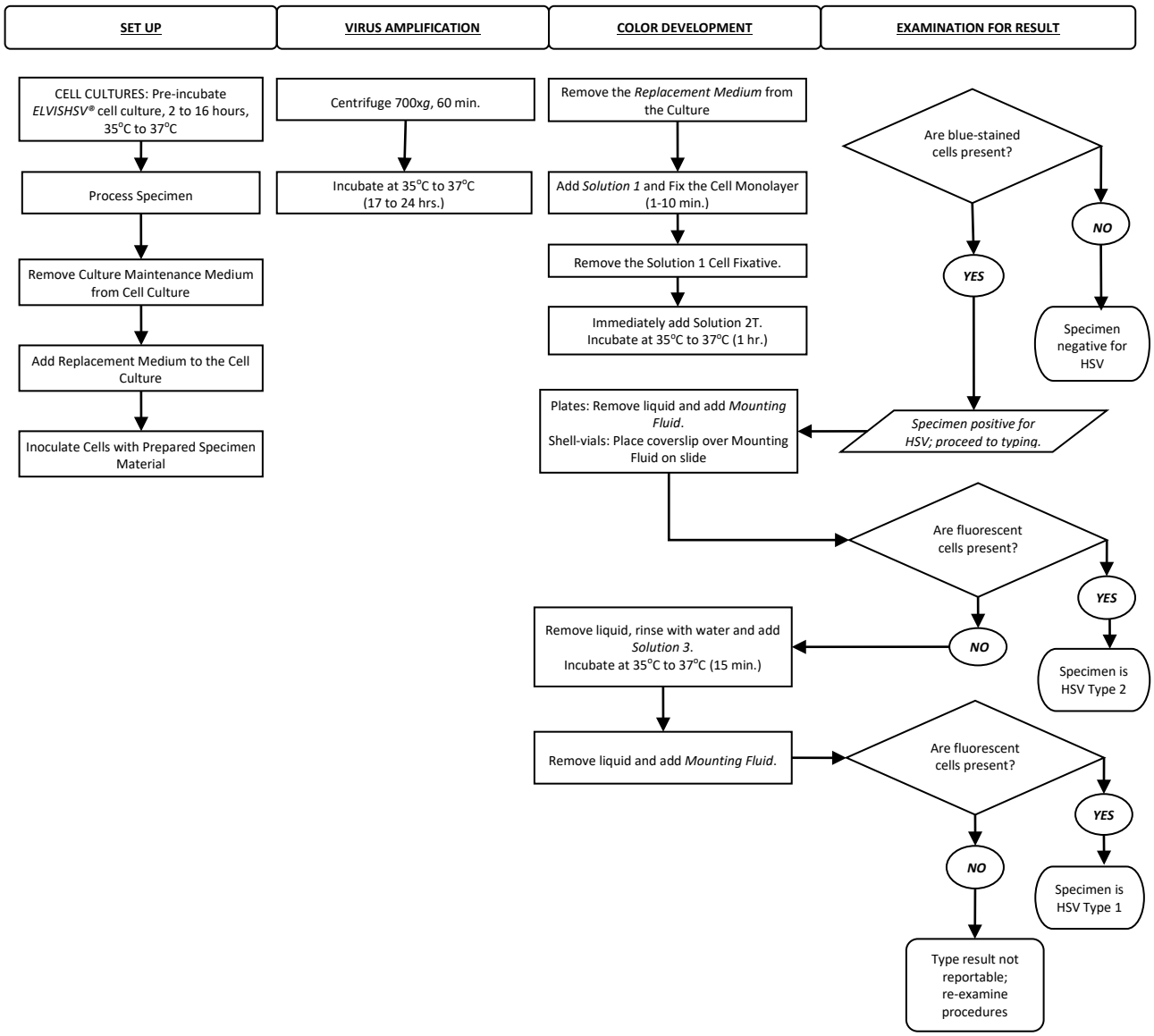
ELVIS HSV Cells are genetically-engineered Baby Hamster Kidney (BHK) cells, which, when infected with either HSV-1 or HSV-2, are induced to generate and accumulate an endogenous, intracellular bacterial enzyme, β-galactosidase. Other related viruses (e.g., Varicella zoster) are not capable of inducing the formation of this enzyme. HSV infection of the ELVIS HSV cells also results in the formation of HSV-type-specific proteins. The presence of these proteins can be detected microscopically when fluorescent labeled HSV-type-specific antibodies are used.

The two Type 1 monoclonal antibodies used in ELVIS are directed against specific epitopes on the HSV-1 protein UL42. The three Type 2 monoclonal antibodies are directed against the HSV-2 glycoproteins C, G and a recombinant glycoprotein G that occur in the cytoplasm of infected cells.

Fresh ELVIS HSV cell cultures are pre-incubated at 35°C to 37°C for 2 to 16 hours. The medium on the cultures is removed and Replacement Medium is added. A specimen swab[9,10] is eluted into a cell culture medium and inoculated onto an ELVIS HSV Cell monolayer. The inoculated cultures are centrifuged and incubated at 35°C to 37°C for a minimum of 17 to 24 hours. The inoculated monolayers are fixed using Solution 1 for 1 to 10 minutes. Solution 1 is then removed and the cells are stained with Solution 2T for 1 hour at 35°C to 37°C. Solution 2T contains the chromogenic substrate for the induced β-galactosidase enzyme, the type-2-specific, fluorescein-labeled monoclonal antibodies and the non-labeled type-1-specific monoclonal antibodies. During this 1-hour incubation period, two reactions will occur if HSV infected cells are present: the accumulated β-galactosidase will react with the substrate to produce a blue precipitate; and monoclonal antibodies will react with virus-specific proteins in the cells. The cell monolayer is examined with standard light microscopy for the presence of blue precipitate containing cells to identify the presence of HSV. Those specimens with no blue cells are HSV negative and can be reported as such. Those specimens identified as HSV positive are examined using a fluorescence microscope for the presence of fluorescent cells which, if present, identify HSV-2 as the infecting virus. If no fluorescent cells are seen, the monolayer is rinsed and then stained for 15 minutes at 35°C to 37°C with Solution 3, which contains fluorescein-labeled goat-antimouse IgG antibodies. The monolayer is re-examined using a fluorescence microscope for the presence of fluorescent cells which, if present, identify HSV-1 as the infecting virus. Due to the high level of assay specificity, background is practically non-existent.[11,12]

The shell-vial culture format allows processing specimens individually. The multi-well plates require no coverslips to handle and manipulate and no stoppering and un-stoppering, with a maximum of 24 specimens being handled at once. After a minimum of 17 hours incubation of the monolayers inoculated with the specimens, they are ready for fixation with Solution 1, and staining using Solution 2T.

Flowchart of ELVIS Procedure



REAGENTS AND MATERIALS PROVIDED

The ELVIS HSV ID and D³ Typing Test System consists of:

ELVIS HSV Cells **55-xxxx**

The ELVIS HSV Cells have a routine use period of 7 days from customer receipt while all other components have a shelf-life of months (see expiration date on label of each component). ELVIS HSV Cells are provided as 75% to 95% confluent monolayers in shell-vials with or without coverslips, or in multi-well plates with or without coverslips, and up to 24 monolayers per plate. Each monolayer is covered by at least 0.75 mL of Eagle's Minimum Essential Medium (EMEM) with fetal bovine serum (FBS), penicillin, and streptomycin. Cells are characterized by isoenzyme analysis and have been tested and found free of Mycoplasma spp. and other adventitious organisms.

ELVIS HSV Replacement Medium **10-220xxx**

Sterile EMEM containing FBS, Glutamine, Penicillin, Streptomycin and Amphotericin B. ELVIS HSV Replacement Medium is for use with ELVIS HSV Shell-Vials and Multi-well Plates.

ELVIS HSV Solution 1 (Cell Fixative) **00-0110xx**

An aqueous acetone solution.

Note: Label text is red as the reagent bottle is the same size as the 40X PBS Concentrate below.

ELVIS HSV Solution 2T (Staining Buffer) **00-0120xx.v2**

A diluted solution of X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside), N,N-Dimethylformamide, iron, sodium and magnesium salts, fluorescein-labeled HSV-2-specific murine MAbs (directed against HSV-2 glycoproteins C, G, and a recombinant glycoprotein G) and non-labeled HSV-1-specific murine MAbs (specific to epitopes on the HSV-1 protein UL42), penicillin, streptomycin, bovine serum albumin and Evans Blue in an aqueous, buffered solution.

ELVIS HSV Solution 3 **00-0130xx.v2**

An aqueous, stabilized, buffered solution containing fluorescein-labeled, affinity purified goat-anti-mouse IgG antibody and Evans Blue with sodium azide as preservative.

ELVIS HSV Mounting Fluid **00-0140xx**

Aqueous, stabilized, buffered glycerol (pH 7.3 +/- 0.5), containing sodium azide as preservative

40X PBS Concentrate **25 mL**

One bottle of a 40X PBS concentrate consisting of 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water).

The following are sold separately from the kit:

- ELVIS HSV Cells
  - 55-0101 (shell-vials without coverslips)
  - 55-0102 (shell-vials with coverslips)
  - 55-24xx* (multi-well plates)

    *up to 24 monolayers per multi-well plate
- ELVIS HSV Replacement Medium
  - 10-220100 (100 mL)
  - 10-220500 (500 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

- Ambient temperature centrifuge with free-swinging rotor and carriers capable of spinning ELVIS HSV cell culture plates or shell-vials at 700xg.
- Sterile disposable 1 mL pipettes, 0.1 mL graduations; one per specimen.
- Sterile 5- or 10-mL pipette for dispensing *Replacement Medium.*
- Pipettes for dispensing Solution 1 and Solution 2T.
- Disposable plate seals.
- Sterile, disposable Pasteur transfer pipettes.
- Bent teasing needle (for removal of coverslip from a shell-vial for the typing portion of the procedure); fashion a 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. This assists user in lifting an ELVIS stained coverslip (and removing with forceps) from shell-vials. Coverslips may be inverted onto mounting medium dotted on a glass slide for ease of interpretation using an immunofluorescence microscope.
- Class II BioSafety cabinet for aseptic handling of cell cultures and specimens.
- Vacuum aspirator with trap containing hypochlorite disinfectant at a minimum concentration of 0.05%.

- Incubator to maintain 35°C to 37°C. For use with:
  - **ELVIS HSV Multi-well Plates**: The incubator must be humidified with an atmosphere of 5% CO_2 (post-inoculation).
  - **ELVIS HSV Shell-Vials**: There are no special requirements when the vials are tightly capped.
- Inverted or standard light microscope of 100X magnification.
- Fluorescence microscope of at least 200X magnification with a filter for fluorescein
  NOTE: A properly functioning fluorescence microscope is critical to achieving correct results when using fluorescent antibodies. Variations in bulb intensity, bulb alignment and quality of the objective lens can affect interpretation of results. Positive controls are mandatory in fluorescence microscopy. We strongly recommend using a mechanical stage on the microscope to assure complete and efficient examination of the monolayer.
- Positive HSV controls: [Herpes simplex type 1 and type 2 virus strains for preparing positive controls are available from various sources. Contact Quidel technical support for options or recommendations.]
- Microscope slides.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Substitution of reagents, cell lines or other culture systems with the ELVIS HSV ID and D³ Typing Test System is prohibited*.*
- Consider all human specimens, blood derivatives, reagents and materials used for processing as capable of transmitting infectious diseases and handle them in a manner which prevents infection of laboratory personnel. No known test method can offer complete assurance that infectious agents are absent.
  - Conduct all procedures in accordance with the OSHA Standard on Bloodborne Pathogens[13]; the manual "Biosafety in Microbiological and Biomedical Laboratories", CDC, 5th edition, 2007; and, the standard, CLSI/NCCLS Approved Guideline, M29-A3, "Protection of Laboratory Workers from Occupationally Acquired Infections".[14]
  - Cell cultures may have potential as biohazards. Personnel working with cultures must be properly trained in safe handling[15] and have proficiency with tissue culture and aseptic techniques before attempting this procedure.
  - Follow Biosafety Level 2 or other appropriate biosafety practices.
  - Decontaminate specimens and cultures using a 1:10 dilution of household bleach.
- ELVIS HSV Cells are not to be passed or used for serial propagation.
- Only individuals competent in cell culture isolation techniques and the interpretation of virus isolation results should use this device.
- The use of reagents and the inoculation of cells must be prior to or on the Expiration Date.
- Use a safety device for all pipetting steps. **Never** pipette by mouth.
- Solution 1 (Cell Fixative) contains acetone, which is flammable. Keep away from flames and other sources of ignition. Avoid contact with eyes, skin and clothing. If contact occurs, flush with water. The solution is supplied at working strength; any dilution will decrease assay sensitivity.
- Solution 2T (Staining Buffer) contains N, N-Dimethylformamide, a potential carcinogen. Avoid inhalation and skin contact. Should skin contact occur, flush the affected area with copious quantities of water. The solution is supplied at working strength; any dilution will decrease assay sensitivity.
- Solution 3 (Anti-mouse IgG/FITC Conjugate) and Mounting Fluid contain sodium azide as a preservative. When discarding into sewage, always flush with copious amounts of water. The solutions are supplied at working strength; any dilution will decrease assay sensitivity.

- The 40X PBS Concentrate contains 4% Sodium Azide.
  - Sodium azide is considered poisonous. If the 40X PBS Concentrate is swallowed, seek medical
  - advice immediately.
  - Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
  - Avoid disposal of this material down sanitary or industrial plumbing systems.
  - Avoid release to the environment.
- Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush immediately with water.
- 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 working this kit.
- 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.

STORAGE

Storage conditions vary for different components of the kit. Upon receipt, components should be stored as follows:

Table 1. Reagent Storage Conditions

ELVIS HSV Cells:	(See notes below)
Shell-Vials	Store upright at 22°C to 28°C in the dark
Sealed Multi-well Plates	Store seal-side up at 22°C to 28°C in the dark
IMPORTANT: DO NOT STORE IN 35°C to 37°C INCUBATOR. Storage of ELVIS HSV Cells in the incubator (above 28°C) results in overgrowth of the monolayers and sub-optimal morphologic interpretation of results	
ELVIS HSV Replacement Medium	Store at 2°C to 8°C.
ELVIS HSV Solution 1	Store at 2°C to 30°C.
ELVIS HSV Solution 2T	Store at 2°C to 8°C in the dark.
ELVIS HSV Solution 3	
ELVIS HSV Mounting Fluid	
40X PBS Concentrate	Store at 2°C to 8°C. 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.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

- ELVIS HSV Cells exhibiting turbidity (contamination) should be discarded and not used.
- Discoloration, turbidity, or precipitation in any of the ELVIS Solutions or the Replacement Medium indicates possible microbial contamination or deterioration and should not be used.
- Solutions or ELVIS HSV Cells showing signs of leakage should not be used.
- Failure of the controls to perform as expected may be indicative of deterioration.

REAGENT PREPARATION

1X PBS Solution

Warm the 40X PBS Concentrate to ambient temperature (20°C to 25°C) and mix to re-dissolve the crystals. Add contents of the fully dissolved 25 mL 40X PBS Concentrate to 975 mL of de-mineralized water. Label the 1X PBS with a 60-day expiration date after reconstitution, and store at ambient temperature.

SPECIMEN HANDLING AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful HSV isolation. Specimen collection, specimen processing, and cell culture isolation of viruses should be attempted only by personnel trained in performing such procedures.[16,17,18] Care should be taken during all specimen collection and handling to avoid generation of aerosols.

Creams, ointments, lotions, ice, alcohol, Betadine solution, zinc, or a recent sitz bath all reduce viral yield significantly. Use of such remedies should be avoided, if possible, prior to specimen collection, or be reported to the physician when the lesion is sampled. Try not to draw blood, if possible, because antibodies present in plasma may inhibit viral replication in cell culture.[19,20,21]

The preparation of the specimen prior to inoculation is very important to achieving proper results with any virus culture procedure. The culture medium is an excellent growth medium; therefore, if the specimen contains microorganisms, as most do, the contaminant can grow to the point of obscuring or preventing the culture of *HSV*. The longer the incubation period used for virus culture, the more likely contamination of the medium will interfere with the test. Thus, the spin-amplified tests, which can be incubated for as little as 17 hours, are much less susceptible to interference from microbial contamination than standard tube cultures that may be incubated for up to 7 days. To help reduce interference from microbial contamination, Replacement Medium provided with the ELVIS HSV Test System contains Penicillin, Streptomycin and Amphotericin B.

Specimen material present on a swab should be eluted by vigorous agitation (i.e., vortexing) of the transport system, or of the swab in a sterile vessel containing of sterile culture medium. The swab should then be discarded as biohazardous waste.

The specimen eluate should be treated by methods previously established by the laboratory to release cell associated virus into the medium; however, only clear supernatant should be used as inoculum.

If microorganism contamination is apparent (perhaps exhibiting turbidity, flocculence or precipitate) or if excessive debris is present, clarify the specimen by centrifugation (700 to 1000xg for 10 minutes) and filter it through a 0.45 or 0.2-micron pore-size sterilizing filter membrane prior to inoculation. Since such procedures may reduce the number of viruses in a specimen, each individual laboratory should establish the efficacy of its specimen preparation procedures.

We recommend rectal and oropharyngeal specimens be clarified by centrifugation and sterile-filtered before inoculation into cell cultures.[22]
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. These temperatures can be attained using cold packs, wet ice, foam refrigerant, or other coolants.

Specimens should be processed and tested as soon as possible but may be stored at 2°C to 8°C for up to 72 hours before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.[23] Freezing and thawing specimens should be avoided since this will result in a loss of viability of viruses leading to decreased sensitivity for cell culture isolation.

PROCEDURE

Preliminary Comments and Precautions

- Do not use any test component beyond its expiration date.
- Shell-vials and multi-well plates are received with cells adhered to the bottoms at densities of 75% to 95% confluence. For optimal viral amplification and test sensitivity, the shell-vials and multi-well plates should be incubated for a period of 2 to 16 hours before specimen inoculation.
- Previous studies have indicated the use of the shell-vial or multi-well plate format yield similar results and are interchangeable in the ELVIS Test system.
- If the number of specimens to be run is insufficient to use all the wells in a plate, specimens may be stored until sufficient numbers are obtained for testing. See *SPECIMEN HANDLING AND PREPARATION*.
- All cultures should be handled in a Class II biosafety cabinet.
- When opening the shell-vials, the stoppers should be placed aside so that they will not become lost or contaminated.
- The sealant is removed from the plates in a Class II biosafety cabinet by grasping a corner of the seal and peeling it back from the plastic plate. Discard the seal.
- Before addition of the ELVIS HSV Replacement Medium, the culture maintenance medium should be removed from each monolayer by aspiration or hand-operated pipette.
  - The vacuum of the aspirator system, if used, should be such that it yields a gentle aspiration of the medium from the monolayer.
  - Holding the tissue culture plate or shell-vial at a 30°C angle, aspirate the medium at the meniscus, following it down almost to the monolayer. By following this procedure, disturbance of the monolayer will be minimized, and the medium can be almost completely removed.
- Cell monolayers **MUST NOT** dry before adding Replacement Medium. Drying of the monolayer causes cell death and will cause a non-diagnostic result. Monolayers that have been allowed to dry will appear toxic when stained.
- The specimens, controls and reagents should be mixed well before use.
- Additions of solutions to the monolayers should be made by touching the pipette tip to the side of the container and allowing the solution to flow down the side. Avoid directing the stream on the monolayer to prevent undue disturbance of the monolayer.
- When inoculating specimens, use a fresh, sterile pipette for each specimen to avoid cross-contamination, which could cause erroneous results.
- During inoculation of specimens in multi-well plates, touch the pipette tip to the inside of the cell well to avoid possible contamination of adjacent wells.
  NOTE: DO NOT "blow out" residual specimen liquid from the pipette tip since it can result in contamination of adjacent wells with the specimen.
- Specified incubation times and temperatures should be observed and recorded in order to ensure proper test performance.
- Cell culture plates should not be exposed to more than one centrifugation step.
- Specimens are incubated for a minimum of 17 hours. Incubation periods of 17 hours were used in the clinical evaluations reported in *SPECIFIC PERFORMANCE CHARACTERISTICS*.
  NOTE: The extended period of incubation beyond 17 hours was previously evaluated to accommodate incubations extending over a weekend. Incubation for more than 24 hours of cells inoculated with high virus titers or toxins that may be present in the specimens will result in the loss of much of the monolayer. If this is observed, it may be necessary to repeat the culture of that specimen after diluting it or reducing incubation time to 17 to 24 hours, or both. For specimens with lower virus titers, the effect will be more and larger foci. There will be no effect on negative, non-toxic specimens.
- **Do not allow** the monolayers to dry during the staining steps of the procedure. Drying of the monolayer could cause a degradation of the β-galactosidase. This degradation will lead to reduced blue-precipitate production and may lead to erroneous results.

- When removing a coverslip to a microscope slide, be sure to place the coverslip onto the drop of Mounting Fluid **cell-side-down** to ensure that cells are properly bathed in the fluid and that they will not dry out.
- It is a good practice to examine the positive and negative controls before examining the clinical specimen monolayers.
- The medium on the inoculated monolayers should be clear and peach to cherry in color after overnight incubation. Turbidity or a color change to yellow indicates possible bacterial contamination and may render a test result unreliable, due either to a technical contamination during the culture setup or to a contaminated specimen. We recommend the original specimen be filtered and re-cultured.
  NOTE: A color change to magenta indicates a pH shift which may prove toxic to the cells. A "toxic" specimen may cause a portion of the monolayer to be lost due to detachment or cell death. This can be caused by toxins present in the specimen, virus overload or improper incubation conditions. If specimen toxicity is apparent based on appearance of the cell monolayer, repeat the Specimen Inoculation and Incubation procedure using a 1:5 dilution of the residual specimen in Replacement Medium.
- Non-specific blue precipitate possibly can occur if a specimen is grossly contaminated with, or contains epithelial cells colonized with bacteria or yeast. Such precipitate is on a different **focal plane** than the monolayer and will also have a quite different appearance than the infected ELVIS HSV Cells. If blue staining debris or epithelial cells are seen, the original specimen should be filtered and re-cultured.
- Light background fluorescent staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A binds to the Fc portions of the conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, e.g., *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots.
- A useful method for examining each monolayer completely for the presence of blue stained cells indicating HSV infection in the cells is to first scan the circumference of the monolayer. Next, starting at the top, scan from one side to the other, drop down a field width and scan back to the other side, drop down a field width, etc. until the bottom of the monolayer is reached. Scanning is most efficiently done using a 40X magnification and switching to 100X for closer examination of suspicious areas.
- This method should be used for examining for fluorescence as well.

Specimen Inoculation and Incubation

1. Pre-incubate ELVIS HSV Cell cultures at 35°C to 37°C for 2 to 16 hours.
2. Following the required pre-incubation, briefly examine the microscopic appearance of the monolayers. Cells should be healthy in appearance, adhering to the bottom of the wells or shell-vials, and either rounded (from contact with neighbors) or stretched and spindle-shaped (characteristic of fibroblasts).
3. Aspirate the medium from each of the monolayers, taking care not to disturb the cells.
4. Add 1 mL of ambient temperature (18°C to 26°C) Replacement Medium to each monolayer to be used in the assay, including positive and negative controls.
5. Add 0.4 mL of the patient specimen to a single monolayer. Freeze the remainder of each specimen at –70°C or lower, for future reference.
6. Inoculate one monolayer each with an HSV-1 and HSV-2 Positive Control and leave one monolayer un-inoculated as a Negative HSV Control for each test run.
7. After all inoculations have been completed, reseal the plate with a disposable tray seal and replace the cover on the plate, or re-cap the shell-vials, and centrifuge at 700xg for 60 minutes at ambient temperature.
8. Incubate the inoculated cells at 35°C to 37°C for 17 to 24 hours.

Cell Fixation and Staining for HSV-positive Monolayers

1. Aspirate the medium completely from each monolayer and add 0.25 mL of Solution 1 (Cell Fixative) to each monolayer.
2. Mix by **rocking to ensure that each monolayer is uniformly covered** with solution.

3. Allow to stand for a minimum of 1 minute and a maximum of 10 minutes.
4. Aspirate *Solution 1* from each monolayer.
5. Add Solution 2T *(Staining Buffer)* to each monolayer: Add 0.25 mL (i.e., 5 drops from a dropper bottle) to each shell-vial or multi-well plate monolayer.
6. Mix by **rocking to ensure that each monolayer is uniformly covered** with solution.
7. Cover the containers and place at 35°C to 37°C for 1 hour.
8. Examine each monolayer entirely for stained cells using a light microscope with a magnification of 100X. See *INTERPRETATION OF ELVIS STAIN RESULTS* for the criteria for a positive, negative or non-diagnostic result.

Typing ELVIS HSV-2 Positive Monolayers

After the ELVIS HSV Cell positive monolayers have been identified, they may be prepared for examination for HSV-2 fluorescence:

Shell-Vials

1. Aspirate the Solution 2T from the monolayer.
2. Add 1 mL 1X PBS.
3. Remove the PBS by aspiration, repeat the rinse and again remove the PBS by aspiration.
4. Add 1 mL of de-mineralized water. Remove by aspiration.
5. Lift the coverslip carefully from the bottom of the shell-vial using a bent teasing needle.
6. Remove the coverslip with forceps. Take care to identify the cell monolayer side of the coverslip.
7. Blot excess water by touching the edge of the coverslip to absorbent paper.
8. Mount the coverslips with the monolayer side down on a drop of Mounting Fluid on a microscope slide that has been marked to identify the specimens. Examine for fluorescence using an immunofluorescence microscope.
9. When interpreting results, refer to *Specimen Inoculation and Incubation*.

Multi-well Plates

1. Mark the HSV-positive wells for later identification.
2. Aspirate Solution 2T from all wells. Take care not to disturb the monolayer when aspirating.
3. Add 1 mL of 1X PBS to the wells.
4. Aspirate the 1X PBS from all wells. Take care not to disturb the monolayer when aspirating.
5. A second time, add 1 mL of 1X PBS to the wells.
6. Aspirate the 1X PBS from all wells. Take care not to disturb the monolayer when aspirating.
7. Add several drops of Mounting Fluid to the positive wells to cover the monolayers.
8. Replace the plate lid, invert the plate (if an inverted fluorescence microscope is not used) and carefully scan for fluorescence at 100X magnification using the fluorescence microscope.
   NOTE: Make sure the inverted plate is oriented correctly so that each well being examined matches the correct specimen number.
9. When interpreting results, refer to Sections *Cell Fixation and Staining for HSV-positive Monolayers* and *INTERPRETATION OF ELVIS STAIN RESULTS*.

Typing ELVIS HSV-1 Positive Monolayers

An HSV-1 infected monolayer will present with blue cells but there will be **NO** fluorescent cells in the monolayer until the following procedure is performed. Blue-cell-positive monolayers with no HSV-2 fluorescence must be stained with Solution 3 by the following procedure to confirm detection of HSV-1.

Shell-Vials

1. Lift the coverslip from the slide using forceps. This is easily accomplished by first "floating" the coverslip by adding a drop of water to the edge of the coverslip.
2. Rinse the Mounting Fluid from the coverslip by several immersions in a beaker of 1X PBS while still holding it with the forceps.
3. Blot excess PBS by touching the edge of the coverslip to absorbent paper.
4. Add 1 drop of Solution 3 to a surface such as a glass microscope slide.
5. Place coverslip, monolayer side down, on the drop of Solution 3.
6. Incubate the coverslip in a humidified incubator or chamber at 35°C to 37°C for 15 minutes.
7. Rinse Solution 3 from the coverslip by several immersions in a beaker of 1X PBS while still holding it with the forceps.
8. Blot excess PBS by touching the edge of the coverslip to absorbent paper.
9. Rinse the coverslip gently by a single immersion in a beaker of distilled water while still holding it with the forceps.
10. Blot excess distilled water by touching the edge of the coverslip to absorbent paper.
11. Add 1 drop of Mounting Fluid to a fresh microscope slide.
12. Place coverslip, monolayer side down, on the drop of Mounting Fluid and examine again for fluorescence.
13. When interpreting results, refer to Sections *Cell Fixation and Staining for HSV-positive Monolayers* and *INTERPRETATION OF ELVIS STAIN RESULTS*.

Multi-well Plates

1. Rinse the monolayer free of Mounting Medium by adding 1 mL of 1X PBS to the well and gently aspirating.
2. Add a second 1 mL of 1X PBS and aspirate. Do not disturb the monolayer.
3. Add 0.25 mL of Solution 3 to the well.
4. Rock the plate to assure that the entire monolayer is covered.
5. Incubate for 15 minutes at 35°C to 37°C in a humidified incubator.
6. Rinse the monolayer free of Solution 3 by adding 1 mL of 1X PBS and then gently aspirate.
7. Repeat the rinse step with a second 1 mL rinse and aspirate.
8. Add several drops of Mounting Fluid and examine again for fluorescence.
9. When interpreting results, refer to Sections *Cell Fixation and Staining for HSV-positive Monolayers* and *INTERPRETATION OF ELVIS STAIN RESULTS*.

Stability of Final Reaction Material

The blue-colored precipitate in the infected cells is stable for at least 7 days when stored at 2°C to 8°C provided Solution 2T is removed, and the monolayer is washed with 1 mL of water and covered with 1 mL of PBS.

The fluorescent stain in infected cells is stable for at least 7 days when stored at 2°C to 8°C and covered with a layer of Mounting Fluid.

QUALITY CONTROL[24]

Guidance on appropriate quality control procedures and practices may be found in the above reference to the CLSI (formerly NCCLS) C24-A, Approved Guideline "Statistical quality control for quantitative measurements: Principles and definitions, 1999", 7.2 (Control materials: Characteristics) and 8.2 (QC applications: Frequency of control measurements).

To assure that the culture, cell fixation and stain development procedures have been properly conducted and to provide a basis for interpreting specimen results, an HSV-infected and a non-infected monolayer should be included with each run.

If the controls do not perform as expected, review the steps and conditions under which the test was performed to determine the cause(s). Do not report results until controls perform properly. For technical assistance call Quidel.

INTERPRETATION OF ELVIS STAIN RESULTS

Characteristics of an HSV-Positive Result

A positive result for the presence of HSV is indicated by the microscopic observation of infected monolayer cells with **intracellular** blue stain precipitate.

NOTES:

- Be familiar with the appearance of non-specific non-cell-associated blue precipitate, as described in *Preliminary Comments and Precautions*. Such blue precipitate is **not** indicative of a positive *HSV* result.
- Monolayers commonly described as "toxic" due to either high virus titers or specimen toxicity factors or to a combination of the two may yield poorly stained cells due to the rapid development of toxicity prior to formation of sufficient amounts of β-galactosidase. If such a condition is seen in which the monolayer appears heavily infected or toxic but blue cells are not seen, the specimen should be diluted 1:5 with Replacement Medium and a fresh ELVIS HSV Cell culture inoculated. The culture should be processed as before, and the result examined for presence of intracellular blue stain precipitate.
- The intensity of cell staining can vary from light to very dark within a focus and within a monolayer.
- The number of stained cells is proportional to the number of virions in the specimen. Thus, a positive result is indicated when only one monolayer cell is stained blue or blue-green.
- Positives that are representative of specimens with very low virus load may have only one stained focus in the monolayer. There may be no evidence of CPE such as syncytial formation, cell toxicity, etc. in these weak positives.

Report: Herpes simplex virus detected

Characteristics of an HSV-Negative Result

A negative result indicating the absence of HSV is when there are no blue stained cells in the monolayer.

NOTES:

- The entire monolayer must be examined microscopically at 100X magnification before a negative result can be reported.
- There can be cells or debris present on the monolayer which at first appear to be stained blue but are **not HSV infected** since:
  - They are not in the same focal plane as the monolayer cells. Non-specific staining can possibly occur if a specimen contains epithelial cells lightly colonized with E. coli.[12] Such cells are in a different focal plane than the monolayer cells and also have a quite different appearance than the ELVIS HSV Cells. (See *Preliminary Comments and Precautions*.)
  - The color is not localized within the monolayer cell.
  - There is no color in the cells; they appear dark simply due to light refraction.

Report: Herpes simplex virus not detected

NOTE: No diagnostic test for HSV, including viral isolation, will yield 100% positive results in populations of patients with typical clinical histories.[25] When a specimen tests negative but the patient history, clinical signs or serology strongly suggest HSV infection, another specimen should be taken and retested.

Characteristics of a Non-Diagnostic Result

- The results of the Negative and/or Positive Control are not as expected
  [Identify the cause and repeat the test.]

- The monolayer has been partially or completely destroyed due to either toxic factors and/or virus overload in the specimen.
  [A 1:5 dilution of the specimen is made and the test repeated on the dilution in order to obtain reportable results on such specimens. See *Preliminary Comments and Precautions*.]

If the repeated test is also toxic or otherwise unreadable, recommend that another specimen be collected, and report the specimen result as follows:

Report: Unable to Determine the Presence or Absence of Herpes simplex virus due to specimen toxicity. Recollect specimen.

- The stained result appears as non-specific non-cell-associated blue precipitate on the monolayer, or the patient specimen contains excessive debris or cells that could mask small foci or individually infected cells
  [The specimen should be re-filtered and re-cultured. See *Preliminary Comments and Precautions*.]

The cause of a non-diagnostic result should be identified. The specimen should be re-tested after the cause has been identified and corrected.

If the cause cannot be found, recommend that another specimen be collected, and report the specimen result as follows:

Report: Unacceptable specimen (with a statement indicating why).

HSV Type 2 Interpretation

Positive monolayers infected with HSV-2, in addition to presenting with blue cells, will also contain cells that show apple-green membrane and cytoplasmic fluorescence when examined using the fluorescence microscope.

Some HSV-2 blue cells, especially those with heavy blue staining, may show a diffuse fluorescent halo or no fluorescence. This is characteristic of the system as the deposit of a heavy blue precipitate which indicates the specific presence of HSV tends to quench the fluorescent signal in its immediate area.

In very low titer positives in which 10 or fewer blue cells are present, care must be taken to examine the entire monolayer using at least 100X magnification. The presence of a **single fluorescent cell** in a blue-cell-positive monolayer indicates HSV-2.

Report: HSV type 2 isolated.

NOTE: A weakly positive HSV-2 specimen whose fluorescence is overlooked after staining with Solution 2T will continue to fluoresce after staining with Solution 3. This oversight could result in falsely reporting an HSV-2 specimen as an HSV-1.

HSV Type 1 Interpretation

The presence of bright apple green fluorescent nuclear staining in blue-positive monolayers that were not fluorescent upon initial examination confirms that the isolate is *HSV*-1.

Report: HSV type 1 isolated.

Non-Typable HSV Interpretation

If, following the confirmation procedure, fluorescent cells still are not observed, the specimen may be reported as positive for HSV, but not typed. It will be necessary to re-culture the specimen to determine the type.

LIMITATIONS OF THE PROCEDURE

- Performance characteristics of the ELVIS HSV ID and D³ Typing Test System for the screening of HSV-asymptomatic pregnant women prior to delivery have not been established. [Refer to CDC 'Sexually transmitted diseases treatment guidelines 2002, especially 'Virologic tests' section within 'Diseases characterized by genital ulcers; Management of patients who have genital ulcers' and the 'Special populations' section.[15,26,27]]
- As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should be made only by the physician after all the clinical and laboratory findings have been evaluated.
- Live HSV virus particles must be present in the specimen when it is inoculated into the cell culture in order for virus amplification to occur. Many factors can affect viability of virus and/or the ability of the virus to culture. These include, but are not limited to, factors such as transport time and conditions, stage of lesion when specimen was taken and inhibitory components present in the patient specimen, which significantly reduce the ability of the cultured cells (modified BHK's) to produce detectable levels of HSV.
- Bloody specimens may contain antibodies that may inhibit viral replication in cell cultures.
- There is the possibility that this culture system may detect replication-defective Herpes simplex virions.[11]
- High virus titers or specimen toxicity factors or a combination of the two may yield lightly stained cells due to the rapid development of toxicity prior to formation of larger amounts of β-galactosidase.
- This test is limited to the qualitative detection of HSV. Performance characteristics for the quantitative determination of HSV virions have not been established.
- This test may not distinguish between a dual type-infection of HSV-1 and HSV-2 and a single infection of either.

EXPECTED VALUES

Clinical studies were performed at three sites with 735 specimens using a legally-marketed device and the ELVIS HSV ID and D³ Typing Test System. Sixteen specimens were either toxic or contaminated in cell culture, leaving 719 specimens for analysis.

Specimens used in the studies were obtained from a variety of sources. Table 2 shows the specimen source distribution at the combined Study Sites:

Table 2. Combined Study Sites Specimen Source Distribution Values

Source	# Positive (based on Subject Device)/Total
Total Specimens	**719**
Unknown +/–	94/175
Genital† +/–	18/50
Penis +/–	14/44
Vaginal +/–	45/105
Labia +/–	23/47
Cervical +/–	18/50
Perineum* +/–	16/40
Vulva +/–	23/66
Urethra +/–	0/12
Face +/–	4/32
Mouth** +/–	9/37
Skin‡ +/–	13/42
Lesion +/–	5/14
Bartholin Cyst +/–	1/1
Wound +/–	0/4

† Genital: specific area of genitalia is unknown
* Perineum: anal, buttock, tailbone, groin
** Mouth: mouth, lip, throat, tongue, nasopharynx
‡ Skin: skin, breast, leg, arm, abdomen, thigh, ankle, back, finger, hand

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Study Data

Studies were performed at three locations using 735 specimens submitted, April through May, 2009, for HSV culture. The number of specimens cultured at each of the three sites: Study site 1 - 299 specimens; Study site 2 - 136 specimens; and Study site 3 - 300 specimens. The specimens were cultured in duplicate and stained concurrently with both devices. The data generated by each site was similar and has been combined for presentation. Of these 735 specimens, 16 were excluded from the final analysis for the reasons listed in Table 3.

Table 3. Combined Study Sites Rejected Specimens/Samples

Exclusion criteria – Toxic to cell culture	13
Exclusion criteria – Contaminated	3
Grand Total	16

Table 4 shows the age and gender distribution for individuals included in the Study:

Table 4. Combined Study Sites – Age and Gender Distribution (720 Specimens)

Age Range	Values are # Positive (based on Subject Device) / Total		
	Male	**Female**	**Total**
0 to 1 month	0/9	1/9	1/18
> 1 month to 2 years	0/1	0/1	0/2
> 2 to 12 years	1/7	4/7	5/14
> 12 to 21 years	4/22	54/110	58/132
22 to 30 years	9/34	71/146	80/180
31 to 40 years	10/37	44/121	54/158
41 to 50 years	8/22	18/64	26/86
51 to 60 years	3/14	15/50	18/64
> 60 years	3/18	9/47	12/65
Unknown age	0/0	0/0	0/0
Grand Total	*38/165*	*216/555*	*254/719*

Table 5 shows the specimen source distribution for the Study:

Table 5. Combined Study Sites – Specimen Source Distribution (719 Specimens)

Source	**# Positive (based on subject device)/Total**
Total Specimens	254/719
Unknown +/–	66/175
Genital +/–	18/50
Penis +/–	14/44
Vaginal +/–	45/105
Labia +/–	23/47
Cervical +/–	18/50
Wound +/–	0/4
Perineum* +/–	16/40
Vulva +/–	23/66
Urethra +/–	0/12
Lesion +/–	5/14
Face‡‡ +/–	4/32
Mouth** +/–	9/37
Skin† +/–	13/42
Bartholin Cyst +/–	1/1

* Perineum: anal, groin, buttock, perianal, tailbone
** Mouth: mouth, lip, throat, NP Wash, Tongue
† Skin: skin, arm, back, breast, finger, foot, leg, thigh, breast, abdomen, hand
‡‡ Face: cheek, chin, eye, nasal

Table 6 shows the comparison of the Subject device with the Predicate device for the isolation and detection of HSV at Study Sites Combined:

Table 6. Combined Study Sites – Subject Device compared to Predicate Device for the isolation of HSV

Specimen (719 specimens)		**Predicate Device** (Current ELVIS Kit Formulation)	
		Pos	Neg
Subject Device (D^3 ELVIS)	Pos	250	5
	Neg	1	463
Positive Percent Agreement (PPA)		99.6% (250/251)	
95% CI-PPA		97.8 to 100%	
Negative Percent Agreement (NPA)			98.9% (463/468)
95% CI-NPA			97.5 to 99.7%

Table 7 shows the comparison of the Subject device with the Predicate device for the identification of HSV-2 at Study Sites Combined:

Table 7. Combined Study Sites - Subject Device compared to Predicate Device for the Typing of HSV-2

Specimen (250 specimens)		**Predicate Device** HSV-2 (Current ELVIS Kit Formulation)	
		Pos	Neg
Subject Device HSV-2 (D^3 ELVIS)	Pos	145	6
	Neg	1	98
Positive Percent Agreement (PPA)		99.3% (145/146)	
95% CI-PPA		96.2 to 100%	
Negative Percent Agreement (NPA)			94.2% (98/104)
95% CI-NPA			87.9 to 97.9%

Table 8 shows the comparison of the Subject device with the Predicate device for the identification of HSV-2 at Study Sites Combined:

Table 8. Combined Study Sites - Subject Device compared to Predicate Device for the Typing of HSV-1

Specimen (98 specimens)		**Predicate Device** HSV-1 (Current ELVIS Kit Formulation)	
		Pos	Neg
Subject Device HSV-1 (D^3 ELVIS)	Pos	90	1
	Neg	0	7
Positive Percent Agreement (PPA)		100% (90/90)	
95% CI-PPA		96.0 to 100%	
Negative Percent Agreement (NPA)			87.5% (7/8)
95% CI-NPA			47.3 to 99.7%

Analytical Sensitivity

Analytical detection limits for HSV-1 and HSV-2 were addressed with results reported in numbers of blue staining cells per cell monolayer. Each master stock (~1e7-$TCID_{50}$ per mL) virus preparation underwent a series of ten-fold dilutions, which were subsequently inoculated into a 96-well ELVIS HSV cell culture plate. The plates were centrifuged at 700xg for 60 minutes, and then incubated at 35°C to 37°C for 17 hours. Each well was stained with the subject and predicate devices then examined at 200X magnification and the number of blue staining cells counted. Table 9 below lists the results for each virus strain tested.

Table 9. Limit of Detection Compared Between ELVIS Subject (D³ ELVIS) and Predicate (Current ELVIS Kit Formulation) Typing Systems

Virus strain	Virus per Inoculum	Blue staining cells/well	
		ELVIS Predicate	ELVIS Subject
HSV-1 Strain F ATCC VR-733	65-$TCID_{50}$	74, 67, 65, 69, 70, 64	76, 70, 63, 68, 72, 71
	6.5-$TCID_{50}$	9, 8, 11, 7, 7, 12	10, 9, 9, 11, 7, 13
	0.65-$TCID_{50}$	1, 2, 1, 1, 3, 3	3, 2, 4, 3, 1, 1
	0.065-$TCID_{50}$	0, 0, 3, 1, 1, 0	0, 0, 1, 2, 0, 0
	0.0065-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
HSV-1 CW0H0062 Clinical Isolate Passage 2	85-$TCID_{50}$	70, 79, 75, 72, 80, 67	82, 77, 72, 65, 76, 85
	8.5-$TCID_{50}$	10, 7, 7, 6, 9, 6	11, 10, 8, 6, 7, 7
	0.85-$TCID_{50}$	0, 1, 3, 0, 0, 1, 0	2, 0, 0, 0, 2, 2
	0.085-$TCID_{50}$	0, 0, 0, 0, 1, 0	1, 0, 0, 0, 1, 0
	0.0085-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
HSV-1 CWOH0085 Clinical Isolate Passage 2	60-$TCID_{50}$	39, 47, 52, 41, 42, 48	46, 48, 37, 42, 47, 50
	6.0-$TCID_{50}$	6, 10, 11, 8, 7, 15	7, 14, 9, 8 ,11, 7
	0.6-$TCID_{50}$	2, 0, 2, 0, 0, 1	1, 1, 0, 0, 0, 1
	0.06-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
HSV-2 G Strain ATCC VR-734	100-$TCID_{50}$	92, 102, 95, 91, 97, 90	95, 96, 97, 98, 89, 103
	10-$TCID_{50}$	12, 11, 17, 9, 9, 10	12, 12, 7, 16, 13, 12
	1.0-$TCID_{50}$	3, 2, 1, 1, 3, 4	5, 1, 2, 2, 1, 3
	0.1-$TCID_{50}$	0, 1, 0, 1, 0, 0	1, 0, 0, 0, 1, 1
	0.01-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
HSV-2 CWOH0082 Clinical Isolate Passage 2	80-$TCID_{50}$	70, 67, 73, 78, 70, 62	76, 77, 64, 80, 70, 69
	8.0-$TCID_{50}$	8, 7, 10, 11, 6, 5	7, 8, 14, 11, 11, 9
	0.8-$TCID_{50}$	1, 0, 3, 3, 2, 2, 1	2, 1, 1, 3, 1, 0
	0.08-$TCID_{50}$	0, 0, 1, 0, 0, 0	0, 1, 0, 0, 0, 0
	0.008-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
HSV-2 CWOH0091 Clinical Isolate Passage 2	55-$TCID_{50}$	53, 61, 55, 62, 67, 65	70, 62, 55, 57, 53, 59
	5.5-$TCID_{50}$	3, 7, 7, 9, 2, 4	4, 4, 7, 8, 10, 3
	0.55-$TCID_{50}$	1, 0, 0, 2, 2, 1	3, 1, 0, 0, 2, 2
	0.055-$TCID_{50}$	0, 0, 0, 1, 0, 0	1, 0, 0, 0, 0, 0
	0.0055-$TCID_{50}$	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

In this study, the detection limit for the test is defined as the lowest inoculum level at which positive wells (i.e., containing blue staining cells) are observed, in terms of $TCID_{50}$. The results presented in Table 9 above indicate that detection limit for both subject and predicate devices averages between 0.65- and 8.5-$TCID_{50}$ for HSV-1 and 1.0- and 8.0-$TCID_{50}$ for HSV-2 depending on the strain.

Cross-Reactivity and Inhibition

The specificity of the MAbs used in the device was assessed using the organisms listed in Table 10. The subject device Solution 2T at 2X concentration was tested in duplicate on the prepared slides. After 1 hour at 37°C, the slides were rinsed with PBS and the subject device Solution 3 secondary stain was added and incubated at 37°C for 15 minutes. After rinsing and applying Mounting Fluid, the slides were examined at 400X using a fluorescence microscope.

Table 9. Respiratory Cross-Reactivity Testing

Organism	Strain or Type	ELVIS HSV Typing Reagent at 2X concentration‡	Concentrations of targets‡‡
Viruses			
Adenovirus	Type 1	–	1000-$TCID_{50}$
	Type 3	–	1000-$TCID_{50}$
	Type 5	–	1000-$TCID_{50}$
	Type 6	–	1000-$TCID_{50}$
	Type 7	–	1000-$TCID_{50}$
	Type 8	–	1000-$TCID_{50}$
	Type 10	–	1000-$TCID_{50}$
	Type 13	–	1000-$TCID_{50}$
	Type 14	–	1000-$TCID_{50}$
	Type 18	–	1000-$TCID_{50}$
	Type 31	–	1000-$TCID_{50}$
Influenza A	Aichi (H3N2)	–	1000-$TCID_{50}$
	Mal (H1N1)	–	1000-$TCID_{50}$
	Hong Kong (H3N2)	–	1000-$TCID_{50}$
	Denver (H1N1)	–	1000-$TCID_{50}$
	Port Chalmers (H3N2)	–	1000-$TCID_{50}$
	Victoria (H3N2)	–	1000-$TCID_{50}$
	New Jersey (HSWN1)	–	1000-$TCID_{50}$
	WS (H1N1)	–	1000-$TCID_{50}$
	PR (H1N1)	–	1000-$TCID_{50}$
Influenza B	Hong Kong	–	1000-$TCID_{50}$
	Maryland	–	1000-$TCID_{50}$
	Mass	–	1000-$TCID_{50}$
	GL	–	1000-$TCID_{50}$
	Taiwan	–	1000-$TCID_{50}$
	JH-001 Isolate	–	1000-$TCID_{50}$
	Russia	–	1000-$TCID_{50}$
RSV	Long	–	1000-$TCID_{50}$
	Wash	–	1000-$TCID_{50}$
	9320	–	1000-$TCID_{50}$
Parainfluenza 1	C-35	–	1000-$TCID_{50}$
Parainfluenza 2	Greer	–	1000-$TCID_{50}$

Organism	Strain or Type	ELVIS HSV Typing Reagent at 2X concentration‡	Concentrations of targets‡‡
Parainfluenza 3	C-243	–	1000-$TCID_{50}$
Parainfluenza 4	M-25	–	1000-$TCID_{50}$
Parainfluenza 4b	CH-19503	–	1000-$TCID_{50}$
CMV	AD169	–	Control Slide
Varicella-zoster	Webster	–	Control Slide
Echovirus 7	ODH-594684	–	Control Slide
Coxsackievirus A9	ODH-36685	–	Control Slide
Coxsackievirus B2	ODH-185	–	Control Slide
Enterovirus 71	ODH 02-89	–	Control Slide
Bacteria*			
Acinetobacter calcoaceticus		–	$3.6x10^9$ CFU
Bordetella bronchiseptica		–	$1.1x10^{10}$ CFU
Bordetella pertussis		–	$4.3x10^9$ CFU
Chlamydia trachomatis	LGV-II	–	Control Slide
Corynebacterium diphtheriae		–	$5.7x10^7$ CFU
Escherichia coli		–	$7.5x10^8$ CFU
Haemophilis influenzae type A		–	$4.1x10^9$ CFU
Klebsiella pneumoniae		–	$1.2x10^9$ CFU
Moraxella cartarrhalis		–	$1.2x10^{10}$ CFU
Mycoplasma hominis		–	$3.5x10^{10}$ CFU
Mycoplasma orale		–	$6.6x10^9$ CFU
Mycoplasma pneumoniae		–	$7.9x10^9$ CFU
Mycoplasma salivarium		–	$7.7x10^8$ CFU
Proteus mirabilis		–	$3.6x10^9$ CFU
Pseudomonas aeruginosa		–	$1.0x10^8$ CFU
Salmonella enteriditis		–	$8.7x10^9$ CFU
Salmonella typhimurium		–	$7.5x10^9$ CFU
Staphylococcus aureus		+†	$6.3x10^9$ CFU
Streptococcus agalactiae		–	$5.5x10^8$ CFU
Streptococcus pneumoniae		–	$6.7x10^9$ CFU
Streptococcus pyogenes		–	$6.9x10^9$ CFU
Yeast*			
Candida glabrata		–	$1.6x10^6$ CFU

‡ Positive (+) or Negative (-) for Reactivity

‡‡ Viruses: $TCID_{50}$ inoculum level; bacteria: CFU

* Turbidity or a color change to yellow indicates possible bacterial contamination and may render a test result unreliable, due either to a technical contamination during the culture setup or to a contaminated specimen. We recommend the original specimen be filtered and re-cultured.

† Light background fluorescent staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A binds to the Fc portions of the conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, e.g., *S. aureus*-bound fluorescence appears as small (~1 micron diameter), bright dots.

Reproducibility Testing

The reproducibility of the device was assessed by creating ten panels of proficiency-level frozen virus suspensions. The panels were processed at each testing site. Each panel was inoculated and stained once according to the ELVIS HSV ID and D³ Typing Test System instructions for use. Two panels per day were tested on separate plates for 5 days (10 total runs).

Panel members were manufactured by diluting high-titered master stocks. The dilutions were made with the same lot of EMEM with 10% Fetal Bovine Serum used as the negative control. These dilutions were frozen at –70°C and sent to the testing labs. The dilution's titer was confirmed pre- and post-freezing and found to fall within the expected infectivity range for the study: low level should exhibit less than 10% of the cells showing fluorescence; high level should exhibit greater than 10% but less than 50% of the cells showing fluorescence.

Table 11. Panel Member Descriptions

Panel Member	Description
HSV-1 low level	SF029* lab adapted QC strain; 200 $TCID_{50}$/mL
HSV-1 high level	SF029 lab adapted QC strain; 1000 $TCID_{50}$/mL
HSV-2 low level	SF028† lab adapted QC strain; 200 $TCID_{50}$/mL
HSV-2 high level	SF028 lab adapted QC strain; 1000 $TCID_{50}$/mL
Negative	EMEM with 10% Fetal Bovine Serum

* Isolate confirmed as HSV-1 by 2 FDA cleared IVD devices
† Isolate confirmed as HSV-2 by 2 FDA cleared IVD devices

Table 12 presents the daily results from each panel member at each site.

Table 12. Daily Results

Panel Member	Site	Day 1		Day 2		Day 3		Day 4		Day 5	
		Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
HSV-1 low level	Site 1	+/–	+/–	+/–	+/–	1+	+/–	+/–	1+	1+	+/–
	Site 2	+/–	1+	1+	1+	+/–	1+	1+	1+	+/–	1+
	Site 3	1+	1+	1+	1+	1+	1+	1+	1+	+/–	1+
HSV-1 high level	Site 1	1+	1+	1+	1+	1 to 2+	1+	1+	1+	1+	1+
	Site 2	1+	1+	1+	2+	1+	1+	3+	2+	1+	2+
	Site 3	2+	2+	2+	2+	2+	2+	2+	3+	1+	2+
HSV-2 low level	Site 1	1+	+/–	+/–	+/–	1+	+/–	+/–	+/–	1+	+/–
	Site 2	+/–	1+	1+	1+	1+	1+	2+	1 to 2+	1+	2+
	Site 3	1+	1+	1+	1+	1+	1+	1+	1+	+/–	1+
HSV-2 high level	Site 1	1+	+/–	1+	+/–	1+	1+	1+	1+	1+	1+
	Site 2	2+	2+	2+	2+	2+	1+	3+	3+	3+	2+
	Site 3	2+	3+	3+	3+	2+	3+	2+	3+	1+	2+
Negative	Site 1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Site 2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Site 3	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

The presence of HSV was reported in 100% (120/120) of the wells in which infected cells were present and the expected type was reported 100% (60/60) for HSV-1 and 100% (60/60) for HSV-2. The absence of HSV was reported in 100% (30/30) of the vials in which no virus was present. Controls performed as expected during each run.

Table 13. Reproducibility Study Summary Results

	Panel Member	HSV-1 SF029 Low Level	HSV-1 SF029 Mid-Level	HSV-2 SF028 Low Level	HSV-2 SF028 Mid-Level	Negative Control	Total Percent Agreement
	Concentration	200 $TCID_{50}$/mL	1000 $TCID_{50}$/mL	200 $TCID_{50}$/mL	1000 $TCID_{50}$/mL	Non-infected cells	
Site 1	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	50/50 (100%)
Site 2	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	50/50 (100%)
Site 3	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	50/50 (100%)
	Total Agreement w/ Expected result	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	30/30 (100%)	150/150 (100%)
	95% CI	88.4 to 100%	88.4 to 100%	88.4 to 100%	88.4 to 100%	88.4 to 100%	97.6 to 100%

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.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. Bryson, Y.J., M. Dillon, M. Lovett, G. Acura, S. Taylor, J.D. Cherry, L. Johnson, E. Weismeier, W. Growdeon, T. Creagh-Kirk and R. Keeney. 1983. "Treatment of first episodes of genital Herpes simplex virus infection with oral acyclovir: A randomized double-blind controlled trial in normal subjects". New Eng. J. Med., 308: 916-921.
2. Dulbecco, R. and H.S. Ginsberg. 1973. "Herpesviruses", Chapter 55 in Microbiology, Third Edition, D.D. Davis, et al. (eds.), Harper and Row, Hagerstown.
3. Lennette, D.A., J.L. Melnick and P.B. Jahrling. 1980. Clinical Virology: Introduction to Methods in Clinical Microbiology, Third Edition, E.J. Lennette, et. al. (eds), pp 760-771, American Society for Microbiology, Washington, D.C.
4. Gleaves, C.A., D.J. Wilson, A.D. Wold and T.F. Smith. 1985. "Detection and Serotyping of Herpes simplex Virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16h post-inoculation". J. Clin. Micro., 21: 29-32.
5. Lyerla, H.C. and F.T. Forrester. 1979. Immunofluorescence Methods in Virology. U.S. Department of Health, Education, and Welfare. Course No. 8231-C, pp 103-111.
6. Moore, D.F. 1984. "Comparison of human fibroblast cells and primary rabbit kidney cells for isolation of Herpes simplex virus". J. Clin. Micro., 19: 548-549.
7. Reeves, E.C., L. Corey, H.G. Adams. L.A. Vontver and K.K. Holmes. 1981. "Risk of recurrence after first episodes of genital herpes: relation to HSV type and antibody response". New Eng. J. Med., 305: 315-319.
8. Ashley, R.L., L. Corey, and W. E. Rawls. 1989. "Herpes simplex Viruses", Chapter 11 in Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. Sixth Edition, N.J. Schmidt and R.W. Emmons, (eds), American Public Health Association, Inc., Washington, D.C.

9. Crane, L.R., P.A. Gutterman, T. Chapel and A.M. Lerner. 1980. “Incubation of swab materials with Herpes simplex Virus”. J. Infect. Dis., 141 (4): 531.
10. Bettoli, E.J., P.M. Brewer, M.J. Oxtoby, A.A. Zaidi and M. E. Guinan, 1982. “The role of temperature and swab materials in the recovery of Herpes simplex Virus from lesions”. J. Infect. Dis., 145:399.
11. Stabell, E.C. and P.D. Olivo, 1992. “Isolation of a cell line for rapid and sensitive histochemical assay for the detection of Herpes simplex virus”. J. Vir. Methods, 38: 195.
12. Stabell, E.C., S.R. O'Rourke, Gregory A. Storch & Paul D. Olivo, 1993. “Evaluation of a genetically engineered cell line and a histochemical ß-galactosidase assay to detect Herpes simplex virus in clinical specimens”. J.Clin. Micro., 31: 2796.
13. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
14. CLSI/NCCLS M29A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, David L. Sewell, Third Edition, Clinical and Laboratory Standards Institute, 01Mar2005.
15. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2007; CDC-NIH manual (Centers for Disease Control and Prevention, and National Institutes of Health). http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
16. "The (herpes) helper", published by the American Social Health Association, Box 100, Palo Alto, CA 94302. Fall 1984, page 7. Adapted from Aultman Hospital Laboratory Report Attachment submitted by John G. Thomas, Ph.D.
17. Lennette, D.A. and E.T. Lennette, 1985. A Users Guide To The Diagnostic Laboratory. University Park Press, Baltimore, MD.
18. Lennette, D.A., 1985. "Collection and Preparation of Specimens for Virological Examination", Chapter 61 in Manual of Clinical Microbiology, Fourth Edition, edited by E.H. Lenette, A. Balows, W.J. Hausler, Jr. and H.J. Shadomy, published by American Society for Microbiology, Washington, D.C., pp. 687-693.
19. Lennette, D.A., J.L. Melnick and P.B. Jahrling. 1980. Clinical Virology: Introduction to Methods in Clinical Microbiology, Third Edition, E.J. Lennette, et. al. (eds), pp 760-771, American Society for Microbiology, Washington, D.C.
20. Lennette, D.A. and E.T. Lennette, 1985. A Users Guide to the Diagnostic Laboratory. University Park Press, Baltimore, MD.
21. Lennette, D.A., 1985. "Collection and Preparation of Specimens for Virological Examination", Chapter 61 in *Manual of Clinical Microbiology*, Fourth Edition, edited by E.H. Lenette, A. Balows, W.J. Hausler, Jr. and H.J. Shadomy, published by American Society for Microbiology, Washington, D.C., pp. 687-693.
22. Isenberg, Henry D., 1992. Clinical Microbiology Procedures Handbook, published by American Society for Microbiology, Washington DC, pg. 8.3.3.
23. Schmidt, N.J. and R.W. Emmons, 1989. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, Sixth Edition, N.J. Schmidt and R.W.Emmons (eds.), published by American Public Health Association, Washington, D.C., pp 9-18,
24. NCCLS Document C24-A, 1991. “Internal Quality Control Testing: Principles and Definitions”. Approved Guidelines. National Committee for Clinical Laboratory Standards, Wayne, PA.
25. Solomon, A.R., et al. 1984. “The Tzanck Smear in the diagnosis of Cutaneous Herpes simplex”. JAMA, 251:633.
26. Centers for Disease Control and Prevention, 2002. “Sexually transmitted diseases treatment guidelines 2002”. MMWR 2002:51(No. RR-6).
27. U.S. Preventive Services Task Force, Screening for Genital Herpes, 2005.

REF SKT-ELVIS-60.v2; SKT-ELVIS-300.v2 – ELVIS HSV ID and D^3 Typing Test System

IVD

Diagnostic Hybrids, Inc. – a subsidiary of Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

PI0500000EN00 (02/19)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	IVD For *In Vitro* diagnostic use
 Contains sufficient for XX determinations	CONT Contents/Contains
CONT NaN_3 Contents/Contains	