

ELVIS[®] HSV ID Test System

**A Test System for the Culture and Identification
of Herpes simplex virus using the
Enzyme Linked Virus Inducible System[®]**












REF: SK-ELVIS-100, SK-ELVIS-200,
SK-ELVIS-500 & SK-ELVIS-1000
For *in vitro* Diagnostic Use

Please contact Diagnostic HYBRIDS Technical Services
for technical assistance regarding this procedure.

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US Patent Nos.: 5,418,132 and patents pending.

Symbols Lexicon/Glossary

 In Vitro Diagnostic Medical Device	 Batch code/lot number
 Temperature limit	 Catalog number
 Consult instructions for use	 Use by YYYY-MON-DD
 Do not reuse	 Manufacturer
 Patent Numbers	
 Contains sodium azide	 Authorized representative in the European Community

I. INTENDED USE

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

II. 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). Since there is an effective antiviral chemotherapeutic agent (acyclovir) available to treat HSV infections, it becomes very important to have a rapid and accurate test for the detection and diagnosis of HSV.

It is widely recognized that the most sensitive method to demonstrate HSV in patient specimens is cell culture. 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. Direct immunofluorescence confirmation of cell culture CPE has been regarded as the standard for confirmation of HSV identification.

Diagnostic Hybrids' ELVIS[®] HSV Test System combines the sensitivity of cell culture amplification with the specificity of HSV activated reporter genes. The ELVIS[®] HSV Test

eliminates the subjective nature of detecting viruses in culture by CPE and reduces turn-around-time from 7 days to <1 day. Thus, negative and positive specimen results can be reported the day after receipt of the specimen. The System produces results which are substantially equivalent to standard virology assays and is offered in two formats: (1) shell vials with and without coverslips and (2) multi-well plates. These different format options provide users with the ELVIS[®] Test best suited for their laboratory requirements, and allow centrifugation, which eliminates the handling of coverslips and substantially reduces processing time. Also, since HSV infection, and only HSV, is signaled by a blue dye deposited in HSV-infected cells, there is no need for a confirmatory fluorescent monoclonal antibody test of positives. All formats are based on transgenic reporter technology and share the same reagents for detection of HSV in clinical specimens.

III. PRINCIPLE OF THE PROCEDURE

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

The technology upon which ELVIS[®] HSV is based is fundamentally different from other viral detection approaches which utilize antibodies or nucleic acid probes. Use of antibodies or probes requires the addition of an exogenous component which first must react specifically with viral-specific antigen or genes, respectively. These reagents are most often tagged with a fluor or an enzyme. When used in high concentrations to promote rapid reactions, the potential exists for nonspecific background signal. Thus, washes are necessary to remove as much nonspecific reactivity as possible, and even then, background signal may be evident which could compromise final detection.

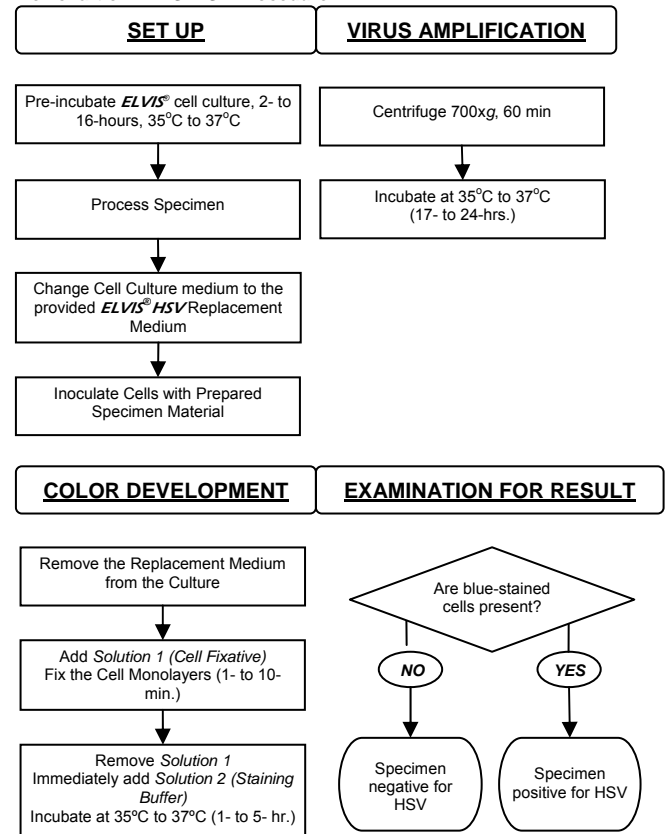
ELVIS[®] HSV Cells are genetically engineered Baby Hamster Kidney (BHK) cells, which, when infected with either HSV-1 or HSV-2, are induced to generate and accumulate an endogenous, intracellular bacterial enzyme, β -galactosidase. Other related viruses (e.g., Varicella-zoster) are not capable of inducing the formation of this enzyme.

Specimens are inoculated onto the ELVIS[®] HSV Cells. After an overnight incubation period (17- to 24-hours) the inoculated monolayers are fixed using solution 1 then treated with Solution 2, which contains the chromogenic substrate for the induced β -galactosidase enzyme. Those cells infected with HSV develop an indigo-blue precipitate, while uninfected cells remain colorless.^{9,10} Due to the high level of assay specificity, background is practically nonexistent.

Monolayers are examined for cells containing this blue precipitate (blue cells) using standard light microscopy. Those monolayers that do not contain blue cells are negative for HSV; those that contain blue cells are positive for HSV.

After this culture amplification period, the supernatant is removed and the cell monolayer fixed. The fixed cells are then incubated in a Staining Buffer containing substrate for the HSV induced enzyme which is specific to Herpes simplex virus, both types 1 and 2. After the staining period, the cell monolayers are examined by light microscopy 1- to 5-hours later to detect positive specimens with their stained cells and negative specimens with no stained cells.

Flowchart of ELVIS[®] HSV Procedure:



IV. REAGENTS

A. The ELVIS[®]HSV Test System consists of:

1. ELVIS[®]HSV Cells: The ELVIS[®] Cells have a routine use period of 7 days from receipt while all other components have a shelf life of months (see expiration date on label of each component). ELVIS[®] 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.
2. ELVIS[®]HSV Replacement Medium: Sterile EMEM containing FBS, Penicillin, Streptomycin and Amphotericin B. ELVIS[®]HSV Replacement Medium is for use with ELVIS[®]HSV Shell Vials and Multi-well Plates.
3. ELVIS[®]HSV Solution 1 (Cell Fixative): an aqueous acetone solution.
4. ELVIS[®]HSV Solution 2 (Staining Buffer): A dilute solution of X-Gal (5-Bromo-4-Chloro-3-Indolyl- β -D-Galactopyranoside), N,N-Dimethylformamide, iron, sodium and magnesium salts; in an aqueous, buffered solution.

B. Warnings

1. For *in vitro* diagnostic use.
2. ELVIS[®]HSV Cells are not to be passed or used for serial propagation. Their use is covered by U.S. Patent Number 5418132 and additional patents.
3. Only individuals competent in cell culture isolation techniques and the interpretation of virus isolation results should use this device.
4. Specimens should be handled according to BioSafety Level 2 practices as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, 'Biosafety in Microbiological and Biomedical Laboratories', 1999 (and specifically Section II. 'Principles of biosafety: Clinical laboratories').²²
5. Assume all specimens are infectious. As with any sample that may contain pathogens, care must be taken to prevent contact with skin or mucus membranes. Process swabs, transport media, cell culture vials and plates, etc., carefully and disinfect with a hypochlorite solution disinfectant (at a minimum concentration of 0.05%) and autoclave or incinerate prior to disposal.
6. Use aseptic technique, sterile equipment and materials throughout the viral culture portion of this procedure. Avoid microbial contamination of the reagents or incorrect results may be obtained.
7. The use of reagents and the inoculation of cells must be limited to the period prior to the Expiration Date.
8. Use a safety device for all pipetting steps. Never pipet by mouth.
9. 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.
10. Solution 2 (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.

C. Storage Instructions

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

TABLE 1: Reagent Storage Conditions	
1. 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	
2. ELVIS [®] HSV Replacement Medium	Store at 2°C to 8°C.
3. ELVIS [®] HSV Solution 1	Store at 2°C to 30°C.
4. ELVIS [®] HSV Solution 2	Store at 15°C to 30°C.

D. Indications of Deterioration

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.

V. SPECIMEN HANDLING

A. Specimen Collection

All specimens should be obtained from the patient by appropriately trained individuals. Specimens collected from lesions in the acute or vesicular stage will yield the largest number of viruses; as the lesion ulcerates, crusts and heals, the number of viable viruses decreases.

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.^{3,12,13,14}

Care should be exercised during specimen collection to avoid contamination from body sites other than the lesion to be sampled. A sterile dry swab should be used to absorb fluid and collect cells from the base of the lesion. Swabs should be delivered to the

laboratory as soon as possible in a suitable transport system. Important to note is that the swab and the transport medium should not be inhibitory to HSV or to BHK cells. Cotton, rayon or Dacron swabs are best; calcium alginate swabs should not be used.^{11,15}

Specimens should be inoculated as soon as possible. The specimen should be stored at 2°C to 8°C until it is processed. If the specimen will not be processed within 48-hours, freeze at -70°C until use.¹⁶

B. Specimen Preparation

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 1.5- to 2-mL 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 (700-1000xg 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 some oropharyngeal specimens be clarified by centrifugation and sterile-filtered before inoculation into cell cultures.²⁰

VI. PROCEDURE

A. Materials Provided

1. ELVIS[®]HSV Cells, in shell vials with or without coverslips, or in multi-well plates with or without coverslips and up to 24 monolayers per plate
2. ELVIS[®]HSV Replacement Medium
3. ELVIS[®]HSV Solution 1 (Cell Fixative)
4. ELVIS[®]HSV Solution 2 (Staining Buffer)

B. Materials Required But Not Provided

1. Ambient temperature centrifuge with free-swinging rotor and carriers capable of spinning ELVIS[®]HSV cell culture plates or shell vials at 700xg.
2. Sterile disposable 1-mL pipets, 0.1-mL graduations; one per specimen.
3. Sterile 5- or 10-mL pipet for dispensing Replacement Medium.
4. pipets for dispensing Solution 1 and Solution 2.
5. Disposable plate seals.
6. Sterile, disposable Pasteur transfer pipets.
7. Bent 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. 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 a microscope.
8. Class II biosafety cabinet for aseptic handling of cell cultures and specimens.
9. Vacuum aspirator with trap containing hypochlorite disinfectant at a minimum concentration of 0.05%.
10. Incubator to maintain 35°C to 37°C. The incubator must be humidified with an atmosphere of 5% CO₂ (post-inoculation) for ELVIS[®]HSV Multi-well Plates. There are no special requirements for ELVIS[®]HSV Shell Vials when the vials are tightly capped.
11. Inverted or standard light microscope of 100X magnification.
12. Positive HSV controls [Herpes simplex type 1 and type 2 virus strains for preparing positive controls are available from various sources. Contact Diagnostic Hybrids, Inc. technical services for options or recommendations.
13. Microscope slides.

C. Preliminary Comments and Precautions

1. Do not use any test component on or beyond its expiration date.
2. Shell vials and 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 plates should be incubated for a period of 2- to 16-hours before specimen inoculation.
3. 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 Section V.
4. 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 pipet.
 - 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° 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 pipet 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 pipet for each specimen to avoid cross-contamination, which could cause erroneous results.
 - During inoculation of specimens in multi-well plates, touch the pipet 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 pipet 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 that are spin-amplified in shell vials or wells are incubated for a minimum of 17-hours. Incubation periods of 17- to 69-hours were used in the clinical evaluations reported in Section X. The extended period of incubation beyond 17-hours was 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 to reduce 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 step of the procedure (for instance when, after the staining period, Solution 2 might be exchanged for PBS as described in Section VI.E.9). 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. 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 (see Section VI.D) 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[®] Cells. If blue staining debris or epithelial cells are seen, the specimen should be filtered and re-cultured.
 - 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.

D. Specimen Inoculation and Incubation

- Pre-incubate ELVIS[®]HSV Cell cultures at 35°C to 37°C for 2- to 16-hours. [The seals should be removed from multi-well plates just prior to pre-incubation, as described in Section VI.C.4.]
- 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).
- Aspirate the medium from each of the monolayers, taking care not to disturb the cells.

- 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.
- Add 0.2-mL of the patient specimen to each of two monolayers. Freeze the remainder of each specimen at -70°C or lower, for future reference.
- Inoculate one monolayer with an HSV-Positive Control and leave one monolayer uninoculated as a Negative Control for each test run.
- 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.
- Incubate the inoculated cells at 35°C to 37°C for 17- to 24-hours. See Section VI.B.10.

E. Cell Fixation and Staining

- Aspirate the medium completely from each monolayer and add 0.25-mL of Solution 1 (Cell Fixative) to each monolayer.
- Mix by rocking to ensure that each monolayer is uniformly covered with solution.
- Allow to stand for a minimum of 1-minute and a maximum of 10-minutes.
- Aspirate Solution 1 from each monolayer.
- Add 0.25-mL Solution 2 (Staining Buffer) to each monolayer.
- Mix by rocking to ensure that each monolayer is uniformly covered with solution.
- Cover the containers and place at 35°C to 37°C for 1- to 5-hours.
- Upon completion of step VI, above, remove coverslips from shell vials (if required) for interpretation as follows:
 - Lift the coverslip carefully from the bottom of the shell vial using a bent teasing needle.
 - Remove the coverslip with forceps. Take care to identify the cell monolayer side of the coverslip.
 - Wash the coverslip gently by several immersions in a beaker of distilled water while still holding it with the forceps.
 - Blot excess water by touching the edge of the coverslip to absorbent paper.
 - 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.
- Examine each monolayer entirely for stained cells using a light microscope with a magnification of 100X. See Section VII for the criteria for a positive, negative or non-diagnostic result.
- The Solution 2 (Staining Buffer) need not be removed. However, if one wishes to retain the stained monolayers, Solution 2 should be removed and 1-mL of Phosphate Buffered Saline (PBS), pH 7.2, should be added, then the monolayers may be stored at 2°C to 8°C for up to one week (be sure to cover the stained monolayers to prevent drying).

F. Note on Typing ELVIS[®]HSV Positive Monolayers

ELVIS[®]HSV monolayers in multiwell plates or on coverslips in shell vials can be typed for HSV-1 and HSV-2 after the ELVIS[®] result is completed.

ELVIS[®]HSV positive monolayers do not need to be fixed again for typing. In order to type positive monolayers, remove Solution 2 by careful aspiration from each well or coverslip. Follow the Manufacturer's suggested procedure for using the fluorescent typing antibody kit on pre-CPE fixed monolayers on coverslips. Identify HSV-1 and HSV-2 in accordance with the Manufacturer's directions. [The coverslip can be easily removed from the shell vial using a bent teasing needle (see Section VI.E.7).

Specimens of low viral titer, indicated by very few blue stained ELVIS[®]HSV Cells, may show no fluorescence with either type 1 or type 2 fluorescent typing antibodies because the blue stain of the positive cells tends to obscure the fluorescence. Such specimens should be reported out as positive for HSV but must be re-cultured and re-tested with typing antibodies before reporting a definitive typing result.

G. Stability of the 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 2 is removed, and the monolayer is washed with 1-mL of water and covered with 1-mL of PBS.

H. Quality Control Procedures¹⁸

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 an uninfected 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 Diagnostic Hybrids, Inc. using the contact information that can be found on the coverage of this product insert.

VII. INTERPRETATION OF ELVIS[®] STAIN RESULTS

A. Characteristics of a 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.

1. Be familiar with the appearance of non-specific non-cell-associated blue precipitate, as described in Section VI.C.18, as well as Section VII.C.3. Such blue precipitate is not indicative of a positive HSV result.
2. 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[®] culture inoculated. The culture should be processed as before, and the result examined for presence of intracellular blue stain precipitate.
3. The intensity of cell staining can vary from light to very dark within a focus and within a monolayer.
4. 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.
5. Positives that are representative of specimens with very low virus load may have only one stained focus in only one of the duplicate monolayers. There may be no evidence of CPE such as syncytial formation, cell toxicity, etc. in these weak positives.

Report: Herpes simplex virus detected

B. Characteristics of a Negative Result

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

1. The entire monolayer must be examined microscopically at 100x magnification before a negative result can be reported.
2. There can be cells or debris present on the monolayer which at first appear to be stained blue but are not HSV infected since:
 - a. 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*.¹⁰ 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 Section VI.C.18]
 - b. The color is not localized within the monolayer cell.
 - c. 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.¹⁷ When a specimen tests negative but the patient history, clinical signs or serology strongly suggest HSV infection, another specimen should be taken and retested.

C. Characteristics of a Non-Diagnostic Result

1. When the results of the Negative and/or Positive Control are not as expected
[Identify the cause and repeat the test.]
2. When 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 Section VI.C.17.]
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.

3. When 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 Section VI.C.18]
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).

VIII. LIMITATIONS OF THE PROCEDURE

1. Performance characteristics of the ELVIS[®]HSV 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.^{21,23,24}]
2. 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.
3. 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 culturability. 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.
4. Bloody specimens may contain antibodies that may inhibit viral replication in cell cultures.
5. There is the possibility that this culture system may detect replication-defective Herpes simplex virions.⁹
6. 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.
7. This test is limited to the qualitative detection of HSV. Performance characteristics for the quantitative determination of HSV virions have not been established.
8. The ELVIS[®]HSV Cells contain a genetic sequence which is inducible by both HSV types 1 and 2. This test cannot differentiate between these two types of Herpes simplex virus.

IX. EXPECTED VALUES

The prevalence of HSV positive specimens for Laboratories 1 and 2, whose specimens were predominantly of genital origin, were virtually the same at 39.0% and 40.4%, respectively. Laboratory 3, whose specimens were chiefly of genital, oral and bronchial-alveolar lavage origin, had an HSV prevalence of 15.6%.

X. SPECIFIC PERFORMANCE CHARACTERISTICS

The ELVIS[®]HSV Test System has been evaluated in six different laboratories using a total of 1737 specimens. The results were compared to those obtained by the respective laboratory's standard culture method. Two laboratories used the shell vial technique and stained 16- to 24-hours post-inoculation without determining CPE. The four remaining laboratories examined cultures periodically for CPE over a period of 7 days and confirmed the presence of HSV infection using a monoclonal antibody. The results are summarized below. [Since these studies were conducted, a new, acetone-containing fixative has been incorporated into the ELVIS[®]HSV Test System.]

A. Effect of Prevalence on Diagnostic Tests

Since no diagnostic test is always 100% accurate, it is important to understand how the Prevalence of a disease can affect the correctness of a test result. Prevalence is the percent of the population studied that has the disease, e.g., HSV infections. The Positive Predictive Value is defined as the percent positive results that are true HSV-positives when the test is applied to both HSV-negative and HSV-positive patients. Likewise, the Negative Predictive Value is defined as the percent negative results that are true HSV-negatives when the test is applied to both HSV-negative and HSV-positive patients.

The positive and negative predictive values (PPV and NPV, respectively) presented in the following Table of Hypothetical Prevalence Values were calculated using Bayes' Theorem¹⁹ for tests with 99% Sensitivity and 98.3% Specificity, the values resulting from the summary of the clinical studies of the ELVIS[®]HSV Test System.

Prevalence (%)	PPV (True Positives, %)	NPV (True Negatives, %)
5	75.5	99.9
15	91.1	99.8
25	95.1	99.7
35	96.9	99.5
45	98.0	99.2
50	98.7	98.7
75	99.4	97.0

As the values in the Table illustrate, where prevalence of the disease is low, the probability that a negative result is correct is higher than that of a positive result. However, when the prevalence is high, the reverse is true.

B. Clinical Specimens: Multi-well Plates

Unless otherwise indicated, duplicate 0.2-mL samples of the same specimens were inoculated into wells for the ELVIS[®]HSV Test System and run according to the Test Procedure.

LABORATORY 1: A large commercial reference laboratory in the Midwest whose specimens were submitted from 8 to 10 neighboring states for HSV testing.

The specimens were predominantly of genital origin. These specimens were submitted by private physicians, clinics, hospitals and other small laboratories. Specimens were collected in commercial transport medium and processed in the following manner: The specimen swab was eluted in 2-mL medium, 0.2-mL inoculated into duplicate shell vials of human embryonic lung (MRC-5) cells, centrifuged for 60 minutes at 900xg, and stained at 16- 24-hours post-inoculation using HSV 1 and 2 typing fluoresceinated antibody reagents.

Of the 435 random, freshly eluted specimens tested, 59 (13.6%) were "toxic" or microbially contaminated in one or both methods and were not included in the study. About 11% of the Standard Method cultures were affected in this fashion while only about 8% of the ELVIS[®]HSV cultures were affected. Of the remaining 376 specimens, 147 were positive and 220 were negative by both methods. Of the 13 discrepant results which were all positive by ELVIS[®], 4 were positive upon repeat shell vial testing, 6 were negative on repeat testing and 3 were not retested. From these last 9 unresolved discrepant results, 6 were positive by ELVIS[®] in only one of the duplicate wells or there were fewer than 4 positive (blue) cells in both wells indicating a very low level of virus present in the specimen which may not have been detected in the single shell vial test for that Herpes type.

The results from this study are summarized as follows:

(376 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	147	0
	Neg	9	220
Sensitivity		100%	
95% CI		97.5% to 100%	
Specificity		96.1%	
95% CI		92.7% to 98.2%	
Positive Predictive Value =		89.8% to 97.3%	
Negative Predictive Value =		98.3% to 100%	

The prevalence of HSV in this population of specimens was 39%; HSV-1 accounted for 34.7% and HSV-2 accounted for 65.3% of the positives. The ELVIS[®]HSV test detected types 1 and 2 with equal sensitivity.

In this and the following studies, one of two methods was used to calculate the 95% Confidence Interval (95% CI) for the clinical sensitivity and specificity. The methods used were the Exact (noncentral, hypergeometric) and the Normal Methods. Baye's Theorem was used to calculate the corresponding positive and negative predictive values.

LABORATORY 2: A large commercial reference laboratory in the Southeast whose specimens were submitted from 8 to 10 neighboring states for HSV testing.

The specimens were predominantly of genital origin. The specimen swab was eluted in 2-mL medium, 0.2-mL inoculated into a single shell vial of human embryonic lung (MRC-5) cells, centrifuged for 45 min at 900xg, and stained at 16- to 24-hours post-inoculation using HSV bivalent fluoresceinated antibody reagent. All 555 specimens tested at this site were fresh eluates. Specimens were processed by the ELVIS[®]HSV kit within 12-hours of inoculating by the shell vial method. This site adhered to the Test Procedure with two exceptions: first, a single well per specimen was used throughout the complete study; second, 227 specimens were processed with 0.2-mL inoculum volume and 328 specimens were processed with 0.4-mL inoculum volume to study the effect of increased specimen volume on sensitivity and repeat testing of "toxics".

The results of this study are summarized as follows:

(719 specimens)		Standard Method					
		0.2mL Specimens		0.4mL Specimens		All Specimens	
		Pos	Neg	Pos	Neg	Pos	Neg
ELVIS	Pos	87	4	133	4	220	8
	Neg	1	135	3	188	4	323
n=		227		328		555	
Sensitivity		98.8%		97.8%		98.2%	
Specificity		97.9%		97.9%		97.6%	

Summary for All Specimens in this Study:

Sensitivity = 98.2% with a 95% CI of 95.9% to 99.5% (Exact Method).
 Specificity = 97.6% with a 95% CI of 95.9% to 99.2% (Exact Method).
 Positive Predictive Value = 93.2% to 98.5%
 Negative Predictive Value = 97.2% to 99.7%

Thus, no differences in sensitivity are seen when 0.4-mL inoculum volume is used instead of 0.2-mL. Using 0.2-mL inoculum volume resulted in 3.0% of the ELVIS[®]HSV monolayers becoming "toxic" or microbially contaminated while 0.4-mL inoculum volume resulted in 6.7%, a value not significantly different from the values other study sites found. In contrast, 9.4% of the standard method cultures were either "toxic" or contaminated and the testing had to be repeated for these specimens.

The prevalence of HSV in this population of specimens was 40.4%.

LABORATORY 3: A tertiary care teaching hospital laboratory in the Midwest whose specimens were submitted for routine HSV culture.

These specimens were chiefly of genital, oral and bronchial-alveolar lavage origin. The standard procedure was to elute the swab in 3-mL of medium, inoculate duplicate 0.25-mL volumes into shell vial cultures of freshly prepared MRC-5, centrifuge 45 minutes at 700xg and examine the cultures for CPE daily for 7 days. Specimens demonstrating CPE were stained using HSV typing reagents. Of the 167 specimens inoculated onto MRC-5 cells, 13 (7.8%) could not be completed because 7 were microbially contaminated and 6 were "toxic"; none of these 13 specimens interfered in this fashion with the ELVIS[®]HSV test. Among the remaining 154 specimens, 23 were positive and 130 were negative by both methods. The single discrepant result was positive in only one vial of MRC-5 cells on the last day (day 7) of incubation, suggesting a very low titer of virus was present in this specimen.

The prevalence of HSV in this population of specimens was 15.6% with HSV-1 accounting for 41.7% and HSV-2 accounting for 58.3% of the positives.

The results from this study are summarized as follows:

(154 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	23	0
	Neg	1	130
Sensitivity		95.8%	
95% CI		78.9% to 99.9%	
Specificity		100%	
95% CI		97.2% to 100%	
Positive Predictive Value =		83.9% to 100%	
Negative Predictive Value =		82.2% to 100%	

LABORATORY 4: A tertiary care pediatric teaching hospital laboratory in the Northeast whose specimens were submitted for routine HSV culture.

The standard procedure was to elute the swab in 2-mL of medium, inoculate 0.2-mL of specimen into each tube of mink lung fibroblasts and of MRC-5 cells and examine the cultures for CPE daily for 7 days. Specimens demonstrating CPE were stained using HSV typing reagents. All 305 specimens had been frozen, with 60% frozen for a period longer than one week and 40% frozen for one week or less. Of the 305 specimens tested, 147 were positive and 152 were negative by both methods. There were 6 discrepant specimens, negative by ELVIS[®]HSV but positive by at least one of the other cultures.

The results from this study are summarized as follows:

(305 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	147	0
	Neg	6	152
Sensitivity		96.1%	
95% CI		91.7% to 98.6%	
Specificity		100%	
95% CI		97.6% to 100%	
Note: Since frozen specimens were identified and selected for the study, true prevalence and predictive values cannot be calculated.			

C. Clinical Specimens: Shell-Vials

LABORATORY A: A tertiary care pediatric hospital laboratory in the Midwest whose specimens were submitted for routine HSV culture.

The standard procedure was to inoculate 0.2-mL of the eluate into one each of A-549 and HFF tube cultures and roll tubes for up to seven days. Cultures were examined daily for CPE. Specimens demonstrating CPE were stained and confirmed using HSV fluorescent antibody reagents. Of the 125 specimens tested by the laboratory's standard method and the ELVIS[®]HSV test, 30 were fresh specimens and 95 were frozen. The fresh specimens consisted of 3 positives and 27 negatives while the frozen specimens consisted of 36 positives and 59 negatives. There were no discrepant results.

The results from this study are summarized as follows:

(125 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	39	0
	Neg	0	86
Sensitivity		100%	
95% CI		91.0% to 100%	
Specificity		100%	
95% CI		95.8% to 100%	
Positive Predictive Value =		83.9% to 100%	
Negative Predictive Value =		82.2% to 100%	

LABORATORY B: A medical school hospital laboratory in the Northeast whose specimens were submitted for routine HSV culture.

These fresh and frozen specimens were chiefly of genital and oral origin. The standard procedure was to elute the swab in 2-mL of medium, inoculate duplicate 0.2-mL volumes into three separate tubes of MRC-5 or WI 38, HNK and RK cells and examine daily for up to seven days for CPE. Specimens demonstrating CPE were stained using fluorescent labeled typing antibodies. Of the 222 specimens tested by the laboratory's standard method and the ELVIS[®]HSV test, 137 were fresh specimens and 85 were frozen.

The results from this study are summarized as follows:

(222 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	109	0
	Neg	5	108
Sensitivity		95.6%	
95% CI		90.1% to 98.6%	
Specificity		100%	
95% CI		96.6% to 100%	

D. Summary of Clinical Results

		Spin Amplification		7-day Culture	
		Pos	Neg	Pos	Neg
ELVIS	Pos	367	17	318	0
	Neg	4	543	12	476
n=931				n=806	
Sensitivity		98.9%		96.4%	
95% CI		97.3% to 99.7% (Exact Method)		94.3% to 98.4% (Normal Method)	
Specificity		97.0%		100%	
95% CI		95.5% to 98.4% (Normal Method)		99.2% to 100% (Exact Method)	

Summary of Results, All 6 Study Sites

(1,737 specimens)		Standard Method	
		Pos	Neg
ELVIS	Pos	685	17
	Neg	16	1019
Sensitivity		97.7%	
95% CI		96.6% to 98.8%	
Specificity		98.4%	
95% CI		97.6% to 99.1%	

E. Analytical Sensitivity

The minimum number of infectious HSV virions that the Rapid format ELVIS®HSV ID Test System can detect after 16-hours of incubation compared to MRC-5 and CV-1 cell cultures after 7 days incubation is one (1) when determined by TCID50 (Tissue Culture Infectious Dose50).

F. Cross Reactivity and Inhibition

The ELVIS®HSV ID Test System provides a highly specific method for detecting Herpes simplex virus in clinical specimens.

ELVIS®HSV detects HSV types 1 and 2 equally well.

- Laboratory 4 inoculated ELVIS®HSV Cells with 49 known HSV type 1 and 49 known HSV type 2 clinical isolates to demonstrate that both types of HSV of clinical origin would activate the cells to produce color. In all 98 isolates, ELVIS®HSV Cells correctly identified the specimen as containing HSV.
- At DHI, stock virus of each viral type was adjusted for equal numbers of plaque forming units (PFU) /mL on African Green Monkey Kidney (CV-1) cells. Twofold dilutions of each of these viruses were tested using the ELVIS®HSV Test Procedure. The number of infected (blue staining) cells per dilution of each type of HSV was substantially equivalent. These results confirm those reported by Stabell and Olivo.⁹

ELVIS®HSV does not detect closely related viruses such as CMV and VZV.

- DHI demonstrated that ELVIS®HSV gave no false positive results using stock CMV and VZV at 10,000 PFU per well.
- Laboratory 3 reported that 14 specimens were positive for viruses other than HSV-1 and HSV-2. These specimens included CMV, VZV, Parainfluenza Virus Type 3, Adenovirus and Influenza A Virus. None of these induced a false positive (blue stained cell) signal in the ELVIS®HSV Cells. These results confirm and extend those reported by Stabell and Olivo.⁹

ELVIS® detection of HSV is not affected by inocula containing relatively large amounts of closely related viruses such as CMV and VZV.

This was demonstrated at DHI by co-infecting ELVIS® monolayers with 100 PFU's of HSV and 10,000 PFU's of either CMV or VZV. There were no significant differences in number of infected cells between the co-infected monolayers and control monolayers which had been infected with 100 PFU's of HSV only.

ELVIS® detection of HSV is not affected by the presence of *Mycoplasma* spp. in the specimen.

This was shown at DHI by co-infecting ELVIS® monolayers with about 100 PFU's of HSV and between 104 and 105 *Mycoplasma* spp. There were no significant differences in number of infected cells between the co-infected monolayers and control monolayers which had been infected with about 100 PFU's of HSV only. Also, *Mycoplasma* infection of ELVIS® monolayers did not elicit color in the ELVIS® Cells.

ELVIS® detection of HSV is not affected by the presence of *Chlamydia* sp. in the specimen.

Tests were conducted with *Chlamydia pneumoniae* and the 15 known serovars of *C. trachomatis* to determine if they would induce a false positive or false negative result in the ELVIS®HSV test since Baby Hamster Kidney cells permit replication of *C. trachomatis*. After 24 and 48 hours of incubation of *Chlamydia*-infected cells, neither species induced color formation in any ELVIS® Cells of the infected monolayers. Likewise, the added *Chlamydia* (which ranged from about 102 to 106 elementary bodies/monolayer), after 24 and 48 hours of incubation did not induce any false negative results in the ELVIS® monolayers co-infected with HSV.

ELVIS® detection of HSV is not affected by the presence of the HSV specific antiviral drug acyclovir.

XI. REFERENCES

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