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

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.

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 (17-24 hours) the inoculated monolayers are fixed using Solution 1 and 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. Due to the high level of assay specificity, background is practically nonexistent.

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. This reagent is 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.

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 a 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 1-5 hours later to detect positive specimens with their stained cells and negative specimens with no stained cells.

Flowchart of ELVIS®HSV Procedure:

SET UP

1. Pre-incubate ELVIS® cell culture, 21- to 24-hours, 35°C to 37°C
2. Centrifuge 700xg, 60 min

VIRUS AMPLIFICATION

Process Specimen

1. Change Cell Culture medium to the provided ELVIS® HSV REPLACEMENT Medium
2. Incubate at 35°C to 37°C (17- to 24-hrs.)

COLOR DEVELOPMENT

1. Add Solution 1 (Cell Fixative) Fix the Cell Monolayers (1- to 10- min.)
2. Remove the Replacement Medium from the Culture

EXAMINATION FOR RESULT

1. Remove Solution 1 Immediately add Solution 2 (Staining Buffer) Incubate at 35°C to 37°C (1- to 5-hr.)
2. Are blue-stained cells present?
3. Are specimen negative for HSV? Specimen positive for HSV

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.

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-different 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 and loss 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.

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 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
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 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 acetic solution.
4. ELVIS® HSV Solution 2 (Staining Buffer): A dilute solution of X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-galactopyranoside) and 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”).
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 (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 acetic, 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>
<thead>
<tr>
<th>TABLE 1: Reagent Storage Conditions</th>
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<tbody>
<tr>
<td>1. ELVIS®HSV Cells:</td>
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<tr>
<td>(See notes below)</td>
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<tr>
<td>Shell-Vials Store at 2ºC to 8ºC in</td>
</tr>
<tr>
<td>the dark</td>
</tr>
<tr>
<td>Sealed Multi-well Plates Store at</td>
</tr>
<tr>
<td>2ºC to 8ºC in the dark</td>
</tr>
<tr>
<td>IMPORTANT: DO NOT STORE in 35ºC to 37ºC INCUBATOR.</td>
</tr>
<tr>
<td>Storage of ELVIS®HSV Cells in the incubator (above 28ºC) results in overgrowth of the monolayers and sub-optimal morphologic interpretation of results</td>
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<tr>
<td>2. ELVIS® HSV Replacement Medium</td>
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<td>Store at 2ºC to 8ºC</td>
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<tr>
<td>3. ELVIS® HSV Solution 1</td>
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<tr>
<td>Store at 2ºC to 30ºC</td>
</tr>
<tr>
<td>4. ELVIS® HSV Solution 2</td>
</tr>
<tr>
<td>Store at 15ºC to 30ºC</td>
</tr>
</tbody>
</table>

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. 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.

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

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.

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., myology 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. Glass 1 bioassay 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 35ºC to 37ºC. 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 testing. See Section V.
13. M-probe slide staining

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
2. Following the required pre-incubation, briefly examine the microscopic appearance of the monolayer. Cells should be healthy in appearance, which the test was performed to determine the cause(s). Do not report results until all measurements are recorded in order to ensure proper test performance.

4. Add 1-mL of ambient temperature (18ºC to 26ºC) Replacement Medium to each monolayer to be used in the assay, including positive and negative controls.

5. Add 0.2-mL of the patient specimen to each of two monolayers. Freeze the remainder of each specimen at -70ºC or lower, for future reference.

6. Inoculate one monolayer with an HSV-Positive Control and leave one monolayer uninoculated as a Negative Control for each test run.

7. After all inoculations have been completed, replace the plate with a disposable tray seal and replace the cover on the plate, or re-cap the shell vials, and centrifuge at 700g for 60 minutes at ambient temperature.

8. Incubate the inoculated plates at 35ºC to 37ºC for 17- to 24-hours. See Section VI.B.10.

E. Cell Fixation and Staining

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 0.25-mL Solution 2 (Staining Buffer) to each monolayer.

6. Mix by rocking to ensure that each monolayer is uniformly covered with solution.

7. Cover the containers and place at 35ºC to 37ºC for 1- to 5-hours.

8. Upon completion of step VI, above, remove coverslips from shell vials (if required) for interpretation as follows:
   a. Lift the coverslip carefully from the bottom of the shell vial using a bent teasing needle.
   b. Remove the coverslip with forceps. Take care to identify the cell monolayer side of the coverslip.
   c. Wash the coverslip gently by several immersions in a beaker of distilled water while still holding it with the forceps.
   d. Blot excess water by touching the edge of the coverslip to absorbent paper.
   e. 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.

9. 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.

10. 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 2ºC to 8ºC 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 as negative for HSV but must be re-examined 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 2ºC to 8ºC; 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 Procedures

Guidance on appropriate quality control procedures and practices may be found in the American Society for Testing and Materials (ASTM) E1417-99a, "Standard Guide for Performing Quality Control Procedures in the Laboratory: Principles and Definitions, 1999", and 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 coverpage 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. 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 stained 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. 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.]
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 culture results. 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 specimen. Thus, a positive result is indicated when only one monolayer cell is stained blue or blue-green.

IX. EXPECTED VALUES

The prevalence of HSV positive specimens for Laboratories 1 and 2, whose specimens were predominantly of genital or oral 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 monolocular 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 a population studied that has the disease, i.e., 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’ Theorem 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.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tbody>
<tr>
<td>5</td>
<td>75.5</td>
<td>99.9</td>
</tr>
<tr>
<td>15</td>
<td>91.1</td>
<td>99.8</td>
</tr>
<tr>
<td>25</td>
<td>95.1</td>
<td>99.7</td>
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<tr>
<td>35</td>
<td>96.9</td>
<td>99.5</td>
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<tr>
<td>45</td>
<td>98.0</td>
<td>99.2</td>
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<tr>
<td>50</td>
<td>98.7</td>
<td>98.7</td>
</tr>
<tr>
<td>75</td>
<td>99.4</td>
<td>97.0</td>
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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 from 8 to 10 neighboring states for HSV testing. Many factors can affect viability of virus and/or culture results. 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 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.

VI. 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.]
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 culture results. 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.
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.
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:

| TABLE 4: Laboratory 2 – Multi-well Plates | Standard Method |
| (719 specimens) | |
| 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% | 97.9% | 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.
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-infecting 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 whether or not 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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