**INTENDED USE**
Focus Diagnostics’ HerpeSelect® 2 ELISA IgG test is intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-2 in human sera. In conjunction with the Focus Diagnostics HerpeSelect® 1 ELISA IgG, the test is indicated for testing sexually active adults or expectant mothers for aiding in the presumptive diagnosis of HSV infection. The predictive value of a positive or negative result depends on the population’s prevalence and the pretest likelihood of HSV-2 infection. The assay can be used manually or in conjunction with an automated system as outlined in the package insert. The user is responsible for assay performance characteristics when an automated system is used. The performance of this assay has not been established for use in a pediatric population, for neonatal screening, or for testing of immunocompromised patients.

**SUMMARY AND EXPLANATION OF TEST**
HSV is a common human pathogen found worldwide which produces a wide variety of diseases. HSV infects neonates, children and adults, and, by the fourth decade, more than 90% of the adult population demonstrate antibodies to HSV. HSV transmission can result from direct contact with infected secretions from either a symptomatic or an asymptomatic host.

Herpes Simplex virus has been characterized into 2 distinct serotypes: HSV-1 and HSV-2. HSV-1 is generally associated with infection in the tongue, mouth, lips, pharynx and eyes, whereas HSV-2 is primarily associated with genital and neonate infection.

In the U.S., most young sexually active persons with genital ulcers have genital herpes. Genital ulcers have been associated with an increased risk for HIV infection. Genital herpes is usually caused by HSV-2, with the minority of first genital episodes (5-30%) caused by HSV-1. Many cases of genital herpes are transmitted by persons who are unaware that they are infected or do not recognize subtle or atypical symptoms. The Centers for Disease Control and Prevention (CDC) states that counseling is an important aspect of managing patients who have genital herpes.

One of the most serious consequences of genital herpes is neonatal herpes. Without therapy, mortality for untreated infants who develop disseminated infection exceeds 70% with half of the survivors developing neurological impairment. Almost all neonate HSV-2 infections are acquired by passage through an infected birth canal. Most mothers (70%) who transmit HSV to their children are asymptomatic at delivery. Transmission rates are much higher when the mother is experiencing a primary or initial genital infection (> 50%) versus a recurrent infection (<5%), and where the mother is not yet producing IgG antibodies to HSV. The CDC recommends that “…prevention of neonatal herpes should emphasize the prevention of acquisition of genital HSV infection during late pregnancy. Susceptible women whose partners have oral or genital HSV infection, or those whose sex partners’ infection status is unknown, should be counseled to avoid unprotected genital and oral sexual contact during late pregnancy.” Mothers are at greater risk for contracting a primary or initial genital HSV infection when they are seronegative to one or both HSV types, and their partner is seropositive.

Primary HSV-1 infections, acquired through direct person-to-person (primarily nongenital) contact, usually occur in the first decade. When the primary HSV-1 infection is clinical, the classic presentation is herpes gingivostomatitis, a serious infection of the gums, mouth, tongue, lip, face and/ or pharynx. Due to virus reactivation, recurrent HSV-1 infection in the form of herpes labialis (fever blisters or cold sores) or ocular herpes occurs in up to 40% of the HSV-1 seropositive group. A previous oral HSV-1 infection does not protect against a genital HSV-2 infection. Primary HSV-2 infections, usually acquired through sexual contact, are rarely found before the onset of sexual activity. When the primary HSV-2 infection is clinical, the classic presentation is herpes genitialis, an infection characterized by bilaterally distributed lesions in the genital area accompanied by fever, inguinal lymphadenopathy and dysuria. HSV-2 infections cause approximately 85% of symptomatic primary genital HSV cases, with HSV-1 infections causing the remainder. Since HSV-1 is unlikely to produce recurrent genital infections, 99% of recurrent genital herpes is due to HSV-2 infection.

After primary HSV infection, the virus colonizes the sensory neurons, and the latent infection may reactivate causing recurrent sub-clinical or clinical infection. Since recurrent infections may be sub-clinical, and asymptomatic virus shedding is a significant reservoir for virus transmission, unexpected virus transmission occurs and is difficult to prevent.

Viral isolation, direct fluorescent antibody (DFA) testing, and serology can be used to diagnose HSV infections. Positive culture and DFA are the most definitive and viral isolation allows typing of the viral isolate. However, length of culture time, specimen collection and transport difficulties, procedural complexity, and other variables are associated with DFA and culture. Most existing serologic methods for assessing HSV serostatus use viral lysate as antigens. However, due to significant cross-reactivity between HSV-1 and HSV-2, the viral lysate assays are unable to differentiate HSV1 infections from HSV-2 infections. Since most adults have had prior HSV-1 infection, often without primary or recurrent symptoms, HSV-2 serostatus is often impossible to determine with confidence using a viral lysate assay. Recently, HSV type-specific serological assays have been developed using the significant difference between the gG-1 protein of HSV-1 and the gG-2 of HSV-2. The Focus Diagnostics ELISA uses purified recombinant type-specific gG2 antigen immobilized on polystyrene microwells.

**TEST PRINCIPLE**
In the Focus Diagnostics HerpeSelect® 2 ELISA IgG assay, the polystyrene microwells are coated with recombinant gG2 antigen. Diluted serum samples and controls are incubated in the wells to allow specific antibody present in the samples to react with the antigen. Nonspecific reactants are removed by washing, and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD). Sample optical density readings are compared with reference cut-off OD readings to determine results.

**MATERIALS SUPPLIED**
The Focus Diagnostics HerpeSelect® 2 ELISA IgG Test kit contains sufficient materials to perform 96 determinations. Allow the supplied reagents to warm to room temperature before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.
Antigen Wells, 96 wells
12 Eight-well polystyrene microwell strips on a frame. Each well is coated with recombinant gG-2 antigen (molecular weight of 80 to 110 kilodaltons). Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.

IgG Conjugate, 16 mL
One vial of affinity-purified and peroxidase-conjugated goat anti-human IgG (heavy chain specific). Contains protein, buffer and preservatives.

IgG High Positive Control, 0.3 mL
1 vial of human serum. Contains preservatives. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

IgG Low Positive Control, 0.3 mL
1 vial of human serum. Contains preservatives. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

IgG Cut-Off Calibrator, 0.3 mL
1 vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see Specimen, Controls and Calibrator Preparation, below).

HerpeSelect® ELISA Sample Diluent, 100 mL
1 vial of protein, surfactant, and preservatives in PBS. Stable at 2 to 8°C until the expiration date stated on the label. Allow to warm to room temperature before use.

10X Wash Buffer, 100 mL
1 vial of surfactant in PBS with preservatives. Prepare a 1X Wash Buffer solution before use.

To prepare a 1X Wash Buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled (or deionized) water and rinse out any crystals. Use only the highest grade purified water for reconstitution of the wash buffer. It has been observed that some sources of deionized water contain materials which can interfere in the assay. Swirl until well mixed and all crystals are dissolved.

Substrate Reagent, 16 mL
1 vial of tetramethylbenzidine (TMB) and organic peroxide in buffer. A dark blue color indicates contamination with peroxidase; and, if this occurs, use a fresh bottle.

Stop Reagent, 16 mL
1 vial 1 M sulfuric acid.

Sealing Tape
2 sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED
1. Distilled or deionized water
2. 250 or 500 mL wash bottle or automated EIA plate washing device
3. 1 L graduated cylinder
4. 12 x 75 mm borosilicate glass test tubes or equivalent
5. 10 µL and 100 µL pipettors with disposable tips (100 µL 8-channel pipettor recommended for runs over 48 wells)
6. 1 mL pipet or dispenser
7. 5 mL pipet
8. Timer
9. Paper towels or absorbent paper
10. Sink
11. Vortex mixer or equivalent
12. ELISA plate spectrophotometer, wavelength = 450nm
13. Automated ELISA Processor (optional)

SHELF LIFE AND HANDLING
1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS
1. This kit is for in vitro diagnostic use only.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
3. The antigen wells are produced with recombinant gG-2 antigens. After adding patient or control specimens, the strips should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, those reagents (see MATERIALS SUPPLIED, above) should be discarded into sewe rage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5. Do not substitute or mix reagents from different kit lots or from other manufacturers.
6. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
7. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle strips carefully to avoid mixing of sera from adjoining wells. Decant carefully.
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
9. Perform the assay at room temperature (approximate range 20 to 25°C).
10. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.
11. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.

SPECIMEN COLLECTION AND PREPARATION

Serum is the specimen source. No attempt has been made to assess the assay’s compatibility with other specimens. Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage. Separated serum should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at −20°C or colder. Thaw and mix samples well prior to use.

Specimen, Controls and Calibrator Preparation

Dilute each specimen, control or calibrator 1:101. For example, label tubes and dispense 1000 µL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1000 µL Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE

1. Bring all reagents to room temperature before use. Remove the Antigen Well packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused antigen wells at 2 to 8°C. (Note: At the end of the assay, retain the frame for use with the remaining strips.)
2. OPTIONAL 5 minute Pre-Soak. If omitting pre-soak, proceed to Step 3.
   Fill wells with 1X Wash Buffer and soak for 5 minutes. Decant (or aspirate) the Antigen Wells and tap vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
3. Dispense 100 µL of the Sample Diluent into the “blank” well and 100 µL of each diluted specimen, control or calibrator (see Specimen, Controls, and Calibrator Preparation, above) into the appropriate wells. (Note: For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL 8 or 12-channel pipettor.)
4. Cover plates with sealing tape (or place in a humid chamber), and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
5. Remove sealing tape (or remove wells from the humid chamber), and empty the contents of the wells into a sink or a discard basin.
6. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
7. Repeat wash (step 6) an additional 2 times.
8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
9. Dispense 100 µL Conjugate to all wells, using a 100 µL 8 or 12-channel pipettor.
10. Cover plates with sealing tape (or place in a humid chamber) and incubate for 30 ± 1 minutes at room temperature (20 to 25°C).
11. Repeat wash steps 5 through 8.
12. Pipet 100 µL of Substrate Reagent to all wells, using a 100 µL 8 or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (Note: Never pour the substrate reagent into the sample trough as was used for the conjugate.)
13. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
14. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL 8 or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.
15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)
16. Measure the absorbance of each well, or correct all ODs by manually subtracting the blank ODs.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and all 3 controls. If multiple plates are run, include the Cut-off Calibrator and all 3 controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of 4 wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-off Calibrator wells must be within 0.100 to 0.700 OD units. All replicate Cut-off Calibrator ODs should be within 0.10 absorbance units from the mean value. Report results as index values relative to the Cut-off Calibrator. To calculate index values, divide specimen optical density (OD) values by the mean of the Cutoff Calibrator absorbance values.

1. The High Positive Control index value should be greater than 3.5.
2. The Low Positive Control index value should be between 1.5 and 3.5.
3. The Negative Control index value should be less than 0.8.

If the Calibrator or controls are not within these parameters, patient test results should be considered invalid and the assay repeated.

The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator for cutoff precision and only ensures reagent functionality. The Cut-off Calibrator and controls may be formulated using the same lots of raw materials. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
INTRODUCTION OF TEST RESULTS

Report all patient results as index values relative to the Cut-off Calibrator.

To calculate index values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values.

| >1.10 | Positive. An index value of > 1.10 is presumptive for the presence of IgG antibodies to HSV-2. |
| ≥0.90 and ≤1.10 | Equivocal. An index value of ≥ 0.90 but ≤ 1.10 is considered an equivocal result. These samples should be re-tested. If on re-testing, the result remains equivocal, a second sample should be drawn 4 to 12 weeks later and testing repeated. If the specimen may be tested using another method such as Western Blot. |
| <0.90 | Negative. An index value of < 0.90 indicates no IgG antibodies to HSV-2 were detected. |

1. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient.
2. The performance of infection will affect the assay’s predictive value.
3. As with other serological tests, negative results do not rule out the diagnosis of herpes simplex disease. The time required to seroconvert following primary infection varies with the individual, the specimen may have been drawn prior to the appearance of detectable antibodies. There are reports of sero-reversion. When appropriate, e.g., in suspected early herpes simplex disease, the test should be repeated or tested with a different assay. If on re-testing, the result remains negative, then a second sample should be drawn 4 to 12 weeks later and testing repeated. False negative results may occur when the infecting virus is gG deficient, or because it is unknown if the assay’s antigen was glycosylated the same as mammalian cells. Liljeqvist reported about 0.2% (5/2400) HSV-2 isolates were gG deficient. As with other serological tests, false positive results may occur. Repeat testing or testing with a different device may be indicated in some settings, e.g., patients with a low likelihood of HSV infection. If the magnitude of the index value above the Cut-off does not indicate the total amount of antibody present.
4. A single positive result only indicates previous immunologic exposure; level of antibody response or class of antibody response may not be used to determine active infection or disease stage.
5. Serology cannot distinguish genital from oral infections. When appropriate, culture is recommended to identify the infection site. However, false negative HSV cultures are common, especially in patients with recurrent infection or with healing lesions.

LIMITATIONS

1. The performance of this assay has not been established for the general population.
2. The performance of this assay has not been established for ruling out diseases with similar symptoms, e.g., Candida albicans, Bacteroides species, G. vaginalis, Mobiluncus species. Instead, use culture or other appropriate methods.
3. The performance of this assay has not been established for matrices other than serum, or visual result determination(s), or monitoring HSV-2 therapy.

EXPECTED VALUES

An outside investigator assessed the device with masked, archived and unselected sera from 1) sexually active adults over the age of 14 (n = 246), and 2) from expectant mothers (n = 241). The reference method was a HSV-2 Western Blot from a Pacific Northwest university. The observed prevalences and the hypothetical predictive values for the 2 populations are shown in the tables below. The positive predictive value will decrease proportionally to the prevalence of HSV infection as reflected in the table below. The calculations are based on Focus Diagnostics HerpeSelect® 2 ELISA IgG having 1) a hypothetical sensitivity of 96.1% and a hypothetical specificity of 97.0% (sexually active adults), and 2) a hypothetical sensitivity of 100% and a hypothetical specificity of 96.1% (expectant mothers).

Observed Prevalence with Sexually Active Adults & Expectant Mothers

<table>
<thead>
<tr>
<th>Population</th>
<th>HSV-2 Sero-status</th>
<th>Observed Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WB</td>
<td>Focus ELISA</td>
</tr>
<tr>
<td>Sexually Active Adults*</td>
<td>Neg</td>
<td>68.5%</td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>31.5%</td>
</tr>
<tr>
<td>Expectant Mothers†</td>
<td>Neg</td>
<td>75.6%</td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>24.4%</td>
</tr>
</tbody>
</table>

* Excludes 5 atypical Western Blots and 1 ELISA equivocal.
† Excludes 3 atypical Western Blots and 1 ELISA equivocal.

Prevalence vs. Hypothetical Predictive Values

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Sexually Active Adults</th>
<th>Expectant Mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPV</td>
<td>NPV</td>
</tr>
<tr>
<td>50%</td>
<td>97.0%</td>
<td>97.0%</td>
</tr>
<tr>
<td>40%</td>
<td>95.5%</td>
<td>98.0%</td>
</tr>
<tr>
<td>30%</td>
<td>93.2%</td>
<td>98.7%</td>
</tr>
<tr>
<td>25%</td>
<td>91.4%</td>
<td>99.0%</td>
</tr>
<tr>
<td>20%</td>
<td>88.9%</td>
<td>99.2%</td>
</tr>
<tr>
<td>15%</td>
<td>85.0%</td>
<td>99.5%</td>
</tr>
<tr>
<td>10%</td>
<td>78.1%</td>
<td>99.7%</td>
</tr>
<tr>
<td>5%</td>
<td>62.8%</td>
<td>99.8%</td>
</tr>
</tbody>
</table>

Note: Sexually active adult and expectant mother populations in different geographic areas may produce different frequency distributions from the table above. Each laboratory should establish frequency distributions for their specific patient populations.

PERFORMANCE CHARACTERISTICS

Setting the Cut-off

In designing the assay, the Cut-off was validated using five serum panels (n = 383) to initially assess performance: 1) clinical lab samples (n = 94); 2) clinical lab samples that were negative by screening ELISAs (n = 37); 3) clinical lab samples having unusual Western Blot or immunoblot reactivity; 4) Spanish STD clinic samples (n = 159); and 5) Pacific Northwest university samples (n = 62). The reference method was a HSV-2 Western Blot from a licensed clinical laboratory. Excluding 7 atypical Western Blots and 4 ELISA equivocals, the Focus Diagnostics HerpeSelect® 2 ELISA IgG and the Western Blot agreed 94.3% (231/245) with positives, and agreed 87.4% (111/127) with negatives.
Relative Sensitivity and Relative Specificity with Expectant Mothers†
An outside investigator assessed the device’s relative sensitivity and relative specificity with sera from expectant mothers (n = 241). The sera were sequentially submitted to the laboratory, archived, and masked. The reference method was a HSV-2 Western Blot (WB) from a Pacific Northwest university. Of 5 atypical WBs, ELISA was 1 equivocal and 2 negatives. Of 58 WB positives, ELISA was 58 positives. Of 180 WB negatives, ELISA was 172 negatives, 7 positives, and 1 equivocal.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (EL/WB)*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity relative to Western Blot</td>
<td>100% (58/58)</td>
<td>93.8-100%</td>
</tr>
<tr>
<td>Specificity relative to Western Blot</td>
<td>96.1% (172/179)</td>
<td>92.1-98.4%</td>
</tr>
</tbody>
</table>

* Excludes 3 atypical Western Blots and 1 ELISA equivocal.
† The word “relative” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.

Relative Sensitivity and Relative Specificity with Sexually Active Adults†
An outside investigator assessed the device’s relative sensitivity and relative specificity with sera from sexually active adults over the age of 14 (n = 246). The sera were sequentially submitted to the laboratory, archived, and masked. The reference method was a HSV-2 Western Blot from a Pacific Northwest university. Of 5 atypical WBs, ELISA was 2 equivocals, 2 negatives and 1 positive. Of 76 WB positives, ELISA was 73 positives and 3 negatives. Of 165 WB negatives, ELISA was 159 negatives, 5 positives, and 1 equivocal.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (EL/WB)*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity relative to Western Blot</td>
<td>96.1% (73/76)</td>
<td>88.9-99.2%</td>
</tr>
<tr>
<td>Specificity relative to Western Blot</td>
<td>97.0% (159/164)</td>
<td>93.0-99.0%</td>
</tr>
</tbody>
</table>

* Excludes 5 atypical Western Blots and 1 ELISA equivocal.
† The word “relative” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.

Relative Sensitivity with Culture Positives†
An outside investigator assessed the device’s relative sensitivity using sera from culture positive patients (n = 63). Reference methods included culture (infection) and a HSV-2 Western Blot (antibody) from a Pacific Northwest university. Of 5 atypical WBs, ELISA was 2 equivocals, 2 negatives and 1 positive. Of 63 culture positives, ELISA was 61 positives and 2 negatives, and WB was 62 positives and 1 negative. Of 62 WB positives, ELISA was 61 positives and 1 negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (EL/WB or Culture)*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity relative to Culture</td>
<td>96.8% (61/63)*</td>
<td>89.0-99.6%</td>
</tr>
<tr>
<td>Sensitivity relative to Western Blot</td>
<td>98.4% (61/62)*</td>
<td>91.3-100%</td>
</tr>
</tbody>
</table>

* Of the 2 ELISA negatives, 1 was WB positive and the other WB negative.
† The word “relative” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.

Agreement with CDC Panel
The following information is from a serum panel obtained from the CDC and tested by Focus Diagnostics. The results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC. The panel consists of 37% positive and 63% negative samples. The Focus Diagnostics HerpeSelect ® 2 ELISA IgG demonstrated 100% total agreement with the CDC information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC. The panel results. Of the results obtained by Focus Diagnostics, there was 100% agreement with the positive specimens and 100% agreement with the negative specimens.

Relative Specificity with a Low Prevalence Population†
An outside investigator assessed the device’s relative specificity using sera from a population of college students claiming to lack sexual experience (n = 81), and having a published HSV-2 antibody prevalence of 2% (4/186). The laboratory reference method was a HSV-2 Western Blot from a Pacific Northwest university. 1 atypical WB was an ELISA negative. Of 78 WB negatives, ELISA was 77 negatives and 1 positive. Of 2 WB positives, ELISA was 2 positives.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (EL/WB or Culture)*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity relative to Western Blot</td>
<td>98.7% (77/78)</td>
<td>93.1-100%</td>
</tr>
<tr>
<td>Sensitivity relative to Western Blot</td>
<td>100% (2/2)</td>
<td>15.8-100%</td>
</tr>
</tbody>
</table>

* Excludes 1 atypical Western Blot.
† The word “relative” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.

Type Specificity with HSV-1 Western Blot Positives†
An outside investigator assessed the device’s type specificity using HSV-1 Western Blot positive and HSV-2 Western Blot negative sera from the above described populations (n = 287): expectant mothers, sexually active adults, low prevalence persons, and HSV-1 culture positives. Of 287 HSV-1 WB positive and HSV-2 WB negative samples, ELISA was 276 negatives, 1 equivocal and 10 positives.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (EL/WB)*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type specificity relative to Western Blot</td>
<td>96.5% (276/286)</td>
<td>93.7-98.3%</td>
</tr>
<tr>
<td>Type cross-reactivity relative to Western Blot</td>
<td>3.5% (10/286)</td>
<td>1.7-6.3%</td>
</tr>
</tbody>
</table>

* Excludes 1 equivocal ELISA result.
† The word “relative” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.
Cross-reactivity with Taxonomically Related Viruses
Focus assessed the device’s cross-reactivity using sera (n = 27) from 1) HSV sero-negative by another manufacturer’s FDA cleared HSV ELISAs, and 2) IFA IgG positive for taxonomically similar viruses including CMV, EBV VCA, HHV6 and VZV. Discrepancies between the FDA cleared HSV ELISAs and the Focus device were analyzed using a type specific Western Blot from a Pacific Northwest university.

Cross-reactivity with Taxonomically Related Viruses (n = 27)

<table>
<thead>
<tr>
<th>IFA IgG Pos</th>
<th>% Agreement Negative*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>91.7% (11/12)</td>
<td>61.5-99.8%</td>
</tr>
<tr>
<td>EBV VCA</td>
<td>90.9% (20/22)</td>
<td>70.8-98.9%</td>
</tr>
<tr>
<td>HHV6</td>
<td>90.9% (20/22)</td>
<td>70.8-98.9%</td>
</tr>
<tr>
<td>VZV</td>
<td>90.5% (19/21)</td>
<td>69.6-98.8%</td>
</tr>
<tr>
<td>Total</td>
<td>90.9% (70/77)</td>
<td>82.2-96.3%</td>
</tr>
</tbody>
</table>

* Excludes 3 Western Blot positives, and 1 discrepant that was not analyzed with the Western Blot because of insufficient volume.

Intra-assay and Inter-assay Reproducibility
An internal investigator assessed the device’s intra-assay and inter-assay reproducibility by assaying 7 samples in duplicate, twice a day, for 20 days, for a total of 40 runs. 2 sets of samples were masked duplicates.

Inter-lot Reproducibility
An internal investigator assessed the device’s inter-lot reproducibility. 5 samples were run on 3 separate days with 3 separate lots. For 1 lot, the samples were run in triplicate, and run in duplicate with the other 2 lots. Each of the 3 lots had a different lot of Antigen Wells.

Inter-laboratory Reproducibility
An internal investigator and 2 off-site laboratories assessed the device’s inter-laboratory reproducibility. Each of the 3 laboratories ran 7 samples in triplicate on 3 different days. 3 points were excluded because an incorrect sample (instead of sample 27) was run 1 day.

Reproducibility

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inter- &amp; Intra-assay</th>
<th>Inter-lot</th>
<th>Inter-lab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Index Mean %CV</td>
<td>Intra-assay %CV</td>
<td>Inter-assay %CV</td>
</tr>
<tr>
<td>21*</td>
<td>0.18</td>
<td>20.5%</td>
<td>16.9%</td>
</tr>
<tr>
<td>26*</td>
<td>0.18</td>
<td>12.9%</td>
<td>12.4%</td>
</tr>
<tr>
<td>22**</td>
<td>1.23</td>
<td>6.3%</td>
<td>6.2%</td>
</tr>
<tr>
<td>27**</td>
<td>1.22</td>
<td>5.2%</td>
<td>6.3%</td>
</tr>
<tr>
<td>23</td>
<td>1.79</td>
<td>4.7%</td>
<td>5.5%</td>
</tr>
<tr>
<td>24</td>
<td>3.42</td>
<td>3.2%</td>
<td>7.9%</td>
</tr>
<tr>
<td>25</td>
<td>8.17</td>
<td>3.0%</td>
<td>6.9%</td>
</tr>
</tbody>
</table>

* #21 & #26 are same material.  ** #22 & #27 are same material.

% Agreement between the Manual and Automated Methods (n = 257)
An internal and an external investigator compared % agreement between the HerpeSelect automated method vs. the manual method as part of a CLIA validation for a major clinical laboratory located in Southern California. The external investigator sequentially selected and manually tested 257 samples. Each sample was assayed by manual method. For 24 of the 257 samples, each sample was assayed twice a day, for 20 days, for a total of 40 runs. 2 sets of samples were masked duplicates.

% Agreement between Manual and Automated Methods

<table>
<thead>
<tr>
<th>Interpretation*</th>
<th>% Agreement</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>99.4% (174/175)</td>
<td>96.4-100%</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0.0% (0/3)</td>
<td>0.0-70.8%</td>
</tr>
<tr>
<td>Positive</td>
<td>98.7% (78/79)</td>
<td>93.1-100%</td>
</tr>
<tr>
<td>Overall</td>
<td>98.1% (252/257)</td>
<td>95.5-99.4%</td>
</tr>
</tbody>
</table>

* Interpretation by manual method.

Reproducibility Using an Automated Instrument
An internal investigator assessed the device’s intra-assay and inter-assay reproducibility with an automated instrument. 10 samples were tested in triplicate on 3 different days. The manual and automated methods agreed 98.9% (89/90). 1 point from Sample 3 was an outlier (162 standard deviations from the mean).

Stability after Opening Reagents
An internal investigator assessed stability after the reagents had been opened and used with an automated instrument. The kit was used in the inter-assay/intra-assay reproducibility study (above), re-closed, stored at 2 to 8°C for at least 30 days, and then used again to re-test the same samples. There was 100% agreement with the index when the reagents were opened.

REFERENCES

This package insert is available in French, German, Italian, and Spanish at www.focusdx.com, and may be available in other languages from your local distributor.