# HSV gG-2 IgG Test System

REF 9Z9781G/SM9Z9781G

IVD

CE

Rx Only

## INTENDED USE

The ZEUS ELISA HSV gG-2 IgG Test System is intended for the qualitative detection of type specific IgG class antibodies to Herpes Simplex Virus Type 2 (HSV-2) in human serum. The assay is intended for testing sexually active individuals or pregnant women for aiding in the presumptive diagnosis of HSV-2 infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-2. The test is not intended for donor screening or for self testing. The performance of this test has not been established for use in a pediatric population, neonates, children, or immuno-compromised patients.

## SIGNIFICANCE AND BACKGROUND

***Herpes Simplex Virus*** infections are caused by two distinct antigenic types, HSV-1 and HSV-2 (1). Both HSV types are common human pathogens. HSV-1 is usually associated with infections in the oropharyngeal area and eyes while HSV-2 causes most genital and neonatal infections (1, 2). However, the tissue specificity is not absolute (3). HSV-2 can be isolated occasionally from the oropharyngeal area and 5 - 10% of primary genital infections may be caused by HSV-1 (1, 4).

HSV infections are transmitted by virus-containing secretions through close personal contact. HSV infections, both primary and recurrent, are often subclinical and asymptomatic. Shedding of the virus is the most important factor contributing to the spread of the virus (2).

Primary HSV-1 infections of the oral mucosa usually occur in children of less than 5 years of age (2). Most infections are asymptomatic. Symptomatic infections are characterized by gingivostomatitis associated with fever, malaise, and tender swollen cervical lymph nodes (2). Numerous small vesicles develop on the oral mucosa, become ulcerated, and heal within about two weeks. The most common form of recurrent HSV-1 is herpes labialis in which vesicles appear on the lips, nostrils or skin around the mouth (1, 2). Symptoms of genital HSV infections are multiple ulcerative lesions accompanied by pain, fever, dysuria, and lymphadenopathy (6).

The most severe complication of genital HSV infection is neonatal disease (2). Of mothers with an active primary infection, the risk of transmission to infants is as high as 40% (5). About 69 - 80% of infants who develop neonatal herpes are born to women who are asymptomatic of genital HSV infection at the time of birth (5). Genital herpes is problematic in sexually active adults as well as the disease is often transmitted in the absence of symptoms (13). HSV antibody testing is indicated for sexually active adults to identify those at risk for acquiring HSV or transmitting HSV to others and for expectant mothers who are at risk for acquiring HSV infections and transmitting neonatal herpes (7, 13).

Detection of disease caused by Herpes Simplex Virus Type 1 and Type 2 has been complicated by the lack of consistently good diagnostic testing. Although culture combined with Direct Fluorescent Antibody (DFA) testing is definitive in making a diagnosis, the timing is critical and cultures must be obtained during periods of active disease to produce optimal recovery (8, 9). Serological procedures may be useful for diagnosis of primary HSV infections, and for determining evidence of past infection with HSV. Many existing serologic methods for determining HSV sero-status, however, are unable to differentiate between HSV-1 and HSV-2 infections (10). Since the type of HSV implicated in disease has ramifications for prognosis (11, 12), it is important to specify the sub-type. HSV type-specific serological assays have been developed using the significant difference between the glycoprotein G envelope protein (gG-1) of HSV-1 and the gG-2 protein of HSV-2 (10). Early application of type-specific serologic testing for HSV-1 and HSV-2 has been shown to benefit in testing first-time, recurrent, and asymptomatic infections as a means to definitive diagnosis and appropriate patient counseling (13). Serologic type-specific assays are useful in establishing or confirming the diagnosis of HSV-1 or 2 infections in asymptomatic people, those with symptomatic but negative culture lesions, and those with atypical presentations (14). Type-specific testing is recommended for sexually active adults and pregnant women as the presence of HSV antibodies is a reliable indicator that an individual may be infected with HSV and capable of transmitting the virus to others (14).

## PRINCIPLE OF THE ASSAY

The ZEUS ELISA HSV gG-2 IgG Test System is designed to detect IgG class antibodies to HSV-2 in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with inactivated affinity purified HSV-2 antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated at room temperature (20 - 25°C) for 25 ± 5 minutes in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. After incubation, the plate is washed five times to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated at room temperature (20 - 25°C) for 25 ± 5 minutes. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. After incubation, the wells are washed five times to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After 10 - 15 minutes, the reaction is stopped and the color intensity of the solution is measured photometrically within 30 minutes. The color intensity of the solution depends upon the antibody concentration in the original test sample.

## TEST SYSTEM COMPONENTS

**Materials Provided:**

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.**

| | | |
|---|---|---|
| PLATE | | 1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with affinity purified HSV-2 antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant. |
| CONJ | | 2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15mL, white-capped bottle. Ready to use. |
| CONTROL | + | 3. Positive Control (Human Serum): One, 0.35mL, red-capped vial. |
| CAL | | 4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial. |
| CONTROL | - | 5. Negative Control (Human Serum): One, 0.35mL, green-capped vial. |
| DIL | SPE | 6. SAVe Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.** |
| SOLN | TMB | 7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use. |
| SOLN | STOP | 8. Stop Solution: One, 15mL, red-capped, bottle containing 1M $H_2SO_4$, 0.7M HCl. Ready to use. |
| WASHBUF | 10X | 9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.** |

**NOTES:**

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

## PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition (15); and OSHA's Standard for Bloodborne Pathogens (16).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

## STORAGE CONDITIONS

| | |
|---|---|
| 2°C – 8°C | Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue. |
| | Conjugate – DO NOT FREEZE. |
| | Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, SAVe Diluent® |
| 2°C – 25°C | Stop Solution: 2 - 25°C<br>Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days.<br>Wash Buffer (10X): 2 - 25°C |

## SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease: Current Edition (17).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.

3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (18, 19). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (20).

## ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

| EXAMPLE PLATE SET-UP | | |
|---|---|---|
| | 1 | 2 |
| A | Blank | Patient 3 |
| B | Negative Control | Patient 4 |
| C | Calibrator | Etc. |
| D | Calibrator | |
| E | Calibrator | |
| F | Positive Control | |
| G | Patient 1 | |
| H | Patient 2 | |

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

**ABBREVIATED TEST PROCEDURE**

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

## QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

| | OD Range |
|---|---|
| Negative Control | ≤0.250 |
| Calibrator | ≥0.300 |
| Positive Control | ≥0.500 |

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.

5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

## INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *Cutoff OD Value:* To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
   *(CF x Mean OD of Calibrator = Cutoff OD Value)*
   c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

| | | | |
|---|---|---|---|
| Example: | Mean OD of Calibrator | = | 0.793 |
| | Correction Factor (CF) | = | 0.25 |
| | Cutoff OD | = | 0.793 x 0.25 = 0.198 |
| | Unknown Specimen OD | = | 0.432 |
| | Specimen Index Value/OD Ratio | = | 0.432/0.198 = 2.18 |

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

| | Index Value/OD Ratio |
|---|---|
| Negative Specimens | ≤0.90 |
| Equivocal Specimens | 0.91 to 1.09 |
| Positive Specimens | ≥1.10 |

   a. An OD Ratio less than or equal to 0.90 indicates no detectable antibody to HSV-2.
   b. Retest specimens with OD Ratio Values in the equivocal range (0.91 – 1.09) in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimens using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.
   c. An OD Ratio greater than or equal to 1.10 indicates that HSV-2 IgG antibodies were detected.
   d. The numeric value of the final result above the Cutoff is not indicative of the amount of anti-HSV-2 IgG antibody present.
   e. Test results should be interpreted in conjunction with the clinical history, epidemiological data and other information available to the attending physician in evaluating the patient.
   f. False positive test results may occur. Repeat testing or testing with a different device may be indicated in some settings e.g., patients with low likelihood of HSV infection.

## LIMITATIONS OF THE ASSAY

1. The ZEUS ELISA HSV gG-2 IgG Test System cannot discriminate active versus past infection with HSV-2 and patients with active infection or very recent infection with HSV-2 may be negative on this test.
2. There is currently no International Standard for HSV-2 IgG, so value assignments for Calibrators and Controls were based on an internal reference preparation.
3. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
4. Performance characteristics of this device have not been established for matrices other than serum.
5. Serological cross-reactivity has not been evaluated in the specimens containing antibody to *Candida albicans*. Caution should be used when interpreting positive results in patients with these antibodies.
6. The performance of this assay has not been established for monitoring HSV-2 therapy.
7. Hemolytic, icteric, or lipemic samples may interfere with the outcome of this assay. Use of these types of specimens should be avoided.
8. Samples collected too early in the course of the infection may not have detectable levels of HSV-2 IgG.
9. HSV serology cannot distinguish genital from non-genital infections.

## EXPECTED RESULTS

1. **Observed Prevalence and Hypothetical Predictive Values:**
Observed prevalence with the Intended Use populations was assessed internally and externally at two sites with unselected, masked and archived sera. With the sexually active individuals (n=336) the observed prevalence with the ZEUS ELISA HSV gG-2 IgG Test System was 21.1% (71/336). In the population of pregnant women (n=252), the observed prevalence with the ZEUS ELISA HSV gG-2 IgG Test System was 28.7% (72/251 - one sample excluded from this analysis due to unknown age). The observed prevalence with the investigational device in each age group tested in the two populations follows in Tables 1 and 2.

**Table 1: Observed Prevalence with Sexually Active Individuals**

| Age | Sex | ZEUS ELISA HSV gG-2 IgG Test System | | | | Observed % Prevalence HSV gG-2 |
|---|---|---|---|---|---|---|
| | | Positive | Equivocal | Negative | Total % | |
| 15-19 | Male | 1 | 10 | 11 | 9.1 | 1 |
| | Female | 2 | 23 | 25 | 8.7 | 2 |
| 20-29 | Male | 2 | 32 | 34 | 5.9 | 2 |
| | Female | 19 | 69 | 88 | 21.6 | 19 |
| 30-39 | Male | 2 | 18 | 20 | 10.0 | 2 |
| | Female | 17 | 43 | 60 | 28.3 | 17 |
| 40-49 | Male | 7 | 22 | 29 | 24.1 | 7 |
| | Female | 5 | 16 | 21 | 23.8 | 5 |
| 50-59 | Male | 7 | 20 | 27 | 25.9 | 7 |
| | Female | 5 | 5 | 10 | 50.0 | 5 |
| 60-69 | Male | 3 | 6 | 9 | 33.3 | 3 |
| | Female | 1 | 0 | 1 | 100.0 | 1 |
| 70 + | Male | 0 | 1 | 1 | 0.0 | 0 |
| | Female | 0 | 0 | 0 | 0.0 | 0 |
| Sub-Total | Male | 23 | 108 | 131 | 17.6 | 23 |
| | Female | 49 | 156 | 205 | 23.9 | 49 |
| | **Total** | 71 | 265 | 336 | 21.1 | 71 |

**Table 2: Observed Prevalence with Pregnant Women**

| Age | ZEUS ELISA HSV gG-2 IgG Test System | | | Observed % Prevalence HSV gG-2 |
|---|---|---|---|---|
| | Positive | Negative | Total | |
| 15-19 | 2 | 22 | 24 | 8.3 |
| 20-29 | 46 | 103 | 149 | 30.9 |
| 30-39 | 15 | 48 | 63 | 23.8 |
| 40-49 | 9 | 6 | 15 | 60.0 |
| Total | 72 | 179 | 251* | 28.7 |

*One sample submitted with age unknown was excluded.

**2. Prevalence vs. Hypothetical Predictive Values:**

The hypothetical predictive values for the two populations are shown in the tables below. The calculations are based on the ZEUS ELISA HSV gG-2 IgG Test System having: a sensitivity of 93.3% and a specificity of 99.6% in sexually active adults; a sensitivity of 98.6% and a specificity of 98.9% in pregnant women.

**Table 3: Prevalence vs. Hypothetical Predictive Values**

| Prevalence | ZEUS ELISA HSV gG-2 IgG Test System | | | |
|---|---|---|---|---|
| | Sexually Active Individuals | | Pregnant Women | |
| | PPV | NPV | PPV | NPV |
| 50% | 99.6% | 93.7% | 98.9% | 98.6% |
| 40% | 99.4% | 95.7% | 98.4% | 99.1% |
| 30% | 99.1% | 97.2% | 97.5% | 99.4% |
| 25% | 98.8% | 97.8% | 96.8% | 99.5% |
| 20% | 98.4% | 98.3% | 95.7% | 99.6% |
| 15% | 97.8% | 98.8% | 94.1% | 99.8% |
| 10% | 96.5% | 99.3% | 90.9% | 99.8% |
| 5% | 92.9% | 100.0% | 82.5% | 100.0% |

## PERFORMANCE CHARACTERISTICS

**1. Comparative Studies:**

The ZEUS ELISA HSV gG-2 IgG Test System was compared to a commercially marketed immunoblot test system for the detection of IgG antibodies to HSV-2. A total of 788 samples were evaluated. Five hundred and fifty-eight (588) samples were from the Intended Use populations of sexually active individuals (n=336) and pregnant women (n=252) with an HSV test requested. One-hundred additional samples from a low prevalence population of 17 - 19 year old blood donors were tested as were 100 samples acquired from the CDC (CDC panel). The clinical sites, amount of samples tested and their demographics are summarized in Table 4.

**Table 4: Number of Samples Tested at Each Site**

| Populations | Site One | Site Two | Site Three | Total |
|---|---|---|---|---|
| Sexually Active Individuals | 100 | 136 | 100 | 336 |
| Pregnant Women | 33 | 155 | 64 | 252 |
| Low Prevalence Population | 33 | 33 | 34 | 100 |
| CDC Panel | 33 | 25 | 42 | 100 |

a. **Performance in the Intended Use Population of Sexually Active Individuals:** A total of 336 prospective, unselected samples from sexually active individuals with an HSV-2 test ordered were tested with the ZEUS ELISA HSV gG-2 IgG Test System and compared with a commercially available immunoblot test, see Table 5. The samples were submitted for HSV-2 antibody testing, sequentially numbered, de-identified and archived.

**Table 5: Sexually Active Individuals**

| | | Immunoblot | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Positive** | **Equivocal** | **Negative** | **Site Total** | | **% Agreement*** | **95% CI** |
| **ZEUS ELISA HSV gG-2 IgG** | **Positive** | 70 | 0 | 1 | 71 | PPA | 93.3 (70/75) | 86.9 - 98.5 |
| | **Equivocal** | 0 | 0 | 0 | 0 | | | |
| | **Negative** | 1 | 4 | 260 | 265 | NPA | 99.6 (260/261) | 97.9 - 100 |
| | **Site Total** | 71 | 4 | 261 | 336 | | | |

*Equivocal results in one test but not the other were treated as discrepant and included in the calculations.

b. **Performance in the Intended Use Population of Pregnant Women:** A total of 252 prospective, unselected samples from pregnant women with an HSV-2 test ordered were tested with the ZEUS ELISA HSV gG-2 IgG Test System and compared with a commercially available immunoblot test. One-hundred and twenty-one (121) samples were from women in the first trimester, 64 from women in the second trimester and 67 from women in the third trimester, see Table 6. The samples were submitted for HSV-1 antibody testing, sequentially numbered, de-identified and archived.

**Table 6: Pregnant Women**

| | | Immunoblot | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Positive** | **Equivocal** | **Negative** | **Site Total** | | **% Agreement*** | **95% CI** |
| **ZEUS ELISA HSV gG-2 IgG** | **Positive** | 70 | 1 | 1 | 72 | PPA | 98.6 (70/71) | 92.5 - 100 |
| | **Equivocal** | 0 | 0 | 0 | 0 | | | |
| | **Negative** | 1 | 0 | 179 | 180 | NPA | 98.9 (179/181) | 96.1 - 99.9 |
| | **Site Total** | 71 | 1 | 180 | 252 | | | |

*Equivocal results in one test but not the other were treated as discrepant and included in the NPA calculations.

c. **Performance in a Low Prevalence Population:** A total of 100 samples collected from 17 - 19 year old blood donors in a non-STD setting were tested with the ZEUS ELISA HSV gG-2 IgG Test System and compared with a commercially available immunoblot test, see Table 7. These samples were purchased from a New York vendor.

**Table 7: Low Prevalence Population**

| | | Immunoblot | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Positive** | **Equivocal** | **Negative** | **Site Total** | | **% Agreement*** | **95% CI** |
| **ZEUS ELISAHSV gG-2 IgG** | **Positive** | 0 | 0 | 0 | 0 | PPA | N/A | N/A |
| | **Equivocal** | 0 | 0 | 2 | 2 | | | |
| | **Negative** | 0 | 0 | 98 | 98 | NPA | 98.9 (98/100) | 93.0 - 99.8 |
| | **Site Total** | 0 | 0 | 100 | 100 | | | |

*Equivocal results in one test but not the other were treated as discrepant and included in the NPA calculations.

d. **CDC HSV-2 IgG Panel:** A total of 100 samples were obtained from the CDC for analysis. The performance of the ZEUS ELISA HSV gG-2 IgG Test System was assessed using a masked, well characterized HSV serum panel from the CDC. The panel consists of 24% HSV-1 and HSV-2 dual-positive samples, 46% HSV-2 positive and 54% HSV-2 negative samples. The results are presented in Table 8 to convey further information on the performance of the assay and do not imply endorsement of the assay by the CDC.

**Table 8: CDC HSV Panel**

| | | CDC HSV-2 Results | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **Positive** | **Equivocal** | **Negative** | **Site Total** | | **% Agreement*** | **95% CI** |
| **ZEUS ELISA HSV gG-2 IgG** | **Positive** | 46 | 0 | 0 | 46 | PPA | 100 (46/46) | 93.7 - 100 |
| | **Equivocal** | 0 | 0 | | 0 | | | |
| | **Negative** | 0 | 0 | 54 | 54 | NPA | 100 (54/54) | 94.6 - 100 |
| | **Site Total** | 46 | 0 | 54 | 100 | | | |

*Equivocal results in one test but not the other were treated as discrepant and included in the NPA calculations.

**2. Precision and Reproducibility**

a. **Precision:** Precision was conducted as follows: twelve samples were identified and/or prepared for use in the study based upon their activity on the ZEUS ELISA HSV gG-2 IgG Test System. Two samples each were selected that were negative, high negative, near cut-off, low positive, moderate positive and high positive. On each day of testing, the samples were aliquoted in duplicate and tested. This was repeated in a second run on the same day by a different technologist for a total of twenty days (2 times x 2 runs x 20 days = 80 replicates per panel member). The precision data was analyzed according to the principles described in the CLSI guidance EP5-A2, revised November 2004. The standard deviation (StD) and percent coefficient of variation (% CV) were calculated. Results are shown in Table 9.

**Table 9: Summary of In-House Precision for the ZEUS ELISA HSV gG-2 IgG Test System**

| | Sample | Mean Index | Within-Run | | Within-Day | | Between-Run | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Panel Member** | **(n)** | **Value** | **StD** | **% CV** | **StD** | **% CV** | **StD** | **% CV** | **StD** | **% CV** |
| High Positive | 80 | 9.096 | 0.22 | 2.4 | 0.34 | 3.7 | 0.29 | 3.2 | 0.41 | 4.5 |
| High Positive | 80 | 5.290 | 0.12 | 2.3 | 0.19 | 3.5 | 0.15 | 2.9 | 0.25 | 4.6 |
| Moderate Positive | 80 | 2.512 | 0.06 | 2.5 | 0.11 | 4.3 | 0.10 | 4.1 | 0.15 | 5.9 |
| Moderate Positive | 80 | 2.308 | 0.07 | 3.0 | 0.10 | 4.3 | 0.09 | 3.9 | 0.12 | 5.3 |
| Low Positive | 80 | 1.377 | 0.06 | 4.5 | 0.07 | 5.2 | 0.04 | 3.3 | 0.08 | 6.0 |
| Low Positive | 80 | 1.288 | 0.05 | 4.0 | 0.07 | 5.1 | 0.04 | 3.3 | 0.09 | 6.7 |
| Near Cutoff | 80 | 1.028 | 0.03 | 3.0 | 0.05 | 5.0 | 0.05 | 4.7 | 0.06 | 6.0 |
| Near Cutoff | 80 | 0.992 | 0.03 | 2.9 | 0.05 | 4.6 | 0.04 | 3.9 | 0.06 | 6.1 |
| High Negative | 80 | 0.702 | 0.02 | 3.3 | 0.03 | 4.6 | 0.02 | 3.4 | 0.04 | 5.8 |
| High Negative | 80 | 0.663 | 0.03 | 3.9 | 0.03 | 5.3 | 0.02 | 3.8 | 0.05 | 7.5 |
| Negative | 80 | 0.205 | 0.01 | 5.2 | 0.02 | 7.6 | 0.01 | 6.6 | 0.02 | 10.2 |
| Negative | 80 | 0.342 | 0.01 | 2.6 | 0.02 | 4.8 | 0.02 | 4.5 | 0.02 | 7.1 |
| Non-Reactive Control | 80 | 0.256 | 0.01 | 4.6 | 0.01 | 5.5 | 0.01 | 3.9 | 0.02 | 7.1 |
| Reactive Control | 80 | 8.903 | 0.13 | 1.5 | 0.21 | 2.4 | 0.19 | 2.1 | 0.34 | 3.8 |

b. **Reproducibility:** Reproducibility was evaluated internally and at two external clinical sites. The study was conducted as follows: twelve samples were identified and/or prepared for use in the study based upon their activity on the ZEUS ELISA HSV gG-2 IgG Test System. Two samples each were selected that were negative, high negative, near cutoff, low positive, moderate positive and high positive. To assess reproducibility, on each day of testing, each sample was aliquoted in duplicate, each aliquot was tested in triplicate in two runs by two operators resulting in twelve results per day. The samples were tested for five days at three sites. The precision data were analyzed according to the principles described in the CLSI guidance EP5-A2, revised November 2004. The standard deviation (StD) and percent coefficient of variation (%CV) were calculated. Results are shown below.

**Table 10: Summary of Reproducibility for the ZEUS ELISA HSV gG-2 IgG Test System**

| | Sample | Mean Index | Within-Run | | Within -Day | | Between-Run | | Between-Site | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Panel Member** | **(n)** | **Value** | **SD** | **% CV** | **SD** | **% CV** | **SD** | **% CV** | **SD** | **% CV** | **SD** | **% CV** |
| High Positive | 180 | 8.270 | 0.33 | 3.9 | 0.38 | 4.5 | 0.24 | 2.8 | 0.43 | 5.2 | 0.68 | 8.3 |
| High Positive | 180 | 5.263 | 0.26 | 4.9 | 0.32 | 6.1 | 0.22 | 4.3 | 0.36 | 6.8 | 0.41 | 7.8 |
| Moderate Positive | 180 | 2.535 | 0.15 | 5.7 | 0.17 | 6.8 | 0.11 | 4.2 | 0.21 | 8.3 | 0.25 | 10.0 |
| Moderate Positive | 180 | 2.237 | 0.10 | 4.6 | 0.12 | 5.5 | 0.08 | 3.6 | 0.14 | 6.3 | 0.18 | 8.1 |
| Low Positive | 180 | 1.350 | 0.08 | 5.8 | 0.09 | 6.8 | 0.06 | 4.3 | 0.10 | 7.5 | 0.11 | 8.4 |
| Low Positive | 180 | 1.320 | 0.08 | 5.9 | 0.10 | 7.5 | 0.07 | 5.6 | 0.11 | 8.0 | 0.14 | 10.7 |
| Near Cutoff | 180 | 1.020 | 0.06 | 5.9 | 0.07 | 7.2 | 0.05 | 4.9 | 0.09 | 9.0 | 0.11 | 10.4 |
| Near Cutoff | 180 | 0.969 | 0.06 | 6.5 | 0.08 | 7.9 | 0.05 | 5.2 | 0.08 | 8.8 | 0.10 | 10.1 |
| High Negative | 180 | 0.668 | 0.05 | 7.0 | 0.05 | 8.2 | 0.03 | 4.9 | 0.06 | 9.3 | 0.07 | 10.3 |
| High Negative | 180 | 0.653 | 0.05 | 8.4 | 0.06 | 9.7 | 0.04 | 5.8 | 0.07 | 11.1 | 0.08 | 12.1 |
| Negative | 180 | 0.352 | 0.03 | 9.3 | 0.04 | 11.3 | 0.03 | 7.5 | 0.05 | 14.0 | 0.05 | 15.5 |
| Negative | 180 | 0.320 | 0.03 | 10.5 | 0.04 | 12.4 | 0.02 | 6.8 | 0.05 | 15.0 | 0.05 | 16.4 |
| Non-Reactive Control | 180 | 0.245 | 0.03 | 12.6 | 0.04 | 14.8 | 0.02 | 7.4 | 0.04 | 17.3 | 0.05 | 18.7 |
| Reactive Control | 180 | 8.869 | 0.51 | 6.0 | 0.59 | 6.8 | 0.34 | 3.9 | 0.73 | 8.3 | 0.87 | 9.9 |

**3. Cross Reactivity and Interfering Substances**

a. **Cross Reactivity:** Studies were performed at the manufacturing facility to assess cross reactivity with the ZEUS ELISA HSV gG-2 IgG Test System using samples that were sero-positive to EBV VCA IgG, ANA, Rubella, VZV IgG, CMV, Measles, *Treponema pallidum*, Gonorrhea, HPV, Chlamydia, RF, *Toxoplasma gondii*, and HSV gG-1. Ten samples for each analyte were tested for a total of 100 samples. Micro-particle and ELISA immunoassay test systems manufactured for commercial distribution were used to determine the sero-positivity of the samples. Additionally, results for HPV, Chlamydia and Gonorrhea were provided from an alternate testing facility. The results presented were obtained by testing the analytes against high concentrations of possible cross reactants and are summarized in Table 11.

**Table 11: ZEUS ELISA HSV gG-2 IgG Test System Cross Reactivity Study**

| Analyte | Positive/Tested | Analyte | Positive/Tested | Analyte | Positive/Tested | Analyte | Positive/Tested |
|---|---|---|---|---|---|---|---|
| **EBV VCA IgG** | 0/10 | **CMV** | 0/10 | **HPV** | 0/10 | ***T. gondii*** | 0/10 |
| **ANA** | 0/10 | **VZV** | 0/10 | **Chlamydia** | 0/10 | | |
| **Measles** | 0/10 | ***T.pallidum*** | 0/10 | **RF** | 0/10 | | |
| **Rubella** | 0/10 | **Gonorrhea** | 0/10 | **HSV gG-1** | 0/10 | | |

b. **Interfering Substances:** The effect of potential interfering substances on assay generated sample results was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. The quantity of analyte in each interfering substance was as follows:

Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 10 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples for HSV gG-2 were chosen based on their performance on the investigational device: positive, borderline and negative. The possible interfering substances were added to the samples. Test and control samples were evaluated in replicates of ten. All positive samples showed a change of signal less than 20%. All borderline samples showed a change of signal less than 20% with the exception of the high spike of intralipid (75.5%). The negative sample showed a change of signal (>20%) with the low spike (42.9%) and high spike of albumin (78.6%), the high spikes of hemoglobin (245.2%), the low spike of intralipid (78.6%), and the low and high spikes of bilirubin (272.7% and 318.2% respectively). The negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

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