**HSV-2 IgG Test System**

**IVD**

CE Mark.bmp

**REF**

**9Z9751G**

**Rx Only**

|  |  |
| --- | --- |
| Institute Name | Date |
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**PRINCIPLE OF THE ASSAY**

The ZEUS ELISA HSV-2 IgG Test System is designed to detect IgG class antibodies to HSV-2 in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with HSV-2 antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed 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. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed 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 a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. 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®.**

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| **PLATE** | | 1. | Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated HSV-2 (strain G) antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant. |
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| **CONJ** | | 2. | Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15mL, white-capped bottle. Ready to use. |
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| **CONTROL** | **+** | 3. | Positive Control (Human Serum): One, 0.35mL, red-capped vial. |
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| **CAL** | | 4. | Calibrator (Human Serum): One, 0.5mL, blue-capped vial. |
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| **CONTROL** | **-** | 5. | Negative Control (Human Serum): One, 0.35mL, green-capped vial. |
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| **DIL** | **SPE** | 6. | SAVe Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. **NOTE: The SAVe Diluent**® **will change color when combined with serum.** |
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| **SOLN** | **TMB** | 7. | TMB: One, 15mL, amber-capped, amber bottle containing 3, 3’, 5, 5’ - tetramethylbenzidine (TMB). Ready to use. |
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| **SOLN** | **STOP** | 8. | Stop Solution: One, 15mL, red-capped, bottle containing 1M H2SO4, 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 within 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, handle these products 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; and OSHA’s Standard for Bloodborne Pathogens (19).
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 upon 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**

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| storage2-8.bmp | 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® |
| storage2-25.bmp | Stop Solution: 2 - 25°C  Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days.  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).
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 (15, 16). 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.

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| **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 |  |

1. 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.**
2. 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.
3. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
4. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
5. Wash the microwell strips 5 times.
   1. **Manual Wash Procedure**:
6. Vigorously shake out the liquid from the wells.
7. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
8. Repeat steps 1. and 2. for a total of 5 washes.
9. 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.
   1. **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.

1. 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.
2. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
3. Wash the microwells by following the procedure as described in step 7.
4. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
5. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
6. 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.
7. 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.

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| **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

1. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
2. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
3. 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:**
2. *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.
3. *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)*

1. *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.

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| 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 |

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

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

1. HSV-1 and HSV-2 share many cross-reacting antigens. Therefore, to fully evaluate the IgG antibody status to HSV, run both the HSV-1 and HSV-2 ELISA tests simultaneously on each sample. Compare and evaluate the results of both tests as follows:

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| **Positive (≥1.10)** | **Equivocal (0.91-1.09)** | **Negative (< 0.90)** | **Interpretation** |
| HSV-1, HSV-2  HSV-1  HSV-2  HSV-1  HSV-2 | HSV-2  HSV-1 | HSV-2  HSV-1 | Positive for IgG antibody to HSV. Indicates a current or previous infection with HSV-1, HSV-2, or both. |
|  | HSV-1, HSV-2  HSV-1  HSV-2 | HSV-2  HSV-1 | Equivocal. Samples should be retested. See note (b) below. |
|  |  | HSV-1, HSV-2 | Negative for IgG antibody to HSV. Indicates no current or previous infection with HSV-1 or HSV-2. See note below. |

1. 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. Test repeatedly equivocal specimens using an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later.
2. Specimens obtained too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, take another specimen in eight to 14 days and tested concurrently in the same assay with the original specimen to determine seroconversion.
3. To evaluate paired (acute and convalescent) sera for seroconversion, test both samples in the same assay. If the acute specimens are negative and the convalescent specimens are positive for IgG antibody to either HSV-1 or HSV-2 or both, seroconversion has taken place and a primary HSV infection is indicated.

**LIMITATIONS OF THE ASSAY**

1. HSV-1 and HSV-2 share many cross-reacting antigens and a majority of the antibody produced in response to an initial infection is to shared antigens (17). Initial infection with HSV-2 in persons with a past infection with HSV-1 will likely produce a significant rise in antibody titer to common antigens as well as to HSV-2 specific antigens.
2. HSV-1 or HSV-2 antibody test results will not indicate the site of infection. The test is not intended to replace viral isolation.
3. The presence of IgG antibodies to HSV-1 or HSV-2 does not necessarily imply protection from future infection with HSV-1 (17). However, persons with a past HSV-1 infection who are subsequently infected with HSV-2 may have a less severe clinical course (17).
4. Do not use the OD ratio of a single serum specimen to determine recent infection. Collect paired samples (acute and convalescent) and test concurrently to demonstrate seroconversion.
5. Interpret test results for demonstration of seroconversion in conjunction with the clinical evaluation and the results of other diagnostic procedures.
6. Specimens containing antibodies to nuclear antigens (as are found in patients with systemic lupus erythematosus) may give false positive results in the ZEUS ELISA HSV-1 and HSV-2 Test Systems.
7. Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, collect a second sample after two to seven weeks and test concurrently with the original specimen to look for seroconversion.
8. Interpret a positive HSV IgG test in neonates with caution since passively acquired maternal antibody can persist for up to six months. A negative test for IgG antibody in the neonate may help exclude congenital infection (18). The most definitive diagnosis of active HSV infection requires viral isolation.
9. The ZEUS ELISA HSV-1 and HSV-2 Test Systems are not intended to be used for diagnosis of current infection in pregnant women. Determine current infection in pregnant women using viral isolation (4).
10. The results of this test are qualitative. Consider the result as either positive or negative for the presence of IgG antibodies to HSV. This test can only detect seroconversion (acute serum negative, convalescent serum positive). Criteria for a significant rise in titer have not been established.

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