

INTENDED USE

The ZEUS IFA Herpes Simplex Virus-2 (HSV-2) IgM Test System is an indirect fluorescent antibody (IFA) assay designed for the qualitative and semi-quantitative detection of IgM antibodies to Herpes Virus Homnis antibody in human serum.

SIGNIFICANCE AND BACKGROUND

Herpes Simplex Virus is a common pathogen of humans. The clinical course of HSV in humans is extremely variable. Primary infection with HSV, either type 1 or 2, is unapparent or subclinical in a majority of cases (1, 2). When clinically apparent, HSV infections can range from minimal stomatitis to a fatal generalized infection.

Some of the major clinical manifestations of HSV infections are acute gingivostomatitis, recurrent herpes labialis, keratoconjunctivitis, eczema herpeticum, encephalitis, and meningitis. Generalized infections are normally confined to individuals with immune deficiency, immunosuppressed patients, or newborns (3 - 6).

There are two distinct types of HSV: Types 1 and 2 (6). HSV-1 and HSV-2 are closely related but they can be separated both serologically and biologically (7). HSV-1 is associated with lesions above the waist (encephalitis, stomatitis, eye infections, and, in some cases, of generalized infections). HSV-2 is primarily an infection of the genitalia and surrounding area. HSV-2 is sexually transmitted and is the cause of a large majority of generalized infections of the newborn. However, a strict relationship of localization and types of HSV is not absolute.

There are a number of serological tests for the detection of HSV antibodies. The IFA test for the detection of HSV-1 and HSV-2 antibodies in human serum is a valid procedure for screening patients for HSV-1 and HSV-2 antibodies (8, 9, and 10). A very distinct advantage of the IFA technique is the ability to determine the specific antibody class involved in the positive reaction (IgG or IgM).

The ZEUS IFA HSV-2 IgM Test System uses a substrate of human fibroblast cells infected with Herpes Simplex Virus type 2. These infected substrates are fixed and, do not contain live virus. Herpes viruses have not been re-isolated from fixed HSV-2 substrates. These substrate slides should, as with all other viral reagents employed in any laboratory, be considered as potentially hazardous material.

The ZEUS IFA HSV-2 IgM Test System is useful in determining:

1. Recent infection with HSV by demonstrating the presence of immunoglobulin M antibody directed against HSV-2.
2. Undiagnosed congenital diseases and etiology of prolonged fever in immunosuppressed patients.

PRINCIPLE OF THE ASSAY

The ZEUS IFA HSV-2 IgM Test System is designed to detect circulating HSV-2 IgM antibodies in human sera. The assay employs HSV-1 infected substrate cells and goat FITC labeled antihuman IgM. The test procedure involves three incubation steps:

1. Test sera are first treated to remove IgG, and rheumatoid factor. (See Limitations of the Assay, #2).
2. Test sera are added to the wells, and incubated. Antigen specific IgM will bind to HSV antigen immobilized on the slide. The slides are washed to remove unbound antibody and other serum components.
3. Fluorescein labeled anti-human IgM conjugate is added to the wells and the slides are incubated. The Conjugate will react with the antigen specific IgM antibodies bound to the slides in step 2. The slides are washed to remove unbound Conjugate. The slides are then mounted with a coverslip and read under a fluorescence microscope.

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: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVE Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •	1. HSV-2 Antigen Substrate Slides: Ten, 10-well substrate slides containing HSV-2 (G strain) infected cells in each well. Also includes absorbent blotter and desiccant pouch.
CONJ	2. Conjugate: Anti-human IgM (μ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL +	3. Positive Control (Human Serum): Will produce positive apple-green staining of the HSV infected cells. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL -	4. Negative Control (Human Serum): Will produce no detectable staining of the HSV infected cells. One, 0.5mL, green-capped, vial. Ready to use.
DIL SPE	5. SAVE Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. NOTE: The SAVE Diluent® will change color when combined with serum.
BUF PBS	6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED	7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVE Diluent® (Product #: FA005CC), Mounting Media (Product #: FA009S), and PBS (Product #: 0008S).
2. Test System also contains a Component Label containing lot specific information inside the Test System box.

PRECAUTIONS

1. This Test System is for Research Use Only.
2. For *In Vitro* diagnostic use.
3. 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.
4. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
5. The Controls are **potentially bio-hazardous 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 Bio-safety 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 (20).

6. 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 their original containers immediately and follow storage requirements.
7. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
8. The SAvE Diluent®, Conjugate, and Controls 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. This preservative may be toxic if ingested.
9. Dilution or adulteration of these reagents may generate erroneous results.
10. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
11. Avoid microbial contamination of reagents. Incorrect results may occur.
12. Cross contamination of reagents and/or samples could cause erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
14. Avoid splashing or generation of aerosols.
15. Do not expose reagents to strong light during storage or incubation.
16. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
17. 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.
18. 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.
19. Do not apply pressure to slide envelope. This may damage the substrate.
20. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
21. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
22. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
23. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).
12. Incubator: 35 - 37°C.
13. IgG Removal System (see Limitations of Assay).

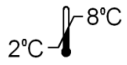
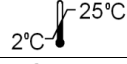
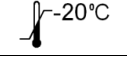
The following filter systems, or their equivalent, have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

Transmitted Light			
Light Source: Mercury Vapor 200W or 50W			
Excitation Filter	Barrier Filter	Red Suppression Filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten – Halogen 100W			
KP490	K510 or K530	BG38	
Incident Light			
Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	Dichroic Mirror	Barrier Filter	Red Suppression Filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten – Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (11, 12). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. 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 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 (19).

STORAGE CONDITIONS

	Mounting Media, Conjugate, SAVe Diluent®, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.
	Slides

ASSAY PROCEDURE

- Remove Slides from frozen storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
- Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted.**
- Diluting Patient Sera:
 - It is recommended that test sera are pre-treated to remove IgG. Precipitation with anti-human IgG is recommended because this procedure is effective in removing all subclasses of human IgG and is less cumbersome to perform than other methods. After the pretreatment step, test sera should be at a 1:10 screening dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS).
 - If patient samples are to be titrated to endpoint, one should pre-treat the serum to remove IgG and then make any subsequent dilutions with SAVe Diluent® or PBS. **NOTE: The SAVe Diluent® will undergo a color change confirming the combination of specimen with Diluent.**
 - As an option, users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
- With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
- Incubate Slides at 35 - 37°C for 60 ± 5 minutes.
- Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
- Wash Slides for two, 5 minute intervals, changing PBS between washes.
- Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe. **CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. Do not allow the Slides to dry during the test procedure.**
- Add 20µL of Conjugate to each well. Incubate Slides at 35 - 37°C for 30 ± 5 minutes.
- Repeat steps 6 through 8.
- Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope. **NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.**

QUALITY CONTROL

- Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
- It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid, see Figure 1.
 - Negative Control - characterized by the absence of nuclear staining and a red, or dull green, background staining of all the cells due to Evans Blue. Use the reaction of the negative control serum as a guide for the interpretation of patient results.
 - Positive Control - characterized by a 2+ to 3+ apple-green fluorescent staining intensity, forming plaques of the nucleus and/or cytoplasm of the cells. Five (5) to 15% staining of the total cell population represents a positive reaction.
- Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. **NOTE: The intensity of the observed fluorescence may vary with the microscope and filter system used.**

INTERPRETATION OF RESULTS

Titer	Clinical Significance
Less than 1:10	Negative: No detectable IgM antibody. This indicates no primary, reactivated infection, or re-infection. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken within 7 days to look for the presence of specific IgM. If the second specimen is positive, a primary, reactivated infection, or reinfection is indicated.
Greater than 1:10	Positive: Detectable IgM antibody. This indicates a primary, reactivated infection, or re-infection. Such individuals are presumed to be at risk of transmitting infection.

LIMITATIONS OF THE ASSAY

- IgG antibodies, if present in the sample, may interfere with determination of IgM titers to the organism. High affinity IgG antibodies may preferentially bind to antigenic determinants leading to false negative IgM titers (13). Also, IgM rheumatoid factor may bind to the antigen specific IgG leading to false positive IgM titers. Both of these problems can be eliminated by removing IgG from the samples before testing for IgM. Several different methods of separating IgG have been used. These include gel filtration, absorption with protein A (14), ion exchange chromatography (15), precipitation of IgG with anti-human IgG serum (16), or the use of **Zorba®**-IgG Removal Reagent (ZEUS Product #: FA003G).
- HSV-1 and HSV-2 share many cross-reacting antigens; therefore, to fully evaluate the IgM antibody status to HSV, both ZEUS IFA HSV-1 IgM and ZEUS IFA HSV-2 IgM Test Systems should be run simultaneously on each sample.
- A negative result does not rule out a primary or reactivated infection with HSV-1 or HSV-2 because samples may have been obtained too early in the course of infection, or IgM titers may have declined below detectable levels.
- Heterotypic IgM antibody responses may occur in patients infected with Epstein-Barr virus and give false positive results in the ZEUS IFA HSV-1 IgM and ZEUS IFA HSV-2 IgM Test Systems.
- HSV specific IgM antibody may reappear during reactivation of HSV infection (7, 17, and 18).
- Results of the ZEUS HSV-1 IgM and HSV-2 IgM IFA Test Systems are not by themselves diagnostic and should be interpreted in light of the patient's clinical condition and the results of other diagnostic procedures.
- In immunocompromised patients, the ability to produce an IgM response may be impaired and HSV specific IgM may be falsely negative during an active infection.

EXPECTED RESULTS

The incidence of HSV infection varies with age, geographic location, sexual behavior and socioeconomic status. IgM antibodies to HSV-1 or HSV-2 appear following both primary and reactivated infections with HSV. IgM antibody to HSV may persist for up to 9 months following a primary infection in some patients (7).

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ZEUS IFA HSV-2 IgM Test System

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