HSV-1 IgG Test System

IVD

REF FA9051G

Rx Only

INTENDED USE

The ZEUS IFA Herpes Simplex Virus-1 (HSV-1) IgG Test System is designed for the qualitative and semi-quantitative detection of Herpes Virus Hominis Antibody in human serum by the indirect fluorescent (IFA) technique and is for *In Vitro* diagnostic use.

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, kerato-conjunctivitis, eczema herpeticum, encephalitis, and meningitis. Generalized infections are normally confined to individuals with immune deficiency, immuno-suppressed 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-1 IgG Test System uses a substrate of human fibroblast cells infected with HSV-1. These infected substrates are fixed and exposed to ultraviolet radiation. The fixed substrates do not contain live virus. Herpes viruses have not been re-isolated from fixed HSV-1 substrates. These substrate slides should, as with all other viral reagents employed in any laboratory, be considered as potentially hazardous material. ZEUS IFA HSV-1 IgG and ZEUS IFA HSV-2 IgG Test Systems are available as separate products.

The ZEUS IFA HSV-1 IgG Test System is useful in determining:

1. The history of a previous infection with HSV.
2. Whether or not previous infections have occurred and therefore help determine the patient's relative susceptibility to a primary infection.
3. Undiagnosed congenital diseases and etiology of prolonged fever in immunosuppressed patients.
4. Recent infection with HSV by demonstrating a four-fold rise or fall in antibody titer or by demonstrating the presence of IgM antibody directed against HSV-1.

PRINCIPLE OF THE ASSAY

The ZEUS IFA HSV-1 IgG Test System is designed to detect circulating HSV-1 antibodies in human sera. The assay employs HSV-1 infected substrate cells and goat FITC-labeled anti-human IgG adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. Step one is the interaction of HSV antibodies in patient's sera with the HSV infected substrate cells.
2. Step two is the interaction of FITC-labeled anti-human IgG with the HSV antibodies attached to the HSV localized in the nucleus and/or cytoplasm of the infected cells.

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-1 Antigen Substrate Slides: Ten, 10-well Slides containing HSV-1 (MacIntyre strain) infected cells in each well. Also includes absorbent blotter and desiccant pouch.
CONJ		2. Conjugate: Goat anti-human IgG (γ 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 (plaques). 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 (plaques). 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 #: FA0009S), and PBS (Product #: 0008S).**
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 Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. 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).

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 their original containers immediately and follow storage requirements.
6. 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.
7. 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 by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. 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.
17. 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.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. 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.
20. 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.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. 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).

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 (14, 15). 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 (17).

STORAGE CONDITIONS

2°C – 8°C	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
2°C – 25°C	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

1. Remove Slides from refrigerated 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.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted**. Prepare a 1:10 dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS) of each patient serum. **The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the Diluent.** Patients should be screened at 1:10 and 1:100. The 1:100 dilution can be prepared by further diluting the 1:10 dilution (1:10) using PBS.

   Dilution Options:
   a. As an option, users may prepare initial sample dilutions using PBS, or Zorba-NS (Zorba-NS is available separately. Order Product Number FA025 – 2, 30mL bottles).
   b. 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.
   c. When titrating patient specimens, initial dilutions should be prepared in SAVe Diluent®, PBS, or Zorba-NS and all subsequent dilutions should be prepared in SAVe Diluent® or PBS only. Titrations should not be prepared in Zorba-NS.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash slides for two, 5 minute intervals, changing PBS between washes.
7. 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**.
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. 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

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. 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.
   a. Negative Control - characterized by the absence of nuclear staining and a red, or dull green, background staining of all cells due to Evans Blue. Use the reaction of the Negative Control serum as a guide for interpretation of patient results.
   b. Positive Control - characterized by a 3+ to 4+ 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.
3. 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

1. 1+ to 4+ apple-green fluorescence in the nucleus and/or cytoplasm of the infected substrate cells represents a positive reaction.
2. All positive test sera should be titered to endpoint. This is accomplished by preparing serial two-fold dilutions of the test sera in PBS (i.e., 1:20, 1:40, 1:80, etc.). The endpoint is the last dilution that produces a 1+ positive apple-green staining. Do not prepare serial dilutions for endpoint titers in **Zorba-NS**.
3. The absence of staining in the infected cells represents a negative reaction.

NOTE: The number of infected cells seen in the positive control test wells should closely approximate the number of infected cells seen in positive patient test wells. The number of uninfected cells in each well serves as a built-in Negative Control, should all the cells in patient test wells fluoresce apple-green in the nucleus and/or cytoplasm, consider an autoimmune staining related to anti-nuclear, anti-mitochondrial, or other autoantibody. It should also be noted at low titers (1:10 - 1:40), staining in the cytoplasm of a cell may be related to HLA or blood group antigen antibody reactions.

Serum Titer	Interpretation
Less than 1:10	Negative for antibodies to HSV-1 dependent on substrate used. (Caution: In patients having an early acute encounter with HSV, detectable antibody levels may not have been reached. A second specimen 14 - 21 days later should be requested and retested).
1:10 to 1:100	Positive for antibodies to HSV. This does not confirm an immune status, but does indicate a prior exposure or infection with HSV. (See Limitations of the Assay).
1:1000 or Greater	Suggestive of recent infection with HSV. **NOTE: If further verification is needed an IgM test should be performed.** IgM specific HSV antibodies occur with primary infections, reach a peak in 6 - 8 weeks, and decline rapidly. A second specimen may be drawn 2 - 3 weeks after the first specimen. Perform tests on both specimens simultaneously to determine if a four-fold rise or fall in titer has occurred. This would be diagnostic of a recent infection.

LIMITATIONS OF THE ASSAY

1. HSV types 1 and 2 share common antigens (10, 11, and 12); therefore, the detection of antibody to HSV-1 may not be diagnostic for HSV-1 infection unless no antibody titers are found for HSV-2.
2. A significant rise in antibody titer does not always accompany recurrent infections, reactivated infections, or infections with HSV-2. In addition, significant rises in HSV antibody titers may be caused by Varicella Zoster virus (chicken pox). Patients infected with VZV who have had past HSV infections may show a rise in antibody titers to HSV-1 and HSV-2 antigens (5).

3. Most individuals in epidemiological population studies have been infected by HSV by the time they are twenty years old (2). Evidence of detectable antibody in patient's sera to either type of HSV is generally not very useful with the exception of demonstrating immunoglobulin M (IgM) antibodies or a four-fold rise or fall in titer in acute and convalescent sera. In comparing acute and convalescent sera, antibodies reach their peak titer 4 - 6 weeks following initial infection. These titers may then, in time, decline and usually persist at stable levels for the life of the individual. IgM response occurs in primary infections with HSV and persists for eight weeks after onset.
4. In patients with persisting antibodies to HSV, reactivation or re-infection with the same or a different type of HSV does not, as a rule, produce significant antibody rises.
5. The presence of HSV-1 and HSV-2 antibody levels in patient's sera may or may not confer immunity. The presence of HSV antibody titers of one type may have a protective effect in reducing the severity of infection with the second type of HSV. Re-infection reactivation of HSV occurs even in the presence of high serum antibody titers.
6. A single serological antibody titer to either of the HSV types should not be used as the only criteria for diagnosis. The patient's clinical data and laboratory test should be carefully reviewed by a medical authority before making a diagnosis.

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