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HSV-1 or HSV-2 Test System

Catalog No. 381010-G or 391010-G

PLEASE READ THIS MATERIAL BEFORE USING THE KIT

INTENDED USE

The Diagnostic Automation HSV-1 or HSV-2 antibody test system is designed for the qualitative and semi-quantitative detection of Herpes Virus Homoinis Antibody in human serum by the indirect fluorescent (IFA) technique and is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Herpes Simplex Virus (HSV) 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 (8,9,10). 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). IgM conjugate is available upon request from Diagnostic Automation (Product No: 0003-1MB).

The Diagnostic Automation indirect immunofluorescent test uses a substrate of human fibroblast cells infected with Herpes Simplex Virus type 1 or type 2. 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 and HSV-2 substrates. These substrate slides should, as with all other viral reagents employed in any laboratory, be considered as potentially hazardous material. HSV-1 and HSV-2 substrates are available as separate kits.

The Herpes Simplex Virus indirect fluorescent antibody test is useful in determining:

1. The history of a previous infection with Herpes Simplex Virus.
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 Herpes Simplex Virus by demonstrating a four-fold rise or fall in antibody titer or by demonstrating the presence of immunoglobulin M antibody directed against HSV-1 or HSV-2.

PRINCIPLE OF THE ASSAY

The Diagnostic Automation fluorescent HSV antibody test system is designed to detect circulating HSV-1 or HSV-2 antibodies in human sera. The system employs HSV-1 or HSV-2 infected substrate cells and goat FITC-labeled anti-human immunoglobulin adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps:

1. The first step is the interaction of HSV antibodies in patient's sera with the HSV infected substrate cells.
2. The second is the interaction of FITC-labeled anti-human immunoglobulin with the HSV antibodies attached to the HSV localized in the nucleus and/or cytoplasm of the infected cells.

KIT COMPONENTS

Reactive Reagents:

- HSV-1 or HSV-2 Antigen Slides: Ten, 10-well substrate slides containing infected cells in each well. (Product No:9052-10, HSV-1) or (Product No:9102-10, HSV-2).
- Goat anti-human immunoglobulin labeled with FITC: Contains 1.25% Bovine Albumin and counterstain. Two, 1.5mL vials, lyophilized. (Product No:9053*, HSV-1) or (Product No:9103*, HSV-2).
- HSV Human Positive Control Serum: Two, 0.5mL vials, lyophilized. Composed of human sera. (Product No:9054*, HSV-1) or (Product No:9104*, HSV-2).
- HSV Human Negative Control Serum: Two, 0.5mL vials, lyophilized. Composed of human sera. (Product No:9055*, HSV-1) or (Product No:9105*, HSV-2).
- **ZORBA-NS®** sample diluent formulated to reduce non-specific staining. (Product No:Z025, 25mL) or (Product No:Z125, 125mL). Contains 0.1% sodium azide as a preservative.

Non-reactive Material:

- Phosphate-buffered-saline (PBS): Sufficient to prepare 4 liters. (Product No:0008)
- Mounting Fluid (Buffered Glycerol): 3.0mL (Product No:0009*)

NOTE: These reactive reagents contain a preservative: thimerosal, mercury derivative 0.04%.

PRECAUTIONS

- For *in vitro* diagnostic use.
- The thimerosal and sodium azide preservative may be toxic if ingested.
- **ZORBA-NS** contains sodium azide as a preservative. 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.
- Remove only the amount of **ZORBA-NS** needed to perform each test run to reduce the possibility of product contamination.
- Use **ZORBA-NS** for screening dilutions only. **DO NOT PREPARE SERIAL DILUTIONS FOR ENDPOINT TITERS IN ZORBA-NS.**
- **ZORBA-NS** should be used only as a diluent for patient specimens.
 - a. Do Not use **ZORBA-NS** to reconstitute the controls or conjugate.
 - b. Do Not use **ZORBA-NS** in any of the wash steps.
- The volume of **ZORBA-NS** supplied has been calculated to provide sufficient material for all the individual test wells included in this kit when used according to the instructions herein. The use of larger volumes for sample preparation will result in insufficient **ZORBA-NS** to allow each test well to be utilized.
- Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, 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 Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (16).

- Dilution or adulteration of these reagents may result in loss of sensitivity.
- Do not substitute reagents from kits with different lot numbers or from other manufacturers.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Small test tubes, 13 x 100 mm or comparable.
3. Test tube racks.
4. Staining dish - A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slips: 24 x 60mm, thickness No. 1.
6. Distilled water.
7. Properly equipped fluorescent microscope assembly.

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

10. Plummer G:A review of the Identification and Titration of Antibodies to Herpes Simplex Virus types 1 and 2 in Human Sera. Cancer Res. 33:1469-1476, 1973
11. Nahmias AJ, DelBuono I, Schneiss KE, Gordon DS, and Theis D:Type specific surface antigens of cells infected with Herpes Simplex Virus 1 and 2. Proc. Soc. Exp. Biol. Med. 138:21-27, 1971.
12. Geder L, and Skinner GRB:Differentiation between type 1 and type 2: two strains of Herpes Simplex Virus by an indirect immunofluorescent technique. J. Gen. Viral. 12:279-282, 1971.
13. Weller TH and Coons AH:Fluorescent antibody studies with agents of Varicella and Herpes Zoster propagated *in vitro*. Proc. Soc. Exp. Biol. Med. 86:789-794, 1954.
14. Procedures for the collection of diagnostic blood specimens by venipuncture - Second Edition: Approved Standard. Published by National Committee for Clinical Laboratory Standards, 1984.
15. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.
16. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens, Final Rule. Fed. Register 56:64175-64182, 1991.

NOTE:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed.
2. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity.

PROCEDURE**Preparation of Reagents:**

1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. HSV human positive and negative control sera. Reconstitute with 0.5mL of distilled water. Represents a 1:10 screening dilution. Use as reconstituted. Do not dilute.
3. Goat anti-human immunoglobulin FITC-labeled conjugate. Reconstitute with 1.5mL of distilled water. Alternately, aliquot in 0.5mL amounts and store at -20°C or lower in small tubes.

NOTE: Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.

Note: The controls are intended to be used undiluted. As an option, users may titrate the positive control(s) to endpoint. In such cases, the control(s) should be diluted two-fold in PBS. When evaluated by Diagnostic Automation an endpoint dilution is established and printed on the positive control vial (\pm one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own mean titer for each lot of controls.

Test Procedure:

1. Remove substrate slides from freezer and allow them to warm to room temperature (20-25°C). Tear open the protective envelope and remove slides containing the HSV-1 or HSV-2 infected cells. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
2. Prepare 1:10 (for example: 20µL of sample plus 180µL of PBS), and 1:100 screening dilutions of test sera in PBS. (Alternatively, you may prepare 1:10 and 1:100 screening dilutions in **ZORBA-NS**). Positive, negative, and buffer controls should be run each time the test is performed.
3. Identify each well with the appropriate patient sera and controls.
4. Spread 20µL of test and control sera over each appropriately labeled well. Be careful not to disturb the substrate cells with pipette tip.
5. Incubate slides in a sealed moist chamber at room temperature for 30 minutes. **DO NOT ALLOW WELLS TO DRY.**
6. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. **DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS.**
7. Place slides in a staining dish and wash in PBS for two, 5 minute intervals with a change of PBS.
8. Remove slides from PBS and blot dry with blotters provided. It is suggested that blotters be placed on a flat surface. Place substrate slide in an inverted position over the blotter. Press firmly on back of slide. **DO NOT ALLOW SUBSTRATE WELLS TO DRY.**
9. Place slides in a moist chamber and add 20µl of conjugate to each well.
10. Incubate slides for 30 minutes at room temperature. **DO NOT ALLOW SLIDES TO DRY.**
11. Repeat steps 6, 7, and 8.
12. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Slides should be examined immediately at a total magnification of 250X.

QUALITY CONTROL

1. A positive and negative control, and a buffer should be run with each assay.
2. It is recommended that the positive and negative controls be read prior to evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described, test results are invalid.
3. The negative control is characterized by the absence of nuclear staining and a red background staining of all the cells due to Evans Blue. The reactions of the negative control may be used as a guide for interpreting patient samples.
4. The positive control will exhibit a 3+ to 4+ apple-green fluorescent staining intensity, forming plaques of the nucleus and/or cytoplasm of the cells. 5-15% staining of the total cell population represents a positive reaction.
5. The intensity of the observed fluorescence may vary with the microscope and filter used.
6. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

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, an autoimmune staining related to anti-nuclear, anti-mitochondrial, or other autoantibody should be considered. 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 Herpes Simplex Virus Type 1 or Type 2 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 to 21 days later should be requested and retested).

1:10 To 1:100:

Positive for antibodies to Herpes Simplex Virus. This does not confirm an immune status, but does indicate a prior exposure or infection with HSV. (See Limitation of Test section).

1:1000 or GREATER:

Suggestive of recent infection with Herpes Simplex Virus. **NOTE:** If further verification is needed an immunoglobulin M (IgM) test should be performed. IgM specific Herpes Simplex Virus 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. Substitution of other reagents for components of this kit is to be avoided. Since the components of this kit have been tested for maximum efficiency, Diagnostic Automation is not responsible for test performance if reagent substitution occurs.
2. Herpes Simplex Viruses types 1 and 2 share common antigens (10, 11,12); therefore, the detection of antibody to Herpes Simplex type 1 may not be diagnostic for Herpes Simplex type 1 infection unless no antibody titers are found for Herpes Simplex type 2.
3. A significant rise in antibody titer does not always accompany recurrent infections, reactivated infections, or infections with type 2 Herpes Simplex Virus. In addition, significant rises in Herpes Simplex Virus antibody titers may be caused by Varicella Zoster virus (chicken pox). Patients infected with Varicella virus who have had past Herpes Simplex Virus infections may show a rise in antibody titers to Herpes Simplex Virus types 1 and 2 antigens (5).
4. Most individuals in epidemiological population studies have been infected by Herpes Simplex Virus by the time they are twenty years old (2). Evidence of detectable antibody in patient's sera to either type of Herpes Simplex 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 to 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 Herpes Simplex Virus and persists for eight weeks after onset.

In patients with persisting antibodies to Herpes Simplex Virus, reactivation or reinfection with the same or a different type of Herpes Simplex Virus does not, as a rule, produce significant antibody rises.

5. The presence of Herpes Simplex Virus type 1 and 2 antibody levels in patients sera may or may not confer immunity. The presence of Herpes Simplex Virus antibody titers of one type may have a protective effect in reducing the severity of infection with the second type of Herpes Simplex Virus. Reinfection reactivation of Herpes Simplex Virus occurs even in the presence of high serum antibody titers.
6. A single serological antibody titer to either of the Herpes Simplex types should not be used as the only criteria for diagnosis. The patients clinical data and laboratory test should be carefully reviewed by a medical authority before making a diagnosis.

PERFORMANCE CHARACTERISTICS

The performance of the Diagnostic Automation HSV-1 or HSV-2 IFA test system with **ZORBA-NS** as sample diluent was compared to the same test system using PBS as the sample diluent (standard methodology).

Seventy-four samples from normal plasma donor population were tested as described above. Forty-nine (49) samples were positive for antibody to HSV-1 when screened at 1:20 in PBS. The same samples were positive when screened in **ZORBA-NS**. Ten samples with endpoint titers ranging from 1:10 to 1:2560 in PBS were also titered in **ZORBA-NS**. Identical endpoint titers were obtained in each diluent.

Twenty-five samples were negative for antibody to HSV-1 when screened in PBS. Twenty-four (24) of 25 samples were also negative when diluted in **ZORBA-NS**. The one discrepant sample was 1+ reactive in **ZORBA-NS**; the reaction in PBS was borderline and partially obscured by nonspecific fluorescence.

When tested for antibodies to HSV-2, 67 samples were positive for antibody to HSV-2 when screened at 1:10 in PBS. The same samples were positive when screened in **ZORBA-NS**. Twelve samples with endpoint titers ranging from 1:10 to 1:2560 in PBS were also titered in **ZORBA-NS**. Nine (9) of 12 samples exhibited identical endpoints. The remaining 3 samples were within \pm one, two-fold dilution.

Seven samples were negative for antibody to HSV-2 when screened in PBS. The same samples were negative when diluted in **ZORBA-NS**.

REFERENCES

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2. Scott TFM: Epidemiology of Herpetic Infections. *Am. J. Ophthal.* 43:134-147, 1957.
3. Nehmias AJ, Alford CA Jr., and Korones SB: Infections in Newborns with Herpes Virus Hominis. *Advan. Pediat.* 17:183-226, 1970.
4. Whilley RJ, Chien LT, and Alford CA Jr.: Neonatal Herpes Simplex Infection. *Int. Ophthal. Clin.* 14:141-149, 1975.
5. Rauls WE: Chap. 11, Viral, Rickettsial and Chlamydial Infections. Ed by Lennette EH, Schmidt NJ, 5th Ed., 1979.
6. Rauls WE: Herpes Simplex Virus in the Herpes Viruses. Kaplan AS (ed). Academic Press, New York, pp 291-325, 1973.
7. Nahmias AJ, and Roizman B: Infection with Herpes Simplex Virus 1 and 2. *N. Eng. J. Med.* 289:667-679, 725-729, 781-789, 1973.
8. Rejcani J, Revingerova E, Kocishova D, and Syanto J: Screening of antibodies to Herpes Simplex Virus in human sera by indirect immunofluorescence. *Acta. Viral.* 17:61-68, 1973.
9. Frazer CEO, Melendez LV, and Simeone T: Specificity differentiation of Herpes Simplex Virus types 1 and 2 by indirect immunofluorescence. *J. Infect. Dis.* 130:63-66, 1974.

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (14,15). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° 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.

STORAGE CONDITIONS

- HSV Substrate Slides: -20°C or lower.
- Goat anti-human immunoglobulin labeled with FITC: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Positive and negative human HSV control sera: 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- **ZORBA-NS**: Store at 2-8°C.
- Phosphate-buffered-saline: Store at 2-25°C. Store reconstituted buffer at 2-8°C.
- Buffered glycerol (mounting media): Store at 2-8°C.



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