



HSV 1 +2 IgG EIA kit

(For in vitro diagnostic use only)

Enzyme-linked immunosorbent assay for the detection of IgG antibody to HSV 1 and 2

INTENDED USE:

Atlas HSV 1+2 IgG kit is intended for use in the detection of IgG antibodies to Herpes Simplex Virus, Type 1 and Type 2 infection.

SUMMARY AND PRINCIPLE OF THE TEST

The heightened awareness of herpes simplex viruses (HSV) by both medical professionals and the public is due to many factors. Five factors seem to be primarily important. First, there is the current epidemic of sexually transmitted herpes infection. Second, there is associated with the increase in sexually transmitted herpes infection a rise in neonatal HSV infections. Third, there has been recent detection of HSV in patients following organ transplantation. Fourth, there has been reported detection of HSV in immunodeficient patients. Finally, as a defensive measure against the future spread of HSV infections, there has been development of antiviral therapy specific to HSV. In all, these factors have contributed to a focus of attention on the herpes simplex virus.

Although the clinical description of herpes labialis occurred during the time of Hippocrates, herpes genitalis was not described until 1736, in France by Astnuc, the King's physician. In modern times, the causative agent has been shown to belong to two closely related yet distinct types. HSV 1 and HSV 2, which differ in their clinical and epidemiological patterns. Both types are characteristically rapidly growing, cytolytic viruses which lie dormant in neural ganglion cells until reactivated. Herpes simplex virus is a member of the herpesvirus group which includes Varicella-zoster, cytomegalovirus and Epstein-Barr virus. Replication of the virus occurs within the cell nucleus and is complete upon lysis of the cell. Distinguishing the members of the herpesvirus group can be accomplished by antigenic analysis and definition of biological properties. In recent times, the subdivision of HSV into specific types has become possible,

HSV 2 is transmitted primarily by way of sexual transmission. HSV 2 infection can affect the oral, genital, perianal and anal regions and is associated with fever, malaise, and anorexia. Lesions from recurrent HSV 2 infections are generally less severe and less involved than primary or first time infections. The most severe complication of genital HSV infection is neonatal infection with fetal wastage, birth defects, or even death.

Purified HSV 1 and 2 antigen is coated on the surface of microwells. Patient serum is added to wells, and the HSV1 and 2 IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and substrate and chromogen are added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

1. 8X 12 well microtiter strip: 1 plate, coated with recombinant HSV 1 and 2-antigen.
2. Negative Control: 1 vial, 0.5 ml
3. Positive Control: 1 vial, 0.5 ml
4. Sample Diluent: Buffer contain detergent 12ml.

5. Enzyme Conjugate: HRP-conjugated-anti IgG 12 ml.
6. Wash Buffer : PBS, Tween. The buffer should be diluted with distilled water 1:20 before use 40 ml.
7. Substrate Solution A: urea peroxide 6ml.
8. Substrate Solution B: TMB 6ml.
9. Stop Solution: 2N Sulfuric Acid 6ml.

Materials required but not provided:

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450 nm wavelength
8. Timer

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated Freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 100 ul of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control and add 100ul Sample Diluent and 100ul Sample Diluent and 5ul of serum or plasma samples into respective wells. (After the addition of samples, the color will become blue.)
3. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
4. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
5. Add 100 ul of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
6. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
7. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
8. Add 50 ul of Substrate Solution A (HRP substrate) to each well, then add 50 ul of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.

9. Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Cut Off : 0.10+ Average OD value of Negative Control

Positive: OD value is equal to or greater than the Cut Off value

Negative: OD value is less than the Cut Off value

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE ASSAY

1. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting along with careful washing and timing of the incubation steps are essential for accurate results.
2. This kit is designed to measure IgG antibody in patient samples. Positive results in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
3. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended that an IgM assay be performed, or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
4. The presence of IgG antibody against HSV1 or HSV 2 does not imply protection from future HSV infection.
5. HSV Types 1 and 2 share common antigens, therefore, infection with one type of HSV when there was previous infection with the other type may produce an anamnestic response of the pre-existing antibody. This may cause a more elevated ISR to the pre-existing type than to the more recently infecting type. This test, therefore, is unable to indicate the type of infecting virus.
6. The results of a single specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
7. Lack of significant rise in antibody level does not exclude the possibility of HSV infection.
8. This test will not indicate the site of infection. It is not intended to replace viral isolation.
9. This test is not intended for the diagnosis of current HSV infection in pregnant women. Current infection should be determined by viral isolation.

RELATED READING MATERIALS

1. Becker T.M., J.H. Blount, and M.F. Guinan. 1985. Genital Herpes Infections in Private Practice in the United States. 1966-1981. *JAMA* . 253: 1601-1603.
2. Sullivan-Bolyai, J., J.E. Hull, C. Wilson, and L. Corey. 1983. Neonatal Herpes Simplex Virus Infection in King County, Washington: Increasing incidence and Epidemiologic Correlates. *JAMA* 250:3059-3062.
3. Pass- R.F., R.J. Whitley, J.D. Whelchel, A.G. Diethelm. D.W. Reynolds and C.A. Alford. 1979. Identification of Patients with Increased Risk of Infection with Herpes Simplex Virus after Renal Transplantation. *J. Infect . Dis.* 140: 487-492.
4. Naraq. S., G.G. Jackson, O. Jonasson, and H.M. Yamashiroya. 1977. Prospective Study of Prevalence, Incidence and Source of Herpesvirus Infections in Patients with Renal Allografts. *J. Infect Dis.* 136:531-540.

5. Meyer, J.D., N. Fluornoy, and E.D. Thomas. 1980. Infection with Herpes Simplex Virus and Cell Mediated Immunity after Bone Marrow Transplant. *J. Infect Dis.* 142: 338-346.
6. Hirsh. M.S. and R.T. Schooley. 1983. Treatment of Herpesvirus Infections. *N. Engl. J. Med.* 309: 963-970.
7. Willy. P. 1973. Herpes: History and Classification. In: *The Herpesviruses*. A.S. Kaplan, ed. New York Academic Press. p. 1.
8. Hutfield, D.C. 1966. History of Herpes Genitalis. *British Journal of Venereal Disease.* 42: 263-268.
9. Gentry. G.A. and C.C. Randall. 1973. The Physical and Chemical Properties of the Herpesviruses. In: *The Herpesviruses*. A.S. Kaplan, ed. New York Academic Press p. 45.
10. Ojeda, V. 1980. Fatal Herpes Simplex Encephalitis with Demonstration of Virus in the Olfactory Pathway. *Psychology*, 12:429-437.

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