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HSV-1 IgG ELISA Kit

Name	HSV-1 IgG ELISA Kit
INTENDED USE	The HSV-1 IgG ELISA Kit is intended for the detection of IgG antibody to HSV-1 in human serum or plasma.
SUMMARY AND EXPLANATION	HSV-1 and 2 are virtually identical, sharing approximately 50% of their DNA and have over 80% of common antigens. Both types infect the body's mucosal surfaces, usually the mouth or genitals, and then establish latency in the nervous system. For both types, at least two-thirds of infected people have no symptoms, or symptoms too mild to notice. However, both types can recur and spread even when no symptoms are present. By the time they're teenagers or young adults, about 50% of Americans have HSV-1 antibodies in their blood. By the time they are over age 50, some 80-90% of Americans has HSV-1 antibodies. By comparison, almost all HSV-2 is encountered after childhood, when people become sexually active. HSV type 1 is the cause of most orofacial herpes and HSV encephalitis; type 2 is the primary cause of initial and recurrent genital herpes and neonatal HSV. Reactivation of latent HSV infection is a frequent complication of immunosuppression due to cancer, transplantation and AIDS. Asymptomatic genital shedding of HSV-2 is more common than HSV-1 and occurs more frequently during the first 3 months after acquisition of primary type 2 disease than during later periods. The presence of HSV IgG antibody is indicative of previous exposure A significant increases in HSV IgG is an indicative of reactivation, current or recent infection. IgM antibody is present after primary HSV infection.
PRINCIPLE OF THE TEST	Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and

the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS PROVIDED	96 Tests
Microwells coated with HSV-1 antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1ml
Positive Control: 1 vial (ready to use)	1ml
Negative Control: 1 vial (ready to use)	1ml
Enzyme conjugate: 1 bottle (ready to use)	12ml
TMB Substrate: 1 bottle (ready to use)	12ml
Stop Solution: 1 bottle (ready to use)	12ml
Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

Distilled or deionized water

Precision pipettes

Disposable pipette tips

ELISA reader capable of reading absorbance at 450nm

Absorbance paper or paper towel

Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8 C.

2. Keep microwells sealed in a dry bag with desiccants.

3. The reagents are stable until expiration of the kit.

4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

Potential biohazardous materials:

	The calibrator and controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984. This kit is designed for In Vitro Diagnostic Use. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
SPECIMEN COLLECTION AND HANDLING	1. Collect blood specimens and separate the serum. 2. Specimens may be refrigerated at 2–8 C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing.
REAGENT PREPARATION	Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25C).
ASSAY PROCEDURE	Bring all specimens and kit reagents to room temperature (20-25C) and gently mix. Place the desired number of coated strips into the holder. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 l of the sample to 200 l of sample diluent. Mix well.

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	Dispense 100 l of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100l sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature. Remove liquid from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l of enzyme conjugate to each well and incubate for 20 minutes at room temperature. Remove enzyme conjugate from all wells. Wash wells three times with 300 l of 1X wash buffer. Blot on absorbance paper or paper towel. Dispense 100 l of TMB substrate and incubate for 10 minutes at room temperature. Add 100 L of stop solution. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter to 600-650 nm.
CALCULATION OF RESULTS	Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value. Example of typical results: Calibrator mean OD = 0.8 Calibrator Factor (CF) = 0.5 Cut-off Value = 0.8 x 0.5= 0.400 Positive control O.D. = 1.2 Ab Index = 1.2 / 0.4 = 3 Patient sample O.D. = 1.6 Ab Index = 1.6 / 0.4 = 4.0
LIMITATIONS OF THE TEST	The test results obtained using this kit can not discriminate between HSV-1 and HSV-2 infection due to high cross

reactivity between the two viruses. The results serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

Lipemic or hemolyzed samples may cause erroneous results.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

The O.D. of the Calibrator should be greater than 0.250.

The Ab index for Negative control should be less than 0.9.

3. The Ab Index for Positive control should fall within the range

specified on the COA/label.

INTERPRETATION

The following is intended as a guide to interpretation of HSV-1 IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation

1.1 Detectable antibody to HSV-1 IgG by ELISA.

Converting of Ab Index to IU/mL

As an option, Ab index may be converted to IU/ml by multiplying Ab index by 100. IU/ml values may then be interpreted as follows:

110 IU/ml Detectable IgG antibody to HSV-1 by ELISA

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

336 patient sera were tested by this HSV-1 IgG ELISA and a reference ELISA method. 270 sera were positive and 56 were negative by both methods (97% agreement). The results are summarized below:

	HSV-1 IgG ELISA	HSV-1 IgG ELISA	HSV-1 IgG ELISA
	+		Total
Reference ELISA Kit +	270	4	274
_	6	56	62
Total	276	60	336

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Precision

Intra Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.23	0.06	4.87
2	16	0.66	0.04	6.10
3	16	0.33	0.02	6.06

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.77	0.15	8.47
2	10	0.93	0.09	9.47
3	10	0.21	0.02	14.2

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