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	See external label	2°C-8°C	Σ=96 tests	REF Cat # 9065-11

HSV 1 IgG
Chemiluminescence

Cat. 9065-11

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 =5 µl / 200 µl		
2	Diluted samples, controls & calibrator	100 µl	30 minutes
3	Washing buffer (3 times)	350 µl	
4	Enzyme conjugate	100 µl	30 minutes
5	Washing buffer (3 times)	350 µl	
6	Substrate A and Substrate B mixture	100 µl	5 minutes
7	Read with Luminometer in 5~30 minutes		

NAME AND INTENDED USE

HSV 1 IgG Chemiluminescence ELISA is intended for use in evaluating a patient's serologic status to herpes simples virus (HSV) infection, or for evaluating paired sera for the presence of a significant increase in herpes specific IgG. For investigational use only.

SUMMARY AND EXPLANATION OF THE TEST

Herpes Simplex Virus is a common pathogen and its primary infection is usually asymptomatic. There are two immunologically distinct types of HSV: Type 1 and Type 2. HSV 1 is generally associated with oral infection and lesions above the waist, and HSV 2 is associated with genital infections and lesions below the waist. Clinical cases primarily are 1) eczema herpeticum with eczematous skin changes with numerous lesions, 2) Gingivo-stomatitis and 3) Herpes sepsis, almost only found in newly born of premature infants. HSV IgG Chemiluminescence ELISA is an accurate serologic method to detect HSV specific antibody in serum sample.

PRINCIPLE OF THE TEST

Purified HSV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the HSV 1 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 A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell Strips: purified *HSV 1* antigen coated wells (12 x 8 wells)
2. Sample Diluent: Blue color solution. 1 vial (22 ml)
3. Washing Concentrate 10x: White Cap. 1 bottle (100 ml)
4. Enzyme Conjugate: Red Color Solution. 1 vial (12 ml)
5. Cut-off Calibrator: Yellow Cap. *HSV 1G* Index = 1.0 (150 µl/vial)
6. Negative Control: Range stated on label. Natural Cap. (150 µl/vial)
7. Positive Control: Range stated on label. Red Cap. (150 µl/vial)
8. Substrate A: H_2O_2 in buffer. Natural bottle. 1 vial (7 ml)
9. Substrate B: Luminol in buffer. Amber bottle. 1 vial (7 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{o}C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as 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

2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.

4. 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 ^{O}C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer:
   Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 ^{O}C)
   And gentle mix.

ASSAY PROCEDURE

1. Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control, and calibrators to 200 µl of sample diluent. Mix well.

2. Place the desired number of coated strips into the holder.

3. Dispense 100 µl of diluted sera, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

4. Remove liquid from all wells and repeat washing three times with washing buffer.

5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.

7. Mix equal volume of Substrate A & Substrate B, then dispense 100 µl of this mixture to each well.

8. Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

Determination of Index values

1. Calculate the mean of duplicate RLU values (B).

2. Calculate the HSV 1G Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C).

For example:

Sample	Well No	RLU (A)	Mean RLU (B)	INDEX B/C
Cut-off Calibrator	A1 B1	336269 331072	333671 (C)	1
Positive Control	C1 D1	661450 661557	661504	1.98
Negative Control	E1 F1	224 250	237	0
Patient Sample 1	G1 H1	399011 380409	389710	1.17
Patient Sample 2	A2 B2	596019 578875	587447	1.76

QUALITY CONTROL

1. In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.
2. The RLU values vary with the different luminometer used.

3. Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

Negative: HSV 1 IgG Index of 0.90 or less are seronegative for IgG antibody to HSV 1.

Equivocal: HSV 1 IgG Index of 0.91 - 0.99 is equivocal. Sample should be retested.

Positive: HSV 1 IgG Index of 1.00 or greater are seropositive.

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity:

A total of 66 patient samples were used to evaluate specificity and sensitivity of the test. HSV 1 IgG Chemiluminescence test results were compared to a commercial ELISA kit results.

		Reference ELISA			
		N	E	P	Total
HSV 1 IgG Chemiluminescence ELISA	N	20 (D)	0	0 (B)	20
	E	0	0	1	1
	P	0 (C)	0	45 (A)	45
	Total	20	0	46	66

Sensitivity = A / (A+B) = 45 / (45+0) =100%

Specificity =D / (C+D) = 20 / (0+20) =100%

Accuracy = (A+D) / (A+B+C+D)

= 45+20 / (45+0+0+20) = 65 / 65 = 100%

Expected value and prevalence

48 random samples were determined with HSV 1 IgG CLIA ELISA. 41 samples were found to be positive (85%) and 7 were found to be negative (15%). Another set of 49 random samples, the positivity were found to be 72%. Prevalence may vary depending on a variety of the factors such as geographical location, age, socioeconomic status, race, type of the test employed, specimen collection and handling procedures, clinical and epidemiological history.

Precision:

The precision of the assay was evaluated by testing three different sera of eight replicates over a period of one week.

The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	8.9%	7.5%	6.8%
Inter-assay	10.3%	8.3%	7.5%

Cross-reactivity:

A study was performed to determine the cross-reactivity of HSV 1 IgG CLIA test with positive IgG samples. The results indicated an absence of cross-reactivity of the test: H. pylori, Rubella, Toxo, CMV, Chlamydia trachomatis and EBV VCA.

LIMITATIONS OF THE PROCEDURE

1. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
2. Samples obtained too early during primary infection may not contain detectable antibody.
3. A single serum sample should not be used to aid in the diagnosis of recent infection. Paired samples should be collected and tested simultaneously to look for seroconversion.

REFERENCE

1. Nahmias, A.J., J. Dannenbarger, C. Wickliffe and M. Muther. Clinical aspects of infection with herpes simplex viruses 1 and 2 in the human herpes viruses. An interdisciplinary Perspective (Nahmias, A.J., W.R. Dawdle and R.F. Schinazi eds) New York, Elsevier, pp 3-9, 1981.
2. Vestergaard, B.F., P.C. Grauballe and H. Spanggaard. Titration of herpes simplex virus antibodies in human sera by the enzyme-link immunosorbent assay (ELISA). Acta Pathol. Microbiol. Scand. Sect. B 85:446-448, 1977.
3. Coleman, R.M., L. Pereira, P.D. Bailey, D. Dondero, C. Wickliffe, and A.J. Nahmias. Determination of herpes simplex virus type-specific antibodies by enzyme-linked immunosorbent assay. J. Clin. Microbiol. 18(1983) 287.

Date Adopted	Reference No.
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