Version 30-12/24

User's Manual

Human Herpes Simplex Virus1 IgA (HSV-1 IgA) ELISA Test kit

REF **DEIA345**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

HSV-1 IgA ELISA kit is intended for the detection of IgA antibodies to herpes simplex virus 1 (HSV 1).

General Description

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. The antibodies present to HSV 1 may be of the IgA, IgM and IgG isotypes. The physiological function of IgA and its clinical implication is still unclear. The Creative Diagnostics ELISA HSV 1 IgA is an accurate and sensitive serologic method to detect HSV 1 antibody IgA isotype.

Principles of Testing

Purified HSV antigen is coated on the surface of microwells. Diluted sample serum is added to wells, and the HSV 1 IgA 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 TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgA specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Reagents And Materials Provided

1. Microwell Strips: purified HSV 1 antigen coated wells. (12 x 8 wells)
2. Absorbent Solution: Black Cap. 1 vial (22 ml)
3. Washing Concentrate, 10 ×: White Cap. 1 bottle (100 ml)
4. TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)
5. Enzyme Conjugate: Red color solution. 1 vial (12 ml)
6. Cut-off Calibrator: Yellow Cap. HSV 1 A Index = 1.0 1 vial (150 µl)
7. Negative Control: Range stated on label. Natural Cap. 1 vial (150 µl)
8. Positive Control: Range stated on label. Red Cap. 1 vial (150 µl)
9. Stop Solution: 2 N HCl. 1 vial (12 ml)

Materials Required But Not Supplied

1. Deionized/distilled water
2. EIA Kit Microplate Washer

3. EIA Kit Microplate Reader at 450

Storage

1. Store the kit at 2 - 8°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.

Specimen Collection And Preparation

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 of serum sample.

Reagent Preparation

1. Prepare 1× washing buffer. Prepare washing buffer by adding distilled or deionized water to 10× wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

Assay Procedure

1. Place the desired number of coated strips into the well holder.
2. Prepare 1:40 dilutions by adding 5 µl of the samples, negative control, positive control, and calibrator to 200 µl of absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl absorbent solution in 1A well position. 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. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of 2 N HCl to stop reaction. Make sure there are no air bubbles in each well before reading.
9. Read O.D. at 450 nm with a microwell reader.

Quality Control

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.

2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The HSV 1 A Index for Negative and Positive Control should be in the range stated on the labels.

Calculation

1. Calculate the mean of duplicate calibrator value xc .
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the HSV 1 A Index of each determination by dividing the mean values of each sample by calibrator mean value, xc .

Example of typical results:

Cut-off Calibrator HSV 1 A Index = 1.0

Calibrator O.D. = 0.358, 0.365 xc = 0.362

Negative control O.D. = 0.158, 0.162 xn = 0.160

HSV 1 A Index = 0.160 / 0.362 = 0.44

Positive control O.D. = 1.305, 1.346 xp = 1.326

HSV 1 A Index = 1.326 / 0.362 = 3.66

Patient sample O.D. = 1.409, 1.459 xs = 1.434

HSV 1 A Index = 1.434 / 0.362 = 3.96

Interpretation Of Results

Negative: HSV 1 A Index less than 0.90 are negative for IgA antibody to HSV 1.

Equivocal: HSV 1 A Index between 0.91-0.99. Sample should be retested.

Positive: HSV 1 A Index of 1.00 or greater are positive for IgA antibody to HSV 1.

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	9.8%	7.5%	5.6%
Inter-assay	10.8%	8.9%	6.7%

Sensitivity

98%

Specificity

98.70%

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.

Limitations

1. To prevent false negative results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. 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.
3. A negative serological test does not exclude the possibility of past infection. Following primary HSV infection, antibody may fall to undetectable levels and then be boosted by later clinical infection with the same or heterologous type. Such a phenomenon may lead to incorrect interpretations of seroconversion and primary infection, or negative antibody status. In addition, samples obtained too early during primary infection may not contain detectable antibody. Some persons may fail to develop detectable antibody after Herpes infection.

References

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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