



EBV-EA IgG Elisa Kit

NAME AND INTENDED USE

Atlas Epstein Barr Virus Early Antigen (EBV-EA) IgG Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection of IgG antibody to Epstein Barr Virus Nuclear Antigen-1 in human serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

Detection of the Epstein-Barr virus was first described in 1964 by Epstein, Achong, and Barr using electron microscopic studies of cultured lymphoblasts derived from patients with Burkitt's lymphoma¹. EBV is classified as a member of the herpes-virus family based upon its characteristic morphology^{2,3}.

EBV infection may demonstrate a wide spectrum of clinical symptoms. The majority of primary EBV infections are transmitted via saliva, occur during childhood, and are subclinical⁴. Antibody titers to specific EBV antigens correlate with different stages of IM. Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak 3 to 4 weeks after primary EBV infection. IgM anti-VCA declines rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBNA) detected by anticomplement immunofluorescence develop from 1 month to 6 months after infection; and, like anti-VCA, persist indefinitely⁶. Antibodies to EBNA indicate that the EBV infection was not recent. EBV early antigen (EA) consists of two components; diffuse (D), and restricted (R). The terms D and R reflect the different patterns of immunofluorescence staining exhibited by the two components. Antibodies to EA may appear transiently for up to three months or longer during the acute phase of IM in 85% of patients⁷. The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children may produce antibodies to the R components. A definitive diagnosis of primary EBV infection can be made with 95% of acute phase serum based on antibody titers to VCA, EBNA, and EA⁷.

Antibodies to EA, usually to the R component, together with antibodies to EBNA and high titers of IgG anti-VCA, may be associated with reactivation of the latent viral carrier state. EBV positive serology associated with reactivation of EBV is found in sera of patients with immunodeficiencies⁸, patients with recurrent parotitis⁹, immunosuppressed patients, pregnant women, and persons of advanced age. Antibodies to the R component may be found at moderate to high levels in patients with Burkitt's lymphoma. In contrast, patients with nasopharyngeal carcinoma may produce high titer antibodies to the D component. Elevated levels of anti-EA and IgG anti-VCA may be detected in patients with chronic or recurrent illness suspected of being caused by EBV⁸. However, a diagnosis of chronic EBV should not be based on the presence of antibodies to EA since elevated anti-EA titers may also be found in patients with other diseases as well as in healthy individuals with past EBV infections⁵.

PRINCIPLE OF THE TEST

Purified EBV-EA antigen is coated on the surface of microwells. Diluted patient serum is added to wells, the anti-EBV-EA specific antibody, if present, will bind 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 specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIAL PROVIDED

1. Microwell strips: EBV-EA antigen coated wells (12 x 8 wells).
2. Sample Diluent: White Cap (1 vial 22 ml).
3. Washing concentrate 10x:White Cap (1 bottle 100 ml).
4. TMB Chromogenic Substrate: Amber bottle (1 vial 15 ml).
5. Enzyme conjugate: Red color solution (1 vial 12 ml).
6. Negative control: Range stated on label. Natural Cap(1 vial 150 µl) .
7. Cut-off calibrator: Yellow Cap.IgG Index = 1.(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).

STORAGE AND STABILITY

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.

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.

IgG index 1 (cut off value) = Mean value + 2 SD

36.7% (79 samples) have IgG index greater than 1.

Mean value = 2.352 SD = 1.324

P / N ratio = Mean of POSITIVE / Mean of NEGATIVE
= 2.352 / 0.509 = 4.6

Expected Values and Prevalence:

215 specimens from random asymptomatic blood donors were tested with Atlas EBV-EA IgG ELISA. 79 were found to be positive (36.7 %) and 136 were found to be negative (63.2 %). Prevalence may vary depending on a variety of factors such as geographical location, age, socioeconomic status, race, type of 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	12.5%	8.5%	5.6%
Inter-assay	14.8%	10.9 %	8.5%

LIMITATIONS OF THE ASSAY

1. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in light of the patient's history, physical findings and other diagnostic procedures.
2. Results from children should be reviewed with caution. 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.
3. Results obtained from immunocompromised individuals should be interpreted with caution.
4. There is a possibility of assay cross-reactivity with specimens containing anti-E.coli antibody.

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