CE

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See external label	2°C-8°C	Σ=96 tests	REF Cat # 1415Z

EBV-EA IgG

Cat # 1415Z

Cat # Number	**1415Z**
Test	**EBV -EA IgG ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**ELISA - Indirect; Antigen Coated Plate**
Detection Range	**Qualitative Positive; Negative control & Cut off**
Sample	**5ul Serum**
Specificity	**100 %**
Sensitivity	**100 %**
Total Time	**~90 min**
Shelf Life	**12 -18 Months**

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

NAME AND INTENDED USE

The DIAGNOSTIC AUTOMATION 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 sera 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 lymphoma[1]. EBV is classified as a member of the herpes-virus family based upon it's 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 subclinical[4]. 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 indefinitely[6]. 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 patients[7]. 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 sera based on antibody titers to VCA, EBNA, and EA[7].
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 immunodeficiencies[8], patients with recurrent parotitis[9],

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 EBV[8]. 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 infections [5].

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: Blue Color solution	1 vial (22 ml)
3.	Calibrator: Factor value (f) stated on label. Red Cap.	1 vial (150 µl)
4.	Negative control: Range stated on label. Natural Cap.	1 vial (150 µl)
5.	Positive control: Range stated on label. Green Cap.	1 vial (150 µl)
6.	Washing concentrate 10x: White Cap.	1bottle (100 ml)\
7.	Enzyme conjugate: Red color solution.	1 vial (12 ml)
8.	TMB Chromogenic Substrate: Amber bottle.	1 vial (12 ml)
9.	Stop solution: 2 N HCl.	1 vial (12 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 an 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 make a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20 - 25^{o} C) and gently mix

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:20 dilutions by adding 10 µl of the samples, negative control, positive control, and calibrator to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent 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. 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 30 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.

CALCULATION OF RESULTS

1. To obtain Cut off OD value: Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the EBV-EA IgG Index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For example:
If Factor (f) value on label = 0.4
Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition EBV-EA IgG Index = 1)
Patient sample O.D. = 0.580
EBV-EA IgG Index = 0.580 / 0.44 = 1.32 (Positive result)
Patient sample O.D. = 0.320
EBV-EA IgG Index = 0.320 / 0.44 = 0.73 (Negative result)

QUALITY CONTROL

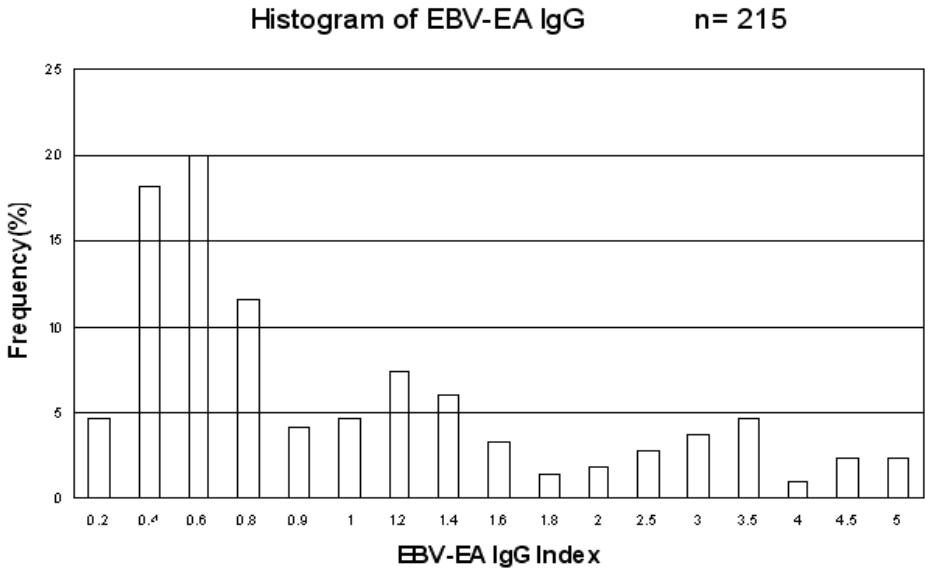
The test run may be considered valid provided the following criteria are met:
1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The EBV-EA IgG Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative:	EBV-EA IgG Index of 0.90 or less.
Equivocal:	EBV-EA IgG Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive:	EBV-EA IgG Index of 1.00 or greater.

PERFORMANCE CHARACTERISTICS

Histogram:
215 random samples are determined with DIAGNOSTIC AUTOMATION Microwell ELISA EBV-EA IgG. The test results are computed as IgG Index using a chosen reference serum as IgG Index 1. The distribution of frequency versus IgG Index value is presented as following:



P / N Ratio
63.2% (136 samples) have IgG index below 1.
Mean value = 0.509 SD = 0.229
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 the DIAGNOSTIC AUTOMATION 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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