EBV-VCA IgM Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAVGM1	EBV-VCA IgM Elisa	96 Tests

Intended Use:

The Epstein-Barr Virus-Viral Capsid Antigen (EBV-VCA) IgM Enzyme linked Immunosorbent Assay (ELISA), is intended for the detection of IgM antibody to Epstein-Barr virus in human serum.

Summary and Principle:

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. EBV is classified as a member of the herpes-virus family based upon its characteristic morphology. 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. In the U.S., 50% of the population demonstrate EBV antibodies before the age of 5 years; 80% by adulthood. Transfusion-associated EBV infections have also been reported. In young adults, EBV infection may be clinically manifested as Infectious Mononucleosis (IM) with typical symptoms of sore throat, fever, and lymphadenopathy.3 College students and military personnel are often cited as a high morbidity incidence population for IM. Following primary EBV infection, it is postulated that the B lymphocyte may continue to harbor the EBV genome and establish a latent infection that may extend through life.4 Reactivation of EBV infection or enhanced EBV activation has been documented in immunodeficient or immunosuppressed patients, i.e., organ transplant patients, individuals with malignancies, pregnant women, and persons of advanced age. Epstein-Barr virus has also been associated in the pathogenesis of two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma. Documentation by means of DNA hybridization studies demonstrates the presence of the EBV genome on biopsy specimens taken from individuals with these carcinomas. Burkitt's lymphoma is primarily observed in Sub-Sahara Africa, especially in African children, and in New Guinea. Malarial infections are usually diagnosed in Burkitt's lymphoma patients and are suggested to be a co-factor. Nasopharyngeal carcinoma is observed in Asia, most notably in Southern China, and may have genetic or environmental influences as the co-factor. In the last two decades, serological methods have progressed from testing for the presence of non-specific heterophile antibodies to measuring levels of IgG or IgM formed against subunits of EBV antigen complexes. One of the best indicators of active EBV infection is antibody to viral capsid antigens, structural proteins necessary for replication of the virus. Viral capsid antigens are present in every cell infected with EBV. The IgM response to VCA is among the earliest detectable humoral immune responses, usually present at the onset of the disease and peaking within four to six weeks. VCA-IgM levels are also transient, declining rapidly and usually becoming undetectable within two to three months from onset of clinical symptoms.

Purified EBV-VCA antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the EBV-VCA IgM specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibodyantigen 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 colour generated is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with Purified EBV VCA Antigens. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 2 months at 2-8°C.
Absorbent solution	22ml	Black cap. Ready to use. Once open, stable for 1 month at 2-8°C.
Calibrator	150ul	Factor value (f) stated on label. Red cap. Ready to use. Once open, stable for 1 month at 2-8°C.
Negative Control	150ul	Range stated on label. Natural cap. Control material negative for EBV VCA IgG. Ready to use. Once open, stable for 1 month at 2-8°C.
Positive Control	150ul	Range stated on label. Green cap. Control Positive for EBV VCA IgG. Ready to use. Once open, stable for 1 month at 2-8°C.
Wash Concentrate (10x)	100ml	PBS-Tween at pH 7.4. 10X concentrate. The concentrate must be diluted with 900 ml of distilled water before use. Once diluted it is stable at room temperature for two months.
TMB Substrate	12ml	*Amber bottle.* Mixture of TMB and Hydrogen Peroxide solution. Ready to use. Once open, stable for 2 months at 2-8°C.
Enzyme conjugate	12ml	*Red colour solution.* 1 vial containing 12ml of HRP labelled Anti Human IgM antibodies in Buffered saline. Once open, stable for 2 months at 2-8°C.
Stop Solution	12ml	Diluted Sulfuric acid solution (1M) Ready to use. Once open, stable for 2 months at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Specimen Collection:

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.

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.

Precautions and Safety:

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.

Procedure:

Reagent preparation:

Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to make a final volume of 1 litre.

Bring all specimens and kit reagents to room temperature 20-25°C and gently mix.

STEP 1

Preparation: Place the desired number of coated strips into the holder.

STEP 2

Addition of the Diluent: Prepare 1:20 dilutions by adding 10ul of the samples, negative control, positive control, and calibrator to 200 ul of absorbent solution. Mix well.

STEP 3

Addition of the Sera, calibrators and controls: Dispense 100ul of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 ul Absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.

STEP 4

Incubation: Incubate for 30 minutes at room temperature.

STEP 5

Washing: Remove liquid from all wells. Repeat washing three times with washing buffer.

STEP 6

Addition of Enzyme Conjugate: Dispense 100ul of enzyme conjugate to each well.

STEP 7

Incubation: Incubate for 30 minutes at room temperature.

STEP 8

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

STEP 9

Addition of TMB Chromogenic Substrate: Dispense 100ul of TMB Substrate into each well and incubate for 15 minutes at room temperature.

STEP 10

Addition of Stop solution: Add 100ul of Stop Solution to stop reaction.

Make sure there are no air bubbles in each well before reading.

STEP 11

Measurement: Read O.D. at 450nm 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 IgM 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
- This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.
- Obtained Calibrator O.D. = 1.100.
- Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition IgM Index = 1)
- Patient sample O.D. = 0.580
- EBV-VCA IgG Index = 0.580 / 0.44 = 1.32 (Positive result)
- Patient sample O.D.= 0.320
- EBV-VCA 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-VCA IgM Index for Negative and Positive Control should be in the range stated on the labels.

Interpretation of results:

Negative: EBV-VCA IgM Index of 0.90 or less are seronegative for IgM antibody to EBV-VCA virus.
Equivocal: EBV-VCA IgM Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: EBV-VCA IgM Index of 1.00 or greater are seropositive. Indicative of current or recent infection.

Limitation 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. The absence of detectable IgM antibody does not rule out the possibility of recent or current infection. A second specimen 5~7 days later should be repeated.
3. The absorbent solution used as sample diluent in this test is to prevent the interferences of specific IgG and rheumatoid factor (RF). However RF larger than 400IU/ml may interfere with the test in the presence of high specific IgG.
4. A positive EBV IgM result is general considered diagnostic for acute IM. To verify the diagnosis, however, it is recommended that the specimen be tested for other EBV antibodies, such as EA-D or EBNA IgG and EBNA IgM to determine predominant antibody.

Performance Characteristics:

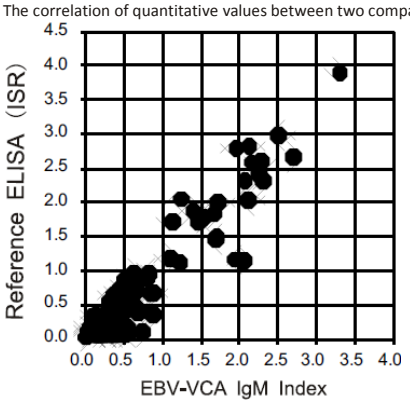
Sensitivity, specificity and accuracy:

A total of 124 random samples from different sources were assayed with Elisa EBV-VCA IgM test and with another commercially available Elisa test kit.

		REFERENCE ELISA		
		N	**P**	**TOTAL**
	N	100 (D)	0 (B)	100
Prestige Elisa	**P**	0 (C)	24 (A)	24
EBV-VCA IgM	**TOTAL**	100	24	124

Sensitivity = A / (A+B) = 24 / 24 + 0 = 100%
Specificity = D / (C+D) = 100 / 100 + 0 = 100%
Accuracy = (A+D) / (A+B+C+D) = 124 / 124 = 100%

The correlation of quantitative values between two comparison methods was summarized:



Cross-Reactivity:

A study was performed to determine the cross-reactivity of EBV-VCA IgM with other member of the HSV family and other IgM antibodies. A total of 44 samples negative for EBV-VCA IgM but positive for IgM for CMV (6), HSV 1 (4), HSV 2 (4), Rubella (10), Toxo (3), RF (7), and ANA IgG (10) by other commercial available kits were assayed. All 4 samples give negative results for EBV-VCA IgM. It indicates and absence of cross-reactiviy of the Elisa EBV-VCA IgM with other members of HSV family and other IgM antibodies.

Precision:

The mean, SD and % CV were calculated inter- and intra- assay:

Intra-assay	**n**	**Mean**	**SD**	**%CV**
Serum 1	8	0.185	0.0141	8.45
Serum 2	8	1.093	0.0200	2.22
Serum 3	8	1.479	0.0362	3.10
Serum 4	8	2.017	0.1356	7.40
Inter-assay	**n**	**Mean**	**SD**	**%CV**
Serum 1	8	0.184	0.015	8.37
Serum 2	8	1.074	0.091	8.46
Serum 3	8	1.506	0.116	7.70
Serum 4	8	2.197	0.188	8.55

References:

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3. Schooley, R.T. and R. Dolin. 1985. Epstein-Barr Virus Infectious Mononucleosis). In: Principles and Practice of Infectious Diseases, 2nd Edition. Mandell, G.L., R.G. Douglas, and J.E. Bennett. (eds). John Wiley and Sons, New York. pp 97-982.
4. Sumaya, C.V. 1985. Serological Testing for Epstein-Barr Virus- Developments in Interpretations. In: J. Inf. Dis. 151 (6):984-987.
5. Tobi, M., and S.E. Straus. 1985. Chronic Epstein-Barr Virus Disease: A Workshop Held by the National Institute of Allergy and Infectious Diseases. In: Ann. Intern. Med. 103 (6 (pt. 1)):951-953.
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7. Pearson, G.R. and Luka, J. Characterisation of the Virusdetermined Antigens. In The Epstein-Barr Virus: Recent Advances. Epstein and Achong, eds. Wiley: New York. 1986.
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REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

CE

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