EPSTEIN-BARR VIRUS-VIRAL CAPSID ANTIGEN (EBV-VCA IgM)

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:20 = 10µl / 200 µl		
2	Diluted samples, calibrator & controls	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	TMB Chromogenic Substrate	100 µl	15 minutes
7	Stop solution	100 µl	
8	Reading OD 450 nm		

NAME AND 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 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]. 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[3]. 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[3].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[4].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[3].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[5,6]. Nasopharyngeal carcinoma is observed in Asia, most notably in Southern China, and may have genetic or environmental influences as the co-factor[5,6].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[7]. 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[8].

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PRINCIPLE OF THE TEST

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 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 IgG 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-VCA antigen coated wells. (12 x 8 wells)
2. Absorbent solution: 1 vial (22 ml)
3. Calibrator: Factor value (f) stated on label. 1 vial (150 µl)
4. Negative Control: Range stated on label. 1 vial (150 µl)
5. Positive Control: Range stated on label. 1 vial (150 µl)
6. Washing Concentrate (H) 20x. 1 bottle (50 ml)
7. Enzyme Conjugate: Red color solution. 1 vial (12 ml)
8. TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)
9. Stop Solution. 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.
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° 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 20x wash concentrate to make a final volume of 1 liter.
3. 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 holder.
2. Prepare 1:20 dilutions by adding 10 µl of 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. 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 Stop solution 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 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
IgM Index = 0.580 / 0.44 = 1.32 (Positive result)
Patient sample O.D. = 0.320
IgM 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

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 of recent infection.

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.

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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 400 IU/ml may interfere with the test in the presence of high specific IgG.
4. A positive EBV IgM result is generally 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 a commercially available ELISA test kit.

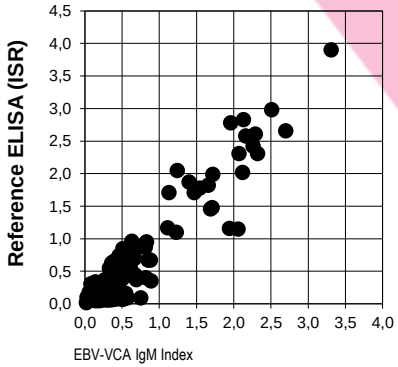
		Reference ELISA		
		N	P	Total
ELISA EBV-VCA IgM	N	100 (D)	0 (B)	100
	P	0 (C)	24 (A)	24
	Total	100	24	124

Relative Sensitivity = A / (A+B) = 24 / 24 + 0 = 100%

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

Relative 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 44 samples give negative results for EBV-VCA IgM. It indicates an absence of cross-reactivity of the ELISA EBV-VCA IgM with other members of HSV family and other IgM antibodies.

PRECISION:

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

Intra-Assay	n	Index M 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	Index M 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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PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET009