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IVD	See external label	2°C-8°C	96 tests	REF 1414-11

EBV-VCA IgA

REF 1414-11

Test	EBV -VCA IgA ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	5ul serum/plasma
Specificity	100 %
Sensitivity	100 %
Total Time	~75 min
Shelf Life	12 Months from the manufacturing date

** 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 DAI Epstein-Barr Virus-Viral Capsid Antigen (EBV-VCA) IgA Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection of IgA antibody to Epstein-Barr virus 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 lymphoma1. EBV is classified as a member of the herpes-virus family based upon it's characteristic morphology2,3.
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. 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 reported3. In young adults, EBV infection may be clinically manifested as Infectious Mononucleosis (IM) with typical symptoms of sore throat, fever, and lymphadenopathy3. College students and military personnel are often cited as a high morbidity incidence population for IM3.
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 life4. 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 age4.
Epstein-Barr virus has also been associated in the pathogenesis of two human cancers, Burkitt's lymphoma and nasopharyngeal carcinoma (NCP). Documentation by means of DNA hybridization studies demonstrates the presence of the EBV genome on biopsy specimens taken from individuals with these carcinomas3.
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-factor5,6. Nasopharyngeal carcinoma is observed in Asia, most notably in Southern China, and may have genetic or environmental influences as the co-factor5,6.
Serological studies have shown that the clinical onset of NPC is preceded by the appearance of a high antibody titer of IgA to viral capsid antigens and early antigens. The titers increase with the total tumor burden and the antibodies decline with the response to therapy. In patients with confirmed clinical remission elevation of IgA serological titers is highly significant for prediction of relapse 7,8,9.

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 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.

MATERIALS PROVIDED

1. Microwell strips: EBV-VCA 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. Brown Cap. 1 vial (150 µl)

6. Washing Concentrate 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 - 8o 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 - 8o 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.
2. Bring all specimens and kit reagents to room temperature (20 - 25o 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 ml of the samples, negative control, positive control, and calibrator to 200 ml of sample diluent. Mix well.
3. Dispense 100 ml of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 ml of 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 ml 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 ml of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 ml 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. This factor (f) is a variable.
It is specific for a lot manufactured and printed on label of Calibrator.
2. Calculate the IgA 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 IgA Index = 1)
Patient sample O.D. = 0.580
IgA Index = 0.580 / 0.44 = 1.32 (Positive result)
Patient sample O.D. = 0.320
IgA Index = 0.320 / 0.44 = 0.73 (Negative result)

QUALITY CONTROL

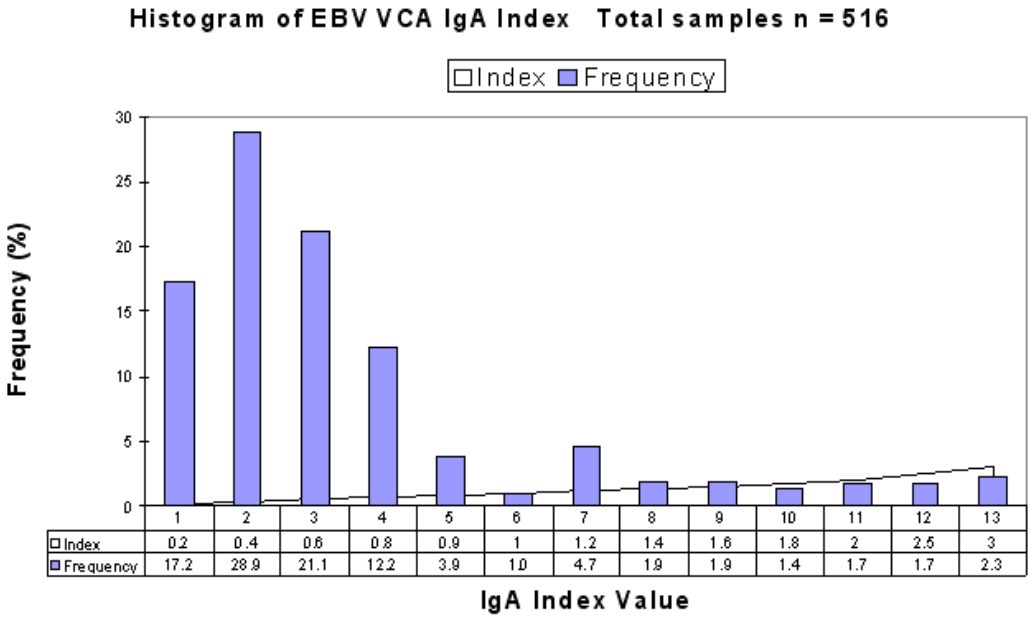
The test run may be considered valid provided the following criteria are met:
1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The EBV-VCA IgA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

INTERPRETATION

Negative: EBV-VCA IgA Index of 0.90 or less is seronegative for IgA antibody to EBV-VCA virus.
Equivocal: EBV-VCA IgA Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: EBV-VCA IgA Index of 1.00 or greater.

PERFORMANCE CHARACTERISTICS

Histogram:
516 random samples are determined with The Diagnostic Automation Inc, microwell ELISA EBV VCA IgA. The test results are computed as IgA Index using a chosen reference serum as IgA Index 1. The distribution of frequency versus IgA Index value is presented as following:



	1	2	3	4	5	6	7	8	9	10	11	12	13
Index	0.2	0.4	0.6	0.8	0.9	1	1.2	1.4	1.6	1.8	2	2.5	3
Frequency	17.2	28.9	21.1	12.2	3.9	1.0	4.7	1.9	1.9	1.4	1.7	1.7	2.3

Validation of cut off value:

84% (435 samples) have IgA index below 1.
Mean value = 0.406 SD = 0.216
IgA index 1 (cut off value) = Mean value + 2.75 x SD

16% (81 samples) have IgA index greater than 1.
Mean value = 1.717 SD = 0.665
P / N ratio = Mean of POSITIVE / Mean of NEGATIVE
= 1.717 / 0.40 = 4.23

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	10.2%	8.5%	7.5%
Inter-assay	12.1%	9.7%	8.4%

LIMITATIONS OF THE PROCEDURE

The results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

REFERENCES

1. Epstein, M.A., B.B. Achong, and Y.M. Barr. 1964. Virus particles in Cultured Lymphoblasts from Burkitt's Lymphoma. In: Lancet 1:702-703.
2. Epstein, M.A., Y.M. Barr, and B.G. Achong. 1965. Studies with Burkitt's Lymphoma. In: Wistar Inst. Sympos. Monogr. 4:69-82.

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.
6. Birx, D.L., R.R. Redfield, and G. Tosarto. 1986. Defective Regulation of Epstein-Barr virus Infection in Patients with Acquired Immunodeficiency Syndrome (AIDS) or AIDS-Related Disorders. In: New Eng. J. Med. 314 (14): 874-879.
7. Mazeron MC value of anti-Epstein-Barr antibody detection in the diagnosis and management of undifferentiated carcinoma of the nasopharynx. Bull Cancer Radiother 1996; 83 (1):3-7.
8. Shimakage M; Dezawa T; Chatani M. Proper use of serum antibody titers against Epstein-Barr virus in nasopharyngeal carcinoma: IgA / virus capsid antigen for diagnosis and EBV-related nuclear antigen – 2 for follow-up Acta Otolaryngol 2000 Jan.; 120(1): 100-4.
9. Dardari R; Khyatti M; et al. Antibodies to the Epstein-Barr virus transactivator protein as a valuable biomarker in young patients with nasopharyngeal carcinoma Int J Cancer 2000 Apr 1; 86(1): 71-5.

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