CE

DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD	See external label	2°C-8°C	Σ=96 tests	REF	Cat# 1405-11

Epstein Barr Virus VCA (EBV, VCA IgG)

REF 1405-11

Test	EBV VCA IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	10ul Serum
Specificity	100 %
Sensitivity	100 %
Total Time	~75 min
Shelf Life	12 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, Inc. Epstein-Barr Virus-Viral Capsid Antigen (EBV-VCA) IgG Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection and quantitative determination of IgG antibody to Epstein-Barr virus in human sera. A single serum specimen may be used to indicate previous infection or immune status with the Epstein-Barr virus.

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].

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 IgG 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.

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. Green Cap.	1 vial (150 µl)
6.	Washing Concentrate 20x: White Cap.	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.
3. If paired sera are to be collected, acute samples should be collected as soon as possible after onset of symptoms and not later than seven days after onset. The second sample should be collected 14 to 21 days after the acute specimen was collected. Both samples must be run in duplicate tests on the same plate to test for a significant rise. If the first specimen is obtained too late during the course of the infection, a significant rise may not be detectable.

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 - 25° C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.

2. Prepare 1:21 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 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 EBV-VCA 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
This factor (f) is a variable.
Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition EBV-VCA IgG 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 0.30, the test is not valid and must be repeated.
3. The EBV-VCA IgG Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: EBV-VCA Index of 0.90 or less are seronegative for IgG antibody to EBV-VCA virus.
Equivocal: EBV-VCA Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: EBV-VCA Index of 1.00 or greater are seropositive. It indicates prior exposure to the EBV-VCA virus.
Significant change in antibody level of the paired sample:
The ratio between the EBV-VCA IgG Index of the second sample and that of the first sample should be greater than 1.3 to be suggestive of a significant rise in antibody level.

LIMITATIONS OF THE PROCEDURE

1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgG antibody and render an EBV-VCA IgG Index result negative.

3. 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.

PERFORMANCE CHARACTERISTICS

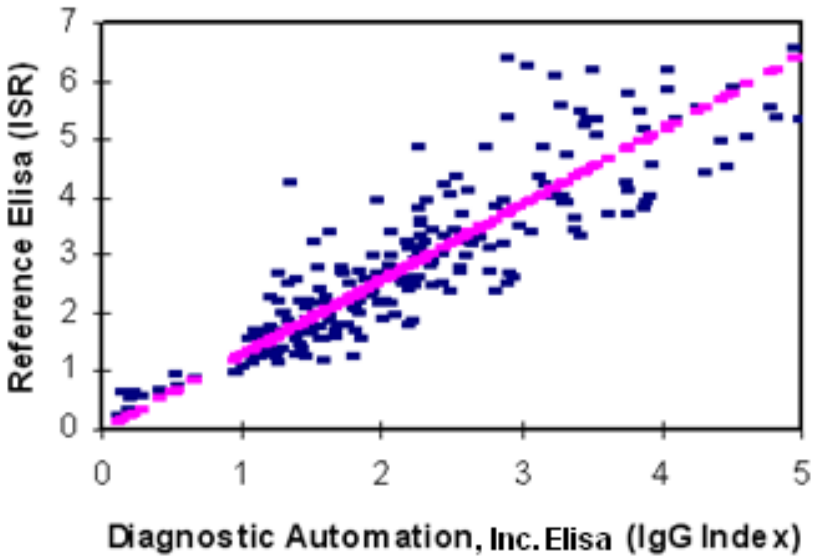
Sensitivity, Specificity, and Accuracy:

A total of 225 random samples from different sources were assayed with Diagnostic Automation, Inc. EBV-VCA IgG test and with a commercially available ELISA test kit.

		Reference ELISA			
		N	E	P	Total
	N	11 (D)	0	0 (B)	11
DIAGNOSTIC	E	0	3	0	3
AUTOMATION, INC	P	0 (C)	0	211 (A)	211
ELISA	Total	11	3	211	225

Sensitivity **= A / (A+B) = 211 / 211 = 100%**
Specificity **= D / (C+D) = 11 / 11 = 100%**
Accuracy **= (A+D) / (A+B+C+D) = 222 / 222 = 100%**

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



Precision:

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

Intra-Assay	n	Index G Mean	SD	% CV
Serum 1	8	0.1705	0.0074	4.39
Serum 2	8	1.274	0.0997	7.83
Serum 3	8	2.330	0.1640	7.04
Serum 4	8	2.481	0.1099	4.43

Inter-Assay	n	Index G Mean	SD	% CV
Serum 1	8	0.170	0.0071	4.20
Serum 2	8	1.296	0.0486	3.75
Serum 3	8	2.278	0.1220	5.36
Serum 4	8	2.444	0.2543	10.41

LIMITATIONS OF THE ASSAY

1. The antibody titer of a single serum specimen cannot be used to determine recent infection. Test results for anti-VCA should be interpreted in conjunction with the clinical evaluation and results of antibody tests for other EBV antigens,
2. Most (80%) of IM individuals have peak anti-VCA IgG titers before they consult a physician. Therefore, testing paired acute and convalescent sera for significant changes in antibody levels is not useful in most patients with IM.

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. Pearson, G.R. and Luka, J. Characterisation of the Virus-determined Antigens. In *The Epstein-Barr Virus: Recent Advances*. Epstein and Achong, eds. Wiley: New York. 1986.
8. Schmitz, H. and Scherer, M. IgM Antibodies to Epstein-Barr Virus in Infectious Mononucleosis. Arch. Ges. Virusforsch. 37:332-39, 1972.

Date Adopted	2016-12-27
REF 1405-11	DA-EBV,VCA IgG

DIAGNOSTIC AUTOMATION, INC.
21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA
Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003

Revision Date: 2016-05-25