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IVD | See external label | 2°C-8°C | Σ=96 tests | REF Cat # 1426Z

EBNA-1 IgM

Cat # 1426Z

Cat # Number	**1426Z**
Test	**EBNA -1 IgM ELISA**
Method	**ELISA: Enzyme Linked Immunosorbent Assay**
Principle	**ELISA - Indirect; Antigen Coated Plate**
Detection Range	**Qualitative Positive; Negative control & Cut off**
Sample	**10 µl 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 Nuclear Antigen-1 (EBNA-1) IgM Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection of IgM 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 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 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].

Infection of the target cells leads to two forms of viral cycles: 1) latent, nonproductive and 2) productive, replicative infections[4]. In both cycles, one of the earliest antigens expressed is lymphocyte-detected membrane antigen, a cell-surface antigen recognized by T-cells. It has been well established that most individuals exposed to EBV develop a heterophile antibody response. Expression of EBNA-1 either follows or parallels membrane antigen at 12 to 24 hours post infection. EBNA-1 is found as nonstructural, intranuclear antigen(s), present in all EBV-transformed cell lines as in tumors from Burkitt's and nasopharyngeal carcinoma patients. In the fully productive, replicative cycle, the synthesis of antigen follows EBNA-1. The viral capsid antigen complex (VCA) appears late in the replicative cycle. It has recently become apparent that EBNA-1 is probably not a single antigenic moiety, but a multicomponent antigen complex, on the basis of reactivities of sera from different classes of patients. The major component EBNA-1 has been purified and sequenced in its entirety[4]. Antibody levels of EBNA-1 IgG, are diagnostic in determining acute and convalescent stages in IM IgG antibodies to EBNA-1 are rarely present in acute IM and rise during convalescence. They will rise to a plateau level in three months to a year and will normally persist for life[5,6]. Antibody levels of EBNA-1 IgM and EBNA-1 IgG, when measured concurrently, are diagnotic in determining acute and convalescent stage in IM.

PRINCIPLE OF THE TEST

Purified EBNA-1 antigen is coated on the surface of microwells. Diluted patient serum is added to wells, the anti-EBNA 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: EBNA antigen coated wells.	(12 x 8 wells)
2.	Absorbent Solution: Black Cap.	1 vial (22 ml)
3.	Washing concentrate 10x : White Cap.	1bottle (100 ml)
4.	TMB Chromogenic Substrate: Amber bottle.	1 vial (15 ml)
5.	Enzyme conjugate: Red color solution.	1 vial (12 ml)
6.	Negative control: Range stated on label. Natural Cap.	1 vial (150 µl)
7.	Calibrator: Factor value (f) stated on label Red Cap.	1 vial (150 µl)
8.	Positive control: Range stated on label. Green Cap.	1 vial (150 µl)
9.	Stop solution: 2 N HCl.	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.
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 10x 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 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 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 the label of Calibrator
2. Calculate the EBNA-1 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
Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 X 0.04 = 0.44 (By definition EBNA-1 IgM Index = 1)

Patient Sample O.D. = 0.580
EBNA – 1 IgM Index = 0.580 / 0.44 = 1.32 (Positive Result)

Patient Sample O.D. = 0.320
EBNA – 1 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 EBNA-1 IgM Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: EBNA-1 IgM Index of 0.90 or less.

Equivocal: EBNA-1 IgM Index of 0.91 - 0.99 are equivocal. Sample should be retested.

Positive: EBNA-1 IgM Index of 1.00 or greater.

PERFORMANCE CHARACTERISTICS

Sensitivity, Specificity, and Accuracy:

A total of 36 random samples from different sources were assayed with DIAGNOSTIC AUTOMATION EBNA-1 IgM test and with a commercially available ELISA test kit.

		Reference ELISA			
		N	E	P	Total
DIAGNOSTIC	N	32 (D)	0	0 (B)	32
AUTOMATION	P	0 (C)	0	4 (A)	4
ELISA	Total	32	0	4	36

Relative Sensitivity = A / (A+B) = 4 / 4 = 100%

Relative Specificity = D / (C+D) = 32 / 32 = 100%

Relative Accuracy = (A+D) / (A+B+C+D) = 36 / 36 = 100%

Expected Values and Prevalence:
57 specimens from random asymptomatic blood donors were tested with the DIAGNOSTIC AUTOMATION EBNA-1 IgM ELISA. 10 were found to be positive (17.5 %) and 47 were found to be negative (82.5 %). 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.

Cross reactivity:
The positive samples of Rubella IgM, Toxo IgM, CMV IgM, HSV 2 IgM, Mump IgM, RF IgM, ANA IgG, Chlamydia trachomatis IgM have been tested with negative results by DIAGNOSTIC AUTOMATION EBNA-1 IgM test.

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	10.8%	6.5%	4.9%
Inter-assay	14.5%	8.6%	7.5%

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. Heterotypic IgM response to EBV may occur in patients infected with CMV and also in patients infected with Varicalla-Zoster.
3. A positive EBNA-1 IgM result is generally consider 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, EBNA-1 IgG, and VCA IgM to determine predominant antibody.
4. Results obtained from immunocompromised individuals should be interpreted with caution.

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.
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