Datasheet for ABIN996985

EBV VCA IgM ELISA Kit

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Overview

Quantity:	96 tests
Target:	EBV VCA IgM
Reactivity:	Epstein-Barr Virus (EBV)
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	Epstein-Barr Virus Viral Capsid Antigen (EBV-VCA) IgM Test System is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative detection of IgM class antibodies to Epstein-Barr Virus Viral Capsid Antigen (EBV-VCA) in human sera.
Sample Type:	Serum
Analytical Method:	Qualitative
Detection Method:	Colorimetric
Specificity:	90.9 %
Material not included:	1. Precision pipettes: 0.04 till approx. 0.4 mL and 0.4 mL . 2. Disposable pipette tips. 3. Distilled water. 4. Vortex mixer or equivalent. 5. Absorbent paper or paper towel. 6. Graph paper. 7. Microtiter well reader.

Target Details

Target:	EBV VCA IgM
Abstract:	EBV VCA IgM Products
Target Type:	Antibody, Antibody

Application Details

Comment:	Quality Control: 1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay. 2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15 % from the mean, discard that value and calculate the mean using the remaining two wells. 3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges: OD Range Negative Control <0.250 Calibrator > 0.300 Positive Control >0.500 a. The OD of the Negative Control divided by the mean OD of the Calibrator should be < 0.9. b. The OD of the Positive Control divided by the mean OD of the Calibrator should be > 1.25. c. If the above conditions are not met the test should be considered invalid and should be repeated. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices. Limitations of procedure: 1. Most (80 %) of IM individuals have peak anti-VCA IgM titers before they consult a physician (4). Therefore, testing paired acute and convalescent sera for significant changes in antibody levels is not useful in most patients with IM (4). 2. The antibody titer of a single serum specimen should not 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, i.e., EBNA, EA, and IgG-VCA. 3. The lack of detectable IgM antibodies does not exclude current EBV infection. The sample may have been collected before development of demonstrable antibody or after the antibody level is no longer detectable. 4. Test results of specimens from immunosuppressed patients may be difficult to interpret. 5. 6. 5. Specific IgM antibodies are usually detected in patients with recent primary infection, but may be found in patients with reactivated or secondary infections, and they are sometimes

	found in patients with no other detectable evidence of recent infection. 6. The anti-IgG absorbent has been shown to functionally remove > 13.9 mg/mL IgG from human serum. Normal adult IgG levels may range from 8 to 16 mg/mL (32). Patients with an IgG level exceeding 14 mg/mL may require additional treatment to neutralize all IgG. 7. Performance characteristics of this device have not been established with EBV-associated disease other than infectious mononucleosis. 8. Test results should be evaluated in relation to patient symptoms, clinical history, and other laboratory findings to establish a diagnosis. Note: 1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease. 2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious. 3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera. 4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2-8 °C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20 °C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.
Sample Volume:	10 µL
Assay Time:	1.5 h
Plate:	Pre-coated
Assay Procedure:	3. Prepare a 1:21 dilution (e.g.: 10 µL of serum + 200 µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum. 4. To individual wells, add 100 mL of each diluted control, calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample. 5. Add 100 µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration. 6. Incubate the plate at room temperature (20-25 °C) for 25 + 5 minutes. 7. Wash the microwell strips 5X. A. Manual Wash Procedure: a. Vigorously shake out the liquid from the wells.

b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of 5 washes.
d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap for mL y to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains.
Collect wash solution in a disposable basin and treat with disinfectant at the end of the days run.

B. Automated Wash Procedure: If using an automated microwell wash system, set the dispensing volume to 300-350 µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped for mL y to remove any residual wash solution from the microwells.
8. Add 100 µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens. Incubate the plate at room temperature (20-25 °C) for 25 + 5 minutes Wash the microwells by following the procedure as described in step
7. Add 100 µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.

EXAMPLE PLATE SET-UP 1 2 A Blank Patient 3 B Neg. Control Patient 4 C Calibrator Etc. D Calibrator E Calibrator F Pos. Control G Patient 1 H Patient 2
Remove the individual components from storage and allow them to warm to room temperature (20-25 °C).
Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run.
A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8 °C.
12. Incubate the plate at room temperature (20-25 °C) for 10 to 15 minutes.
13. Stop the reaction by adding 50 µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450 nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

Application Details

Calculation of Results:	A. Calculations: 1. Correction Factor A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box. 2. CutoffOD Value To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above. (CF x mean OD of Calibrator = cutoffOD value) 3. Index Values or OD Ratios Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2. 4 5 6 Example: Mean OD of Calibrator = 0.793 Correction Factor (CF) = 0.25 Cut off OD 0.793 x 0.25 = 0.198 Unknown Specimen OD = 0.432 Specimen Index Value or OD Ratio = 0.432 / 0.198 = 2.18 B. Interpretations: Index Value or OD Ratio Negative Specimens <0.90 Equivocal Specimens 0.91 to 1.09 Positive Specimens > 1.10 An OD ratio <0.90 indicates no significant amount of IgM antibodies to EBV-VCA detected. A negative result indicates no active infection with EBV and should be reported as non-reactive for EBV-VCA IgM antibody. 1 An OD ratio > 1.10 indicates that IgM antibodies specific to EBV-VCA were detected. A positive test result indicates a current or reactivated infection with EBV-VCA, and should be reported as reactive for EBV-VCA IgM antibody. . Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. valuate repeatedly equivocal specimen by an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later. If the second specimen is positive, consider the patient to have an active infection. . The numeric value of the final result above the cutoff is not indicative of the amount of anti-EBV-VCA IgM antibody present. Comparative Study Clinical studies were conducted to demonstrate the clinical efficacy of the DAI ELISA EBV-VCA IgM Test System as an aid in the diagnosis of EBV-associated infectious mononucleosis. Evaluation occurred at two clinical sites. Site One was an independent laboratory located in northeastern U.S. Site Two was a commercial serum/serum component vendor located in southeastern U.S. Testing of a total of 305 specimens tested took place, 158 at Site One, and 147 at Site Two. Specimens tested at Site One included 119 samples sent to a

reference laboratory for normal EBV serology, 19 specimens previously characterized as EBV negative, and 20 specimens previously characterized as EBV-VCA IgM positive. Specimens tested at Site Two included 100 specimens tested for routine EBV serology, 27 specimens previously characterized as VCA IgM positive, and 20 previously characterized as VCA IgM negative. Serologies performed at each site included: Heterophile, EBV-VCA IgG, EBNA, and the DAI ELISA EBV-VCA IgM Test System. The criteria for determining assay specificity and sensitivity was as follows: all clinical specimens were classified as to the stage of EBV infection and therefore their probable IgM antibody status based primarily upon their profile with respect to the Heterophile and EBNA results. Specifically, there were four such profiles: (1) Heterophile negative, EBNA positive, (2) Heterophile negative, EBNA negative, (3) Heterophile positive, EBNA negative, and (4) Heterophile positive, EBNA positive. The suspected EBV-VCA IgM serologies of these four profiles, along with the results of this study have been summarized in Tables 1 through 3 below: cc Table 1: Clinical Site One Heterophile/EBNA Profile Stage/IgM Activity Positive Negative Equivocala Total Heterophile-, EBNA + Past Infection IgM Negative 9 90 3 102 96/102 (94 %), VCA IgG Positive 0/102 (0 %), VCA IgG Equivocal 6/102 (6 %), VCA IgG Negative Heterophile-, EBNA - Never Infected IgM Negative 0 33 0 33 2/33 (6 %), VCA IgG Positive 3/33 (9 %), VCA IgG Equivocal 28/33 (85 %), VCA IgG Negative Heterophile +, EBNA - Acute Infection IgM Positive 19 1 1 21 8/21 (38 %), VCA IgG Positive 5/21 (24 %), VCA IgG Equivocal 8/21 (38 %), VCA IgG Negative Heterophile +, EBNA + Reactivation IgM Positive 1 1 0 2 1/2 (50 %), VCA IgG Positive 1/2 (50 %), VCA IgG Equivocal 0/2 (0 %), VCA IgG Negative aEquivocal specimens were retested according to the Package Insert. Specimens that were repeatedly equivocal or not retested due to insufficient volume appear in this column. These remaining equivocal specimens were not used in any calculations for sensitivity or specificity. Of the 158 specimens tested at site 1, there were initially 11 equivocal samples. Seven repeated as negative, three repeated as equivocal, and one was not repeated due to insufficient volume. Assay Specificity: 123/132 = 9

3.2 % (88.9 % to 97.5 %) b Assay

The presence of EBV-VCA-IgM antibodies as determined by the ELISA method is highly suggestive of acute EBV infection since such antibodies are found early on in the illness in approximately 90 % of cases and are not usually present in the general population (31). To demonstrate this, the frequency of IgM antibody to EBV-VCA was evaluated using 74 normal blood donor specimens from southeastern United States. Of the 74 specimens, three were reactive (4.0 %), and 71 were non-reactive (96.0 %). A frequency distribution of the actual results appears below:

Restrictions: For Research Use only

Handling

Storage:	4 °C
Expiry Date:	12-18 months

