EBV-VCA IgM Test System

REF 9Z9201M/SM9Z9201M
9Z9201MB

Σ96 Σ480

IVD

CE

Rx Only

INTENDED USE

The ZEUS ELISA 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. The Test System is intended to be used for the diagnosis of EBV-associated infectious mononucleosis when used in conjunction with other EBV serological assays. This test is for *In Vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Epstein - Barr virus (EBV) is a ubiquitous human virus which causes infectious mononucleosis (IM), a self-limiting lymphoproliferative disease (1). By adulthood virtually everyone has been infected and has developed immunity to the virus. In underdeveloped countries, seroconversion to the virus takes place in early childhood and is usually asymptomatic (2). In more affluent countries, primary EBV infections are often delayed until adolescence or later, and manifest as IM in about 50% of this age group (3 - 5).

Following seroconversion, whether symptomatic or not, EBV establishes a chronic, latent infection in B lymphocytes which probably lasts for life (6). EBV replicates in oropharyngeal epithelial cells and is present in the saliva of most patients with IM (7). In addition, 10-20% of healthy persons who are EBV antibody positive shed the virus in their oral secretions (6 - 8). Reactivation of the latent viral carrier state, as evidenced by increased rates of virus shedding, is enhanced by immunosuppression, pregnancy, malnutrition, or disease (8, 9). Chronic EBV infections, whether latent or active, are rarely associated with disease. However, EBV has been implicated at least as a contributing factor in the etiology of nasopharyngeal carcinoma, Burkitt's lymphoma, and lymphomas in immunodeficient patients (4, 8).

The Paul-Bunnell-Davidsohn test for heterophile antibody is highly specific for IM (10). However, 10-15% of adults and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies (11). There is a need for EBV-specific serological tests to differentiate primary EBV infections that are heterophile negative, from mononucleosis-like illnesses caused by other agents such as cytomegalovirus, adenovirus, and *Toxoplasma gondii* (4).

Antibody titers to specific EBV antigens correlate with different stages of IM (4, 10 - 12). Both IgM and IgG antibodies to the Viral Capsid Antigen (VCA) peak three to four weeks after primary EBV infection. IgM anti-VCA decline rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. Antibodies to EBV Nuclear Antigen (EBNA) develop from one to six months after infection and, like anti-VCA, persist indefinitely (11, 12). Antibodies to EBNA indicate that the infection was not recent (11).

EBV Early Antigens (EA) consists of two components; diffuse (D), and restricted (R). The terms D and R reflect the different patterns of immunofluorescence staining exhibited by the two components (13, 14). Antibodies to EA appear transiently for up to three months during the acute phase of IM in 85% of patients (15, 16). The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children produces antibodies to the R component (5, 11). A definitive diagnosis of primary EBV infection can be made with 95% of acute phase sera based on the detection of antibodies to VCA, EBNA, and EA (12). High levels of anti-VCA together with anti-EBNA and anti-EA-R are associated with reactivation of the latent viral carrier state (16, 17). Research shows high levels of IgG anti-VCA in sera of patients with immunodeficiencies (6, 18), recurrent parotitis (19), multiple sclerosis (20), and nasopharyngeal carcinoma (21); as well as immunosuppressed patients (8, 22), pregnant women (23), and persons of advanced age (17).

Screening for the presence of antibodies to VCA and related antigens of EBV can provide important information for the diagnosis of EBV infection. Indirect immunofluorescence has been the serologic method most commonly used to detect antibodies to EBV antigens (11). However, the ELISA procedure, first described by Engvall and Perlman (24, 25), may be a sensitive and reliable method for detection of antibodies to EBV antigens (26, 27). The ELISA procedure allows an objective determination of antibody status to be made on a single dilution of the test specimen and is suitable for screening large numbers of patient samples.

PRINCIPLE OF THE ASSAY

The ZEUS ELISA EBV-VCA IgM Test System is designed to detect IgM class antibodies to EBV IgM in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with EBV antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains antihuman IgG that precipitates and removes IgG and rheumatoid factor from the sample leaving IgM free to react with the immobilized antigen. During sample incubation any antigen specific IgM antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgM is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and Sample Diluent.**

Component		Σ96	Σ480	Description
PLATE		1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with 125kD capsid peptide purified from induced P3-HR1 cells. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific) in 15mL, white-capped bottle(s). Ready to use.
CONTROL	+	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s).
CAL		1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s).
CONTROL	-	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s).
DIL	SPE	1	4	Sample Diluent: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Purple Solution. Ready to use.
SOLN	TMB	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	1	3	Stop Solution: 15mL, red-capped, bottle(s) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped, bottle(s) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably within the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA Plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. ource materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (33).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA Plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent
2°C – 25°C	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.

3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (28, 29). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera and samples that contain high levels of IgG. High levels of IgG have been shown to reduce reactivity to VZV IgM antibody.
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 a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (34).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank 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 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
4. To individual wells, add 100µL 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 100µL 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 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly 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 day's run.
   b. **Automated Wash Procedure**:
      If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/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 firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. 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 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
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, Positive Control, and Negative Control 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.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *Cutoff OD 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 = Cutoff OD Value)
   c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

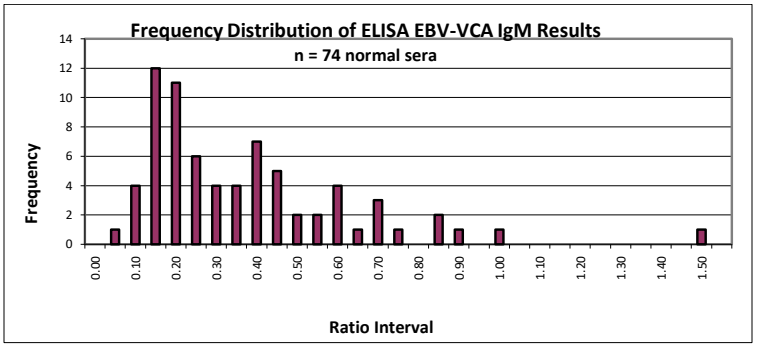
   a. An OD ratio ≤0.90 indicates no significant amount of IgM antibodies to EBV-VCA detected. A negative result indicates no active infection with EBV and should be reported as non-reactive for EBV-VCA IgM antibody.
   b. An OD ratio ≥1.10 indicates that IgM antibodies specific to EBV-VCA were detected. A positive test result indicates a current or reactivated infection with EBV-VCA, and should be reported as reactive for EBV-VCA IgM antibody.
   c. 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.
   d. The numeric value of the final result above the cutoff is not indicative of the amount of anti-EBV-VCA IgM antibody present.

LIMITATIONS OF THE ASSAY

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. Do not use the antibody titer of a single serum specimen to determine recent infection. Interpret test results for anti-VCA 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. Specific IgM antibodies are usually detected in patients with recent primary infection, but may be found in patients with reactivated or secondary infections, and 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 eight 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 have not been established with EBV-associated disease other than infectious mononucleosis.
8. Evaluate test results in relation to patient symptoms, clinical history, and other laboratory findings to establish a diagnosis.

EXPECTED RESULTS

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:



PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

Clinical studies were conducted to demonstrate the clinical efficacy of the ZEUS 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 ZEUS 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:

Table 1: Clinical Site One

Heterophile/EBNA Profile	Stage/IgM Activity	Positive	Negative	Equivocal[a]	Total
Heterophile-, EBNA + 96/102 (94%), VCA IgG Positive 0/102 (0%), VCA IgG Equivocal 6/102 (6%), VCA IgG Negative	Past Infection IgM Negative	9	90	3	102
Heterophile -, EBNA - 2/33 (6%), VCA IgG Positive 3/33 (9%), VCA IgG Equivocal 28/33 (85%), VCA IgG Negative	Never Infected IgM Negative	0	33	0	33
Heterophile +, EBNA - 8/21 (38%), VCA IgG Positive 5/21 (24%), VCA IgG Equivocal 8/21 (38%), VCA IgG Negative	Acute Infection IgM Positive	19	1	1	21
Heterophile +, EBNA + 1/2 (50%), VCA IgG Positive 1/2 (50%), VCA IgG Equivocal 0/2 (0%), VCA IgG Negative	Reactivation IgM Positive	1	1	0	2

[a] Equivocal 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 = 93.2% (88.9% to 97.5%) [b] **Assay Sensitivity: 20/22 = 90.9% (70.8% to 98.9%) [c]**

Percent Agreement: 143/154 = 92.9% (88.8% to 96.9%) [b]

[b] Expressed as a 95% confidence interval calculated using the normal method.

[c] Expressed as a 95% confidence interval calculated using the exact method.

Table 2: Clinical Site Two

Heterophile/EBNA Profile	Stage/IgM Activity	Positive	Negative	Equivocal[a]	Total
Heterophile-, EBNA + 65/72 (90.3%) VCA IgG Positive 1/72 (1.4%), VCA IgG Equivocal 6/72 (8.3%) VCA IgG Negative	Past Infection IgM Negative	13	55	4	72
Heterophile -, EBNA - 4/38 (10%) VCA IgG Positive 1/38 (3%) VCA IgG Equivocal 33/38 (87%) VCA IgG Negative	Never Infected IgM Negative	7	31	0	38
Heterophile +, EBNA - 5/26 (19%), VCA IgG Positive 1/26 (4%), VCA IgG Equivocal 20/26 (77%) VCA IgG Negative	Acute Infection IgM Positive	25	1	0	26
Heterophile +, EBNA + 6/11 (55%) VCA IgG Positive 0/11 (0%) VCA IgG Equivocal 5/11 (45%) VCA IgG Negative	Reactivation IgM Positive	11	0	0	11

[a] Equivocal 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 147 specimens tested at site 2, there were initially seven (7) equivocal samples. One repeated as negative, two repeated as positive, and four were not repeated due to insufficient volume.

Assay Specificity: 86/106 = 81.1% (73.7% to 88.6%) [b] **Assay Sensitivity: 36/37 = 97.3% (85.8% to 99.9%) [c]**

Percent Agreement: 122/143 = 85.3% (79.5% to 91.1%) [b]

[b] Expressed as a 95% confidence interval calculated using the normal method.

[c] Expressed as a 95% confidence interval calculated using the exact method.

Table 3: Clinical Sites One & Two Combined

Heterophile/EBNA Profile	Stage/IgM Activity	Positive	Negative	Equivocal[a]	Total
Heterophile-, EBNA + 161/174 (92.5%) VCA IgG Positive 1/174 (0.6%), VCA IgG Equivocal 12/174 (6.9%),VCA IgG Negative	Past Infection IgM Negative	22	145	7	174
Heterophile -, EBNA - 6/71 (8.4%), VCA IgG Positive 4/71 (5.6%),VCA IgG Equivocal 61/71 (85.9%),VCA IgG Negative	Never Infected IgM Negative	7	64	0	71
Heterophile +, EBNA - 13/47 (27.7%), VCA IgG Positive 6/47 (12.8%), VCA IgG Equivocal 28/47 (59.6%), VCA IgG Negative	Acute Infection IgM Positive	44	2	1	47
Heterophile +, EBNA + 7/13 (53.8%), VCA IgG Positive 1/13 (7.7%), VCA IgG Equivocal 5/13 (38.5%), VCA IgG Negative	Reactivation IgM Positive	12	1	0	13

[a] Equivocal 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.

Assay Specificity: 209/238 = 87.8% (83.7% to 92.0%) [b] **Assay Sensitivity: 44/46 = 95.6% (85.2% to 99.5%) [c]**

Percent Agreement: 253/284 = 89.1% (85.5% to 92.7%) [b]

[b] Expressed as a 95% confidence interval calculated using the normal method.

[c] Expressed as a 95% confidence interval calculated using the exact method.

2. **Reproducibilty**

Reproducibility studies were conducted at both clinical sites. Briefly, six specimens were tested; three strong positive specimens, two moderately positive specimens (close to the cutoff), and one negative specimen. Each specimen was tested in triplicate each day, for a total of three days. The resulting data was used to calculate both intra -assay and inter-assay reproducibility. This has been summarized in Table 4 below:

Table 4: Summary of Reproducibility

		Intra-Assay									Inter-Assay		
		Mean Ratio			Standard Deviation			% CV					
Sample	**Site**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Mean**	**Std**	**% CV**
VM1	1	3.62	3.71	3.49	0.05	0.05	0.02	1.4	1.3	0.6	3.61	0.10	2.8
	2	3.75	3.29	2.98	0.15	0.18	0.59	4.0	5.6	19.9	3.34	0.43	14.6
	3	2.90	2.78	3.13	0.21	0.15	0.23	7.2	5.3	7.4	2.93	0.25	8.4
VM3	1	1.19	1.12	1.09	0.08	0.05	0.05	6.6	4.2	5.0	1.13	0.08	6.7
	2	1.23	1.03	1.06	0.16	0.11	0.10	13.0	11.1	9.2	1.11	0.16	14.0
	3	1.00	0.90	1.00	0.24	0.06	0.26	24.0	6.7	25.5	1.00	0.21	21.5
VM5	1	3.9	4.31	3.64	0.03	0.05	0.04	0.9	1.2	1.2	3.96	0.28	7.0
	2	3.6	3.75	4.19	0.12	0.51	0.14	3.3	13.5	3.4	2.87	0.38	9.9
	3	3.7	3.43	3.47	0.10	0.09	0.16	2.6	2.7	4.5	3.55	0.19	5.4
VM6	1	2.34	2.38	2.23	0.07	0.10	0.01	3.0	4.3	0.6	2.32	0.10	4.2
	2	2.13	2.16	2.50	0.16	0.01	0.08	7.6	0.4	3.3	2.30	0.20	8.7
	3	1.87	1.86	1.82	0.07	0.04	0.12	3.6	2.2	6.4	1.85	0.08	4.5
VM7	1	1.27	1.24	1.20	0.02	0.03	0.03	1.7	2.7	2.6	1.24	0.04	3.2
	2	0.98	1.10	1.30	0.03	0.04	0.05	3.0	4.2	4.0	1.10	0.15	13.4
	3	0.93	0.92	0.91	0.03	0.03	0.09	2.9	2.8	10.1	0.92	0.06	6.2
VM10	1	0.03	0.02	0.03	0.06	0.03	0.03	25.7	14.3	17.1	0.02	0.05	22.9
	2	0.09	0.10	0.12	0.02	0.05	0.01	18.2	54.9	10.7	0.10	0.03	33.8
	3	0.05	0.09	0.07	0.01	0.03	0.02	30.1	27.2	23.3	0.07	0.03	39.3

3. **Cross Reactivity/Interfering Substances**

A. Effect of Rheumatoid Factor (RF):
Experimentation was conducted to demonstrate the effectiveness of the diluent at removing potentially interfering RF antibodies. Briefly, twelve specimens which were RF positive and EBV-VCA IgG positive were tested with and without the anti-IgG absorbent included in the ZEUS ELISA EBV-VCA IgM Test System. The results of this study are shown in Table 5.

B. Effective Removal of Competing IgG Antibody:
Specimens which were positive for IgG antibody and IgM antibody to EBV-VCA were tested with and without treatment to demonstrate the effectiveness of the diluent in removing IgG. The results of the study have been summarized in Table 6.

C. Cross Reactivity with Anti-viral IgM Antibodies:
Samples negative for EBV-VCA IgM antibody and positive for IgM antibodies to various viruses such as CMV, Herpes, and Rubella were tested on the ZEUS ELISA EBV-VCA IgM Test System. One specimen with anti-HSV-1/2 IgM antibody produced an equivocal result. All of the remaining samples were negative. The results of this study have been summarized in Table 7.

Table 5: Effect of Diluent on RF Positive, EBV-VCA IgG Positive and EBV-VCA IgM Negative Specimens.

	ZEUS ELISA EBV-VCA IgM Result (Ratio)				
Sample ID	**With Test System Diluent**		**Diluent without Anti-IgG**		**RF Result [a]**
NA1	0.209	Neg.	0.686	Neg.	0.96
NA4	0.040	Neg.	0.102	Neg.	2.1
NB6	0.109	Neg.	0.259	Neg.	1.7
NC6	0.185	Neg.	0.487	Neg.	1.5
ND3	0.178	Neg.	0.425	Neg.	1.5
ND7	0.145	Neg.	0.360	Neg.	1.3
NF4	0.280	Neg.	0.079	Neg.	0.82
S69	0.120	Neg.	0.240	Neg.	2.5
CBB2	0.132	Neg.	7.871	Pos.	3.1
CBB11	0.184	Neg.	8.446	Pos.	3.1
CBB19	0.899	Neg.	2.262	Pos.	2.7
CBB15	0.302	Neg.	6.235	Pos.	3.1

[a] RF-IgM result determined using a commercial RF ELISA test kit.

RF Interpretation: < 0.80 = Negative 0.80 - 0.99 = Equivocal ≥ 1.00 = Positive

Table 6: Effect of Diluent on EBV-VCA IgG Positive Specimens; Functional Removal of IgG Antibody

	ZEUS EBV-VCA IgG Optical Density (450nm)	
Sample ID	**With Test System Diluent**	**Diluent without Anti-IgG**
VM1	0.010	0.422
VM2	0.012	0.279
VM5	0.019	0.194
VM6	0.014	0.249
15287	0.027	0.335
15288	0.000	0.255
10847	0.030	0.294

NOTE: Human serum samples (n=7) with total IgG concentrations ranging from 4.5 to ≥ 13.9 mg/mL were diluted using the diluent according to the directions within this insert. Following treatment, IgG was not detected in any of the specimens. IgG concentrations were determined using a commercial, quantitative radial immunodiffusion detection test system.

Table 7: ZEUS Scientific Results of Cross Reactivity Testing

IgM Reactivity [a]

Sample ID	ZEUS ELISA EBV-VCA IgM Result (Ratio)	Viral Marker	Result (Ratio)
CMV-3	0.053	CMV IgM	1.150
CMV-4	0.058	CMV IgM	1.497
CMV-7	0.515	CMV IgM	1.261
CMV-10	0.074	CMV IgM	1.422
CMV-13	0.047	CMV IgM	1.532
CMV-14	0.042	CMV IgM	0.781
CMV-18	0.536	CMV IgM	7.576
RUB-1	0.271	Rubella IgM	2.490
RUB-2	0.191	Rubella IgM	1.230
RUB-4	0.063	Rubella IgM	2.340
RUB-7	0.090	Rubella IgM	2.340
RUB-8	0.063	Rubella IgM	1.290
RUB-12	0.159	Rubella IgM	1.090
RUB-19	0.085	Rubella IgM	1.240
RUB-20	0.143	Rubella IgM	1.830
HSV-1	0.287	HSV 1/2	3.43/2.77
HSV-2	0.180	HSV 1/2	1.44/1.33
HSV-3	0.233	HSV 1/2	0.91/0.78
HSV-4	0.600	HSV 1/2	1.99/1.88
HSV-5	0.962	HSV 1/2	1.72/2.71
HSV-6	0.770	HSV 1/2	1.99/0.46

[a] Results of the various specimens using the respective ZEUS ELISA test system. For all ELISA Test Systems, a ratio of less than 0.900 is negative, and a ratio of greater than 1.10 is positive.

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