

### INTENDED USE

The ZEUS ELISA Epstein Barr Virus - Early Antigen (EBV-EA) IgG Test System provides a means for the qualitative detection of IgG class antibodies to Epstein-Barr Virus (EBV) Early Antigen (EA) in human sera. This test is intended to aid in the diagnosis of infectious mononucleosis (IM) when used with other EBV serological tests. The performance characteristics have not been established to aid in the diagnosis of acute IM. This test is intended for *In Vitro* diagnostic use only.

### SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus (EBV) causes infectious mononucleosis; a self-limiting lymphoproliferative disease (1). EBV is a ubiquitous human virus. 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, 4, and 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). Also, 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 (13). However, 10 - 15% of adults, and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies (14). EBV specific serological tests are needed 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, 13, and 14). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak three to four weeks after primary EBV infection. IgM anti-VCA antibodies decline rapidly and is usually undetectable after 12 weeks. IgG anti-VCA antibody titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBV-NA) detected by anti-complement immunofluorescence develop from one month to six months after infection; and, like anti-VCA, persist indefinitely (15, 16). Antibodies to EBV-NA indicate that the EBV infection was not recent (14).

EBV early antigen (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 (17). Antibodies to EA may appear transiently for up to three months or longer during the acute phase of IM in 85% of patients (28). The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children may produce antibodies to the R components (14). A definitive diagnosis of primary EBV infection can be made with 95% of acute phase sera based on antibody titers to VCA, EBV-NA, and EA (28).

Antibodies to EA, usually to the R component, together with antibodies to EBV-NA and high titers of IgG anti-VCA, may be associated with reactivation of the latent viral carrier state (19, 20). EBV positive serology associated with reactivation of EBV is found in sera of patients with immunodeficiencies (21), patients with recurrent parotitis (22), immunosuppressed patients (8, 23), pregnant women (24), and persons of advanced age (20). Antibodies to the R component may be found at moderate to high levels in patients with Burkitt's lymphoma (4). In contrast, patients with nasopharyngeal carcinoma may produce high titer antibodies to the D component (18).

Elevated levels of anti-EA and IgG anti-VCA may be detected in patients with chronic or recurrent illness suspected of being caused by EBV (1 - 12, 21, and 27). However, a diagnosis of chronic EBV should not be based on the presence of antibodies to EA since elevated anti-EA titers may also be found in patients with other diseases as well as in healthy individuals with past EBV infections (6, 20, 25, 26, and 28).

### PRINCIPLE OF THE ASSAY

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

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific 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 IgG is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted 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 SAVE Diluent®.**

PLATE	1. Plate: 96 wells configured in twelve, 1x8-well, strips coated with recombinant EBV-EA antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	2. Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). One, 15mL, white-capped bottle. Ready to use.
CONTROL +	3. Positive Control (Human Serum): One, 0.35mL, red-capped vial.
CAL	4. Calibrator (Human Serum): One, 0.5mL, blue-capped vial.
CONTROL -	5. Negative Control (Human Serum): One, 0.35mL, green-capped vial.
DIL SPE	6. SAVE Diluent®: One, 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use. <b>NOTE: The SAVE Diluent® will change color when combined with serum.</b>
SOLN TMB	7. TMB: One, 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	8. Stop Solution: One, 15mL, red-capped, bottle containing 1M H <sub>2</sub> SO <sub>4</sub> , 0.7M HCl. Ready to use.
WASHBUF 10X	9. Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. One, 100mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). <b>NOTE: 1X solution will have a pH of 7.2 ± 0.2.</b>

**NOTES:**

- 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. SAVE Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.
- Test System also contains a Component Label containing lot specific information inside the Test System box.

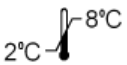
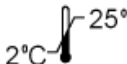
**PRECAUTIONS**

- For *In Vitro* diagnostic use.
- 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.
- The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
- The Controls are **potentially biohazardous materials**. Source 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 (31).
- 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.
- 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.
- The SAVE 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 on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
- 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.
- The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
- The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
- Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
- Dilution or adulteration of these reagents may generate erroneous results.
- Do not use reagents from other sources or manufacturers.
- 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.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross contamination of reagents and/or samples could cause erroneous results.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Avoid splashing or generation of aerosols.
- Do not expose reagents to strong light during storage or incubation.
- Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
- 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.
- Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
- Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
- 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.
- 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**

- 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.**
- Pipettes capable of accurately delivering 10 - 200µL.
- Multichannel pipette capable of accurately delivering 50 - 200µL.
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite).

**STORAGE CONDITIONS**

	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, SAVE Diluent®
	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**

- 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).
- No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.

- Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (29, 30). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
- 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 (32).

### ASSAY PROCEDURE

- Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
- 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	

- Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVE Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVE Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
- 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.
- Add 100µL of SAVE Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
- Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
- Wash the microwell strips 5 times.
  - Manual Wash Procedure:**
    - Vigorously shake out the liquid from the wells.
    - Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
    - Repeat steps 1. and 2. for a total of 5 washes.
    - 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.
  - 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.
- 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.
- 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 100µL of TMB to each well, including the 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 10 - 15 minutes.
- 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.
- 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

- Dilute Serum 1:21.
- Add diluted sample to microwell - 100µL/well.
- Incubate 25 ± 5 minutes.
- Wash.
- Add Conjugate - 100µL/well.
- Incubate 25 ± 5 minutes.
- Wash.
- Add TMB - 100µL/well.
- Incubate 10 - 15 minutes.
- Add Stop Solution - 50µL/well - Mix.
- READ within 30 minutes.

### QUALITY CONTROL

- 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.
- 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.
- The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	<u>OD Range</u>
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

- The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
  - The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
  - 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, but 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 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.  
( $CF \times \text{Mean OD of Calibrator} = \text{Cutoff OD Value}$ )
- 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	=	$0.793 \times 0.25 = 0.198$
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	$\leq 0.90$
Equivocal Specimens	0.91 - 1.09
Positive Specimens	$\geq 1.10$

- a. An OD ratio  $\leq 0.90$  indicates no significant amount of IgG antibodies to EBV-EA detected.
- b. An OD ratio  $\geq 1.10$  indicates that IgG antibodies specific to EBV-EA were detected.
- 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. Evaluate repeatedly equivocal specimen using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

## LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis on the basis of ZEUS ELISA EBV-EA IgG Test System alone. Interpret test results for anti-EBV-EA in conjunction with the clinical evaluation and the results of other diagnostic procedures. Consider test results for VCA and EBNA when evaluating patient specimens for EBV serological status.
2. This test detects both R and D components of EA. The test system is not designed to differentiate between antibodies to the R and D components.
3. Avoid the use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens. Erroneous results may occur.
4. The assay performance characteristics have not been established for matrices other than sera.
5. The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated to IFA titers.
6. Assay performance characteristics have not been established for visual result determinations.
7. Use caution when evaluating samples obtained from immunosuppressed patients.
8. Prevalence of the analyte will affect the assay's predictive value.
9. The performance characteristics of this assay have not been established for Burkitt's Lymphoma, nasopharyngeal carcinoma, and lymphoproliferative disorders. The performance has been established for the aid in the diagnosis of EBV-associated infectious mononucleosis.

## EXPECTED RESULTS

Scientists analyzed the data, 250 specimens, tested at the two clinical sites to establish/estimate the expected reactivity rate. This represented two groups of specimens; 150 clinical specimens, sent to the laboratory for routine EBV serological analysis, and 100 random normal donor specimens. With respect to the clinical population, 43/150 (28.7%) were positive, 101/150 (67.3%) were negative, and 6/150 (4.0%) were equivocal. With respect to the normal population, 18/100 (18%) were positive, 76/100 (76%) were negative, and 6/100 (6%) were equivocal.

## PERFORMANCE CHARACTERISTICS

### 1. Comparative Study

A comparative study was performed to demonstrate the equivalence of the ZEUS ELISA EBV-EA IgG Test System to the ZEUS IFA EBV-EA Test System when evaluating specimens submitted for the diagnosis of IM. The performance of the ZEUS ELISA EBV-EA IgG Test System was evaluated in a three-site clinical investigation. Briefly, there were a total of 273 specimens tested; 125 at Site One, 125 at Site Two, and 23 at Site Three. Clinical specimens tested at Site One and Two consisted of a mixture of routine specimens which were sent to a reference laboratory in Southwestern United States for normal EBV serological analysis, and normal donor specimens. Repository specimens were tested at Site Three. These had been previously tested and were found to be positive for antibody to EBV-EA. Equivocal specimens were excluded from any further analysis. Table 1 below shows the results from Site One. Table 2 shows the results from Site Two. Results from Site Three have not been shown separately, but are included in Table 3, which shows the results of all three clinical sites combined.

**Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site One**

		ZEUS ELISA EBV-EA IgG Test System			
		Positive	Negative	Equivocal	Total
ZEUS IFA EBV-EA IgG Test System	Positive	22	9	2	33
	Negative	2	73	3	78
	Equivocal	5	5	4	14
	Total	29	87	9	125

Relative Sensitivity =  $22/31 = 71\%$  (95% Confidence Interval\* = 55 to 87%)

Relative Specificity =  $73/75 = 97\%$  (95% Confidence Interval\* = 94 to 100%)

Relative Agreement =  $95/106 = 90\%$  (95% Confidence Interval\* = 84 to 95%)

\* 95% confidence intervals calculated using the exact method.

**Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site Two**

		ZEUS ELISA EBV-EA IgG Test System			
		Positive	Negative	Equivocal	Total
ZEUS IFA EBV-EA IgG Test System	Positive	25	6	4	35
	Negative	7	83	0	90
	Equivocal	0	0	0	0
	Total	32	89	4	125

Relative Sensitivity =  $25/31 = 81\%$  (95% Confidence Interval\* = 67 to 94%)

Relative Specificity =  $83/90 = 92\%$  (95% Confidence Interval\* = 87 to 98%)

Relative Agreement =  $108/121 = 89\%$  (95% Confidence Interval\* = 84 to 95%)

\* 95% confidence intervals calculated using the exact method.

**Table 3: Calculation of Relative Sensitivity, Specificity and Agreement; All Three Sites Combined**

		ZEUS ELISA EBV-EA IgG Test System			Totals
		Positive	Negative	Equivocal	
ZEUS IFA EBV-EA IgG Test System	Positive	67	15	7	89
	Negative	11	156	3	170
	Equivocal	5	5	4	14
	Totals	83	176	14	273

Relative Sensitivity =  $67/82 = 82\%$  (95% Confidence Interval\* = 67 to 94%)

Relative Specificity =  $156/167 = 93\%$  (95% Confidence Interval\* = 90 to 97%)

Relative Agreement =  $223/249 = 90\%$  (95% Confidence Interval\* = 86 to 93%)

\* 95% confidence intervals calculated using the exact method.

In Table 3, above, there were a total of 26 discrepant samples. There were 11/273 (4.0%) samples which were positive on the ELISA and negative by IFA. There were a total of 15/273 (5.6%) samples which were negative on the ELISA and positive by IFA. Of this group of 15 samples, 1/15 had an endpoint titer on IFA of 1:160, 4/15 samples had an endpoint titer of 1:20, and the remainder (10/15) had an endpoint titer on IFA of 1:10. **NOTE: Be advised that relative refers to the comparison of this assay's results to that of a similar assay.** There was not an attempt to correlate the assay's results with disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

All 273 specimens tested during the clinical study were also tested for EBV-VCA IgG, EBV-VCA IgM, EBNA-1 IgG, and heterophile antibody. The results of this ancillary testing is depicted in Table 4.

**Table 4: Results of the ELISA EBV-EA IgG by Category of EBV Serological Status.**

		Acute	Seropositive	Seronegative
		VCA IgM Positive Heterophile Positive EBNA Negative	VCA IgG Positive EBNA Positive VCA IgM Negative Heterophile Negative	VCA IgG Negative VCA IgM Negative EBNA Negative Heterophile Negative
ZEUS ELISA EBV-EA IgG Results	Positive	0	54	1
	Negative	2	106	29
	Equivocal	0	10	1
	Total	2/273	170/273	31/273

## 2. Reproducibility

Reproducibility was evaluated as outlined in the FDA guidance document; [Review Criteria for In Vitro Diagnostic Devices for Detection of IgM antibodies to Viral Antigens](#). Reproducibility studies were conducted at both clinical sites using the same specimens. Briefly, six specimens were tested, two relatively strong positive specimens, two specimens near the cut-off, and two which were clearly negative. Additionally, the kit's negative control and positive control were included as additional panel members at site one, for a total of eight specimens. On each day of testing, each of the eight specimens were assayed in eight replicate wells. Testing was performed for a total of three days at each site. A summary of this investigation appears in Table 5 below:

**Table 5: Summary of Precision Testing Conducted at Clinical Sites One and Two**

Specimen	Site	Mean Ratio	Result	Standard Deviation	Overall % CV
1	1	3.46	Positive	0.55	16.0
	2	3.06		0.31	10.0
2	1	2.78	Positive	0.60	21.7
	2	2.76		0.30	10.9
3	1	1.81	Near Cutoff	0.34	18.7
	2	1.69		0.28	16.6
4	1	1.15	Near Cutoff	0.19	16.5
	2	1.13		0.26	23.0
5	1	0.27	Negative	0.06	24.0
	2	0.37		0.07	17.8
6	1	0.28	Negative	0.08	30.1
	2	0.75		0.10	13.5
Positive Control	1	3.71	Positive	1.00	27.0
	2	3.49		0.47	13.4
Negative Control	1	0.28	Negative	0.06	23.6
	2	0.28		0.03	12.2

## 3. Cross Reactivity

A study was performed to investigate the possibility of cross-reactivity with other viruses. In this study, ten specimens were evaluated. Two of the specimens were strongly reactive to Rubella, two to Rubeola, two to HSV-1, two to HSV-2, and two to CMV. None of the ten specimens were reactive on the ZEUS ELISA EBV-EA IgG test system, indicating that there is little potential for cross-reactivity with such patient specimens.

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