INSTRUCTIONS FOR USE

EN

Anti-EBV EBNA-1 IgG

REF 9Z9461G
SM9Z9461G

IVD

CE

Rx Only

Σ 96

INTENDED USE

The Anti-EBV EBNA-1 IgG provides a means for the qualitative detection of IgG antibodies to the nuclear antigen-1 of Epstein-Barr virus (EBNA-1) in human sera. When performed according to these instructions, the results of this test together with other testing, such as the heterophile test, and the EBV-VCA IgG and IgM tests, may aid in the diagnosis of, and provide information on infectious mononucleosis (IM), that may be of value in patient management and treatment. It is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Epstein-Barr Virus (EBV) causes infectious mononucleosis (IM), 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 lasts probably 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). Recent reports suggest that EBV may cause a chronic fatigue syndrome in some persons (9 - 12).

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). 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, 13 - 15). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak three to four weeks after primary EBV infection. IgM anti-VCA declines rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking and last indefinitely. Antibodies to the D component of early antigen (EA) may appear transiently for up to three months during the acute phase of IM in 85% of patients (15). Antibodies to the R component of EA may appear transiently during late convalescence (15). Antibody titers to EA, usually to the R component, may be associated with reactivation of the latent viral carrier state (9, 15 - 20).

Unlike antibodies to VCA and EA, antibodies to EBV nuclear antigen (EBNA) are rarely present during the acute phase of IM but develop during convalescence (15, 21). Antibodies to EBNA gradually increase in titer and, after three months to one year, reach a plateau level where they persist for life in most individuals (15, 21). Therefore, the presence of antibodies to EBNA indicates that the EBV infection was not recent.

Although the classical anticomplementary immunofluorescence (ACIF) assay (27) has been employed to measure EBNA antibodies in human serum, ELISA methods have recently become available. The ELISA methods utilize purified EBNA antigens that have been reported to be of higher titers than ACIF assays, and to be undetectable by ACIF in some IM patients (28, 29, and 30).

PRINCIPLE OF THE ASSAY

The Anti-EBV EBNA-1 IgG is designed to detect IgG class antibodies to Epstein-Barr Virus Nuclear Antigen in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with EBNA-1 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®.

Kit Component	Quantity (Σ 96)	Description
PLATE	1	Plate: 96 wells configured in twelve, 1x8-well, strips coated with recombinant EBNA-1 peptide. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). 15mL, white-capped bottle. Ready to use.
CTRL +	1	Positive Control (Human Serum): 0.35mL, red-capped vial. 21X concentrate.
CAL	1	Calibrator (Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	Negative Control (Human Serum): 0.35mL, green-capped vial. 21X concentrate.
DIL SPE	1	SAVe Diluent®: 30mL, green-capped, bottle containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN TMB	1	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle 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.**

NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with 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.

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 remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, 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

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**. 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 (30).
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 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 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.

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.
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 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 (31).

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 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.**
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 SAVe 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 - 1.09
Positive Specimens	≥1.10

a. An OD ratio ≤0.90 indicates no significant amount of IgG antibodies to EBNA-1 detected. A negative result indicates no current or previous infection with EBV. Presume that such individuals are susceptible to primary infection.

b. An OD ratio ≥1.10 indicates that IgG antibodies specific to EBNA-1 were detected, denoting a previous infection with EBV.

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 anti-EBNA results alone. Interpret test results for anti-EBNA in conjunction with results of antibody tests for other EBV specific antigens; VCA IgG and IgM antibodies and Early Antigen (EA) antibodies.
2. Interpret test results in conjunction with the clinical evaluation and the results of other diagnostic procedures.
3. Patients with severe immunocompromised conditions may show negative results for EBNA antibodies even if antibodies to VCA are present (28). Likewise, an anti-EBNA response may fail to develop in patients who have an immunodeficiency disease or who are immunosuppressed (22, 26).
4. This assay detects antibody to the EBNA-1 antigen and not any other EBNA antigens.
5. 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 IM.

EXPECTED RESULTS

In classical EBV associated IM, both IgG and IgM VCA antibodies usually rise rapidly following the onset of disease and reach peak titers concurrently with clinical symptoms EBNA antibodies are normally absent during the acute phase of IM or are present at very low titers (14, 15). EBNA antibodies reach peak titers from three to 12 months after onset of IM and persist for life in most individuals (21). Titers may vary with tests employed. Antibodies to EBNA, coupled with IgG antibodies to VCA and the absence of IgM antibodies to VCA, indicate a past EBV infection (14, 15). Primary acute EBV infection is indicated by the presence of IgG antibodies to VCA, anti-EA and/or IgM VCA antibodies, and the absence of EBNA antibodies. Although EBNA antibodies during the first three months of IM are predominantly directed against EBNA-2, most cases of IM are also associated with EBNA-1 with rare exception (31). EBNA-1 antibodies appear late in the course of illness and persist for years. Nearly all human sera containing antibodies to EBNA recognize the EBNA-1 antigen (30, 31). The presence of EBNA antibodies in the general population varies with age. EBV infections occur primarily before age three or during adolescence (age 13 - 20) depending primarily on socioeconomic conditions. The incidence of infection between the ages of three and 13 is sporadic and is rare after age 30. The prevalence of EBNA antibodies is virtually 100% by the age of 30 (33). A study was conducted to determine reactivity rates using two asymptomatic populations (n=72 and n=85) from southeastern United States. Positivity rates were 97.2% and 91.8% respectively. Additionally, Figure One shows a frequency distribution of the results from the clinical investigation described below.

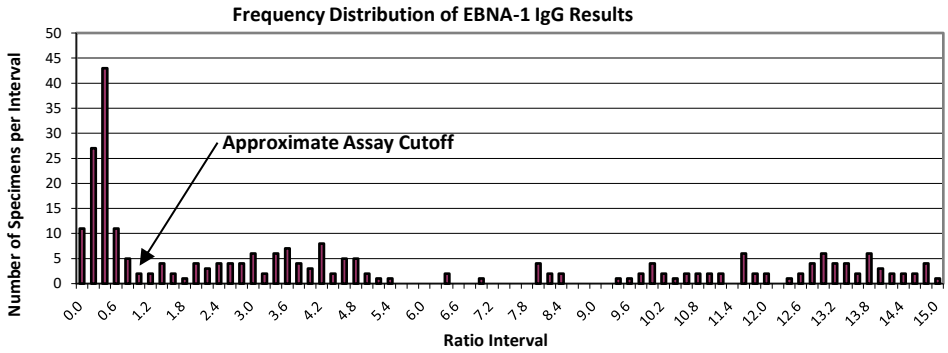

Figure 1: Frequency Distribution of Results from Clinical Investigation.

PERFORMANCE CHARACTERISTICS

1. Comparative Study

Comparative studies were performed to demonstrate the equivalence of the Anti-EBV EBNA-1 IgG to another ELISA test system currently in commercial distribution using a two-site clinical investigation. Briefly, there were a total of 271 specimens tested; 144 at Site One, and 127 at Site Two. Specimens tested at Site One included 124 samples sent to a reference laboratory for routine EBV serology, and 20 repository pediatric samples previously characterized as EBNA negative. Specimens tested at Site Two included 100 specimens tested for routine EBV serology, 7 specimens previously characterized as EBV-VCA IgM positive, and 20 specimens previously characterized as EBV negative. Table 1 summarizes the results of this comparative study. Table 2 highlights the heterophile positive sub-population from this clinical study. Included within the table are also results for EBV-VCA IgG and EBV-VCA IgM. EBNA-1 results are indicated for both the ZEUS ELISA, and the commercial EBNA-1 IgG ELISA.

Table 1: Summary of Initial Sensitivity and Specificity		**Anti-EBV EBNA-1 IgG**			
		Positive	Negative	Equivocal*	Total
Commercial EBNA ELISA Test System	Positive	169	10*	3	182
	Negative	1*	86	0	87
	Equivocal*	0	2	0	2
	Total				271

*Equivocal and discrepant results. All percentages expressed using 95% confidence intervals by the exact method

Relative Sensitivity = 169/179 = 94.4 (90.0% to 97.3%) Relative Specificity = 86/87 = 98.9% (93.8% to 100%)

% Agreement = 225/226 = 95.9 (92.7% to 97.9%)

Analysis of Discrepant Results

Thirteen specimens were positive on the commercial ELISA, and ZEUS ELISA negative (n=10), or equivocal (n=3). Four of 13 were confirmed discrepant and found to be EBNA ACIF negative. Four of 13 were confirmed discrepant and found to be EBNA ACIF positive. Two of 14 were initially ZEUS ELISA equivocal; however, repeat testing resulted in a positive final ZEUS ELISA result. One of 14 was repeatedly ZEUS ELISA equivocal, and therefore was excluded from final calculations. Two of 14 were not resolved due to insufficient volume, and therefore were excluded from final calculations. One specimen was negative on the commercial ELISA, and ZEUS ELISA positive. It was confirmed as a discrepant and found to be EBNA ACIF negative. Two specimens were initially equivocal on the commercial ELISA and upon retesting 1 out of 2 agreed; and therefore did not require IFA testing. One of two was confirmed as a discrepant and found to be EBNA ACIF positive. **NOTE: Resolution of the discrepant specimens resulted in a total of 176 positive specimens on the commercial test system; of which, 171 were also positive on the Anti-EBV EBNA-1 IgG.** Ninety-two specimens were negative on the commercial test system; of which, 91 were also negative on the Anti-EBV EBNA-1 IgG. As a result, there was agreement between the two test systems for 262 of the 268 specimens.

Table 2: Results of all Heterophile Positive Specimens from the Clinical Study:

Anti-EBV EBNA-1 IgG	Commercial EBNA ELISA	Anti-EBV VCA IgG	Heterophile Results	Commercial VCA IgM ELISA
Negative	Negative	Negative	Positive	Equivocal
Negative	Negative	Positive	Positive	Positive
Negative	Negative	Positive	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Equivocal[a]	Positive	Positive	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative[b]	Positive	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative[c]	Positive	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative[d]	Positive	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Negative	Positive	Positive
Negative	Negative	Positive	Positive	Negative
Positive	Positive	Positive	Positive	Negative
Positive	Positive	Equivocal	Positive	Positive

[a] Sample was repeatedly equivocal [b] EBNA ACIF Positive at 1:5 [c] EBNA ACIF Negative [d] EBNA ACIF Negative

2. Reproducibility

Reproducibility was evaluated as outlined in document number EP5-T2: Evaluation of precision Performance of Clinical Chemistry Devices - Second Edition, as published by Clinical Laboratory Standards Institute (CLSI), Villanova, PA. Reproducibility studies were conducted at both clinical sites, as well as at ZEUS Scientific, in Raritan, NJ. Briefly, six specimens were tested: two strong positive specimens, two moderately positive specimens, and two negative specimens. Each specimen was tested in duplicate, two times per day (AM and PM), on each of 20 days. The resulting data was used to calculate the within-run precision estimate, the total precision estimate, and the coefficient of variation where appropriate. Daily results, which included ratios resulting from suspected technical error, were eliminated, and the above-mentioned statistics were recalculated. These results are summarized in Table 3. Table 4 shows the reproducibility of the three replicates of the calibrator for the 20 day reproducibility study. The Calibrator was testing in triplicate for every run. For each run, the OD of the calibrator was converted to an index value. It depicts the highest and lowest index values of the 60 different OD values, the highest and lowest CV of the three replicates for the 20 runs, and the overall CV of all 60 index values of the Calibrator.

Table 3: Summary of Reproducibility Testing

ID	(Site)	Mean Ratio	Swr[a]	S_t[b]	Days Tested	% CV	Total Observations
Strong Positive 1	(1)	3.59	0.23	0.52	19	14.54	76
	(2)	4.08	0.19	0.36	20	8.71	80
	(3)	4.01	0.23	0.41	20	10.23	80
Strong Positive 2	(1)	3.66	0.36	0.59	19	16.18	76
	(2)	4.17	0.20	0.35	20	8.35	80
	(3)	3.98	0.15	0.32	19	8.18	76
Weak Positive 1	(1)	2.37	0.09	0.35	19	14.81	76
	(2)	2.67	0.14	0.21	20	7.76	80
	(3)	1.88	0.17	0.20	18	10.67	72
Weak Positive 2	(1)	2.12	0.18	0.30	19	14.08	76
	(2)	2.32	0.15	0.20	20	8.71	80
	(3)	2.18	0.11	0.17	16	7.69	64
Negative 1	(1)	0.03	0.03	0.05	19	N/A	76
	(2)	0.01	0.02	0.02	20	N/A	80
	(3)	0.02	0.02	0.04	20	N/A	80
Negative 2	(1)	0.14	0.05	0.08	19	N/A	76
	(2)	0.27	0.02	0.04	20	N/A	80
	(3)	0.18	0.03	0.06	20	N/A	80

[a] Point estimate of within run precision standard deviation.
[b] Point estimate of total precision standard deviation.

Table 4: Reproducibility of the Triplicate Calibrator

Site	High Ratio	Low Ratio	High %CV	Low %CV	Overall %CV
Site 1, 20 AM Runs	2.46	1.98	7.92	0.99	4.25
Site 1, 20 PM Runs	2.54	1.87	12.26	0.37	4.44
Site 2, 20 AM Runs	2.49	1.85	12.07	1.62	6.66
Site 2, 20 PM Runs	2.68	1.93	14.67	1.56	6.01
ZEUS, 20 AM Runs	2.44	1.94	9.19	0.55	4.22
ZEUS, 20 PM Runs	2.46	2.01	7.43	0.45	3.49

3. Cross Reactivity

Studies were performed to assess interference in the Anti-EBV EBNA-1 IgGusing sera which were negative for antibodies to EBNA and EBV-VCA and which demonstrated antibodies to the following:

HSV-1	n = 5
HSV-2	n = 4
VZV	n = 5
CMV	n = 2
Antinuclear Antibodies	n = 5

This study with the 21 sera listed above resulted in no detectable cross reactivity with these various IgG antibodies and the Anti-EBV EBNA-1 IgG.

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
CE	Conformity with Directive 98/79		

ZEUS Scientific
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111, Option 2
International: +1 908-526-3744
Fax: +1 908-526-2058
Website: www.zeusscientific.com

EC REP

EMERGO EUROPE
Westervoortsedijk 60
6827 AT Arnhem
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