INSTRUCTIONS FOR USE

EN

Anti-EBV VCA IgG

REF 9Z9201G
SM9Z9201G

IVD

CE

Rx Only

Σ 96

INTENDED USE

The Anti-EBV VCA IgG is an ELISA-based test system designed for the qualitative detection of IgG class antibodies to Epstein-Barr Virus Viral Capsid Antigen in human serum. The Test System is intended to be used to evaluate serologic evidence of previous infection with Epstein-Barr Virus and is for *In Vitro* diagnostic use.

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 with 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 antibodies decline rapidly and are usually undetectable after 12 weeks. IgG anti-VCA antibody titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBNA) develop from one month to six months after infection and, like anti-VCA antibodies, persist indefinitely (11, 12). Antibodies to EBNA indicate that the infection was not recent (11).

EBV early antigens (EA) consist of two components; diffuse (D), and restricted (R). The terms D and R reflect the different patterns of immunofluorescent 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 antibodies together with anti-EBNA and anti-EA-R antibodies 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 based on a single dilution of the test specimen and is suitable for screening large numbers of patient samples.

PRINCIPLE OF THE ASSAY

The Anti-EBV VCA IgG is designed to detect IgG class antibodies to Epstein-Barr Virus in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with EBV antigen. The 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 inactivated Epstein-Barr Virus VCA antigen. 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.**

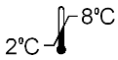
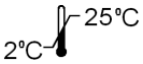
NOTE: The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ZEUS ELISA Test System utilizing Product No. 005CC.

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

	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.
	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 (28).
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 (27, 28). 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 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

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 to 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates no significant amount of IgG antibodies to EBV-VCA 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 EBV-VCA were detected. A positive test result indicates a current or 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.
   d. Values for Calibrators and Controls are assigned based on an internal reference preparation because there are no International Standards for EBV-VCA IgG.

LIMITATIONS OF THE ASSAY

1. Most (80%) of IM individuals have peak anti-VCA IgG titers before they consult a physician (4). Therefore, testing paired acute and convalescent sera for significant changes in antibody levels is not useful in most patients with IM (4).
2. The antibody titer of a single serum specimen cannot be used to determine recent infection. Test results for anti-VCA should be interpreted in conjunction with the clinical evaluation and results of antibody tests for other EBV antigens (*i.e.,* EBNA, EA, and IgM-VCA).

EXPECTED RESULTS

All immunocompetent persons infected with EBV produce antibodies to VCA (6). Both IgM and IgG antibodies to VCA appear rapidly following infection and reach peak titers within three to four weeks (4). IgG antibodies to VCA decline slowly after peaking but persist indefinitely (15). Indications of a primary acute EBV infection are the presence of IgG antibodies to VCA, coupled with anti-EA, and/or IgM anti-VCA antibodies, and the absence of antibodies to EBNA (4, 11). The presence of IgG anti-VCA and anti-EBNA indicates the infection was not recent (4, 11). The incidence of EBV infection varies with age and socioeconomic status (6). In the underdeveloped countries, most persons acquire EBV in early childhood and the infection is usually unapparent (2, 4). In one study in the United States, about 50% of college freshmen were seropositive for EBV (30). In a study conducted by ZEUS Scientific technicians using 135 normal samples from the Southeastern United States, 134/135 samples were positive by both the Anti-EBV VCA IgG and the ZEUS IFA EBV-VCA IgG Test System. In another study consisting of 32 normal pediatric samples, 32/32 samples were negative using both the Anti-EBV VCA IgG , and the ZEUS IFA EBV-VCA IgG Test System. The number of individuals with IgG antibody to EBV-VCA varies with age and socioeconomic status. ZEUS Scientific recommends that each laboratory establish their expected values based upon the population type typically tested.

PERFORMANCE CHARACTERISTICS

1. Comparative Study

A comparative study was conducted to compare the Anti-EBV VCA IgG to another commercial ELISA for the detection of IgG antibodies against EBV-VCA. A total of 199 serum specimens were obtained from two plasma donor centers and a reference laboratory. Below is a results summary.

Table 1: Anti-EBV VCA IgG vs. Commercial EBV-VCA IgG ELISA

		Anti-EBV VCA IgG			
		Positive	Negative	Equivocal*	Total
Commercial EBV-VCA IgG ELISA	Positive	104	8**	3	115
	Negative	10**	46	4	60
	Equivocal*	11	12	1	24
	Total	125	66	8	199

Relative Sensitivity: 104/112 = 92.9% Relative Specificity: 46/56 = 82.1% Percent Agreement: 150/168 = 89.3%

*Equivocal samples not included in calculations. **Discrepant results.

See Table 2 below for the results of the retesting of the discrepant samples using the ZEUS IFA EBV-VCA IgG Test System:

Table 2: Analysis of Discrepant Results

Sample ID	Anti-EBV VCA IgG	Commercial VCA-IgG ELISA	ZEUS IFA EBV-VCA IgG Test System
26	0.251	1.16	-
29	0.261	1.00	-
38	0.358	1.00	-
39	0.368	1.13	-
41	0.391	1.30	-
53	0.582	1.05	-
63	0.767	1.10	+
34	0.797	1.30	+
81	1.243	0.65	+
83	1.270	0.70	+
89	1.399	0.73	+
93	1.449	0.39	-
101	1.614	0.57	+
111	1.859	0.60	+
133	2.216	0.48	+
137	2.254	0.75	+
139	2.320	0.77	+
152	2.730	0.20	+

Summary:

a. Eight samples were negative by ZEUS ELISA and positive by the commercial VCA IgG ELISA. ZEUS IFA confirmed six of these eight samples to be negative.

b. Ten samples were positive by ZEUS ELISA and negative by the commercial VCA IgG ELISA. ZEUS IFA confirmed nine of these ten samples to be positive.

c. Recalculation of the relative sensitivity, relative specificity, and percent agreement, based upon the resolution of the discrepant samples by IFA, provided the results are shown in Table 3:

Table 3: Recalculation of Relative Sensitivity, Specificity, and Percent Agreement

Relative Sensitivity:	113/115 = 98.3%
Relative Specificity:	52/53 = 98.1%
Percent Agreement:	165/168 = 98.2%

2. Reproducibility

Technicians tested five serum samples ranging from positive to negative, using two different master lots of product, to determine intra-assay and inter-assay variation. On each of three days, a technician tested each specimen once a day, eight times each, on each master lot. A responsible party then calculated the mean OD ratio and coefficient of variation from the resulting data. Table 4 (Lot A), and Table 5 (Lot B) summarize the intra-assay and inter-assay results. Table 6 summarizes the overall test variability combining the data points from the lot-to-lot and day-to-day comparison.

Table 4: Anti-EBV VCA IgG Intra-Assay and Inter-Assay Variability Testing Summary

Lot A	Day 1			Day 2			Day 3					
Sample	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV
1. Equivocal	0.96	0.04	4.2	0.77	0.04	5.2	0.99	0.04	4.0	0.91	0.10	11.0
2. Low Positive	1.53	0.12	7.8	1.29	0.11	8.5	1.51	0.07	4.6	1.44	0.15	10.4
3. Low Positive	1.33	0.11	8.3	1.12	0.10	8.9	1.25	0.04	3.2	1.23	0.12	9.8
4. Low Positive	1.52	0.12	7.9	1.12	0.09	8.0	1.43	0.12	8.4	1.36	0.21	15.4
5. Negative	0.15	0.03	20.0	0.11	0.02	18.2	0.15	0.02	13.3	0.14	0.03	21.4

Table 5: Anti-EBV VCA IgG Intra-Assay and Inter-Assay Variability Testing Summary

Lot B	Day 1			Day 2			Day 3					
Sample	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV	Mean Ratio	StD	% CV
1. Equivocal	1.03	0.06	5.8	0.97	0.06	6.2	1.11	0.07	6.3	1.04	0.08	7.7
2. Low Positive	1.38	0.09	6.5	1.38	0.03	2.2	1.29	0.08	6.2	1.35	0.09	6.7
3. Low Positive	1.48	0.10	6.8	1.44	0.02	1.4	1.34	0.05	3.7	1.42	0.09	6.3
4. Low Positive	1.69	0.14	8.3	1.55	0.12	7.7	1.49	0.10	6.7	1.58	0.15	9.5
5. Negative	0.28	0.02	7.1	0.22	0.02	9.1	0.24	0.03	12.5	0.25	0.03	12.0

Table 6: Anti-EBV VCA IgG Variability Testing Summary - Combination of Lot A and Lot B (n=48) *

Sample	Mean Ratio	StD	% CV
1. Equivocal	0.97	0.11	11.9
2. Low Positive	1.40	0.13	9.5
3. Low Positive	1.32	0.14	10.9
4. Low Positive	1.47	0.21	14.5
5. Negative	0.19	0.06	32.2

* Variability was tested by running eight wells per sample on two different lots, on three different days. These data represent a compilation of data from Tables 4 and 5.

3. Cross Reactivity

Studies were performed to assess interference in the Anti-EBV VCA IgG using sera which were negative for antibodies to EBV-VCA and positive for antibodies to nuclear antigens (n=9) and the following Herpes viruses:

HSV-1 IgG	(n=8)
HSV-2 IgG	(n=6)
VZV IgG	(n=10)
CMV IgG	(n=6)

These studies indicate that interference with the test procedure by the above list is minimal.

REFERENCES

1. Rapp CE, and Heweston JF: Infectious mononucleosis and the Epstein-Barr virus. Am. J. Dis. Child. 132:78, 1978.
2. Biggar RJ, Henle W, Fleisher G, Bocker J, Lennette ET, and Henle G: Primary Epstein-Barr virus infections in African infants. I: Decline of maternal antibodies and time of infection. Int. J. Cancer. 22:239, 1978.
3. Fry J: Infectious mononucleosis: Some new observations from a 15 year study. J. Fam. Prac. 10:1087, 1980.
4. Lennette ET: Epstein-Barr virus. In: Manual of Clinical Microbiology, 4th edition. Lennette ET, Balows A, Hausler WJ, Shadomy HJ, eds. Washington DC, American Society for Microbiology, p. 326, 1987.
5. Fleisher G, Henle W, Henle G, Lennette ET, and Biggar RJ: Primary infection with Epstein-Barr virus in infants in the United States: Clinical and Serological Observations. J. Infect. Dis. 139:553, 1979.
6. Merlin TL: Chronic mononucleosis: Pitfalls in the laboratory diagnosis. Hum. Path. 17:2, 1986.
7. Sixbey JW, Nedrud JG, Raab-Traub N, Hanes RA, Pagano JS: Epstein-Barr virus replication in oropharyngeal epithelial cells. New Eng. J. Med. 310:1225, 1984.
8. Chang RS, Lewis JP, Reynolds RD, Sullivan MJ, Neuman J: Oropharyngeal excretion of Epstein-Barr virus by patients with lymphoproliferative disorders and by recipients of renal homografts. Ann. Intern. Med. 88:34, 1978.
9. Jones JF, Ray G, Minnich LL, Hicks MJ, Kibler R, Lucus DO: Evidence of active Epstein-Barr virus infection in patients with persistent, unexplained illness. Elevated anti-early antigen antibodies. Ann. Intern. Med. 102:1, 1985.

10. Evans AS, Neiderman JC, Cenabre LC, West B, and Richards VA: A prospective evaluation of heterophile and Epstein-Barr virus-specific IgM antibody tests in clinical and subclinical infectious mononucleosis: Specificity and sensitivity of the tests and persistence of antibody. J. Infect. Dis. 132:546, 1975.
11. Henle W, Henle GE, and Horowitz CA: Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. Hum. Path. 5:551, 1974.
12. Lennette ET, and Henle W: Epstein-Barr virus infections: Clinical and serological features. Lab Management. p. 23, June, 1987.
13. Reedman BM, and Klein G: Cellular localization of an Epstein-Barr virus (EBV) associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer 11:499, 1973.
14. Henle G, Henle W, and Horowitz CA: Antibodies to Epstein-Barr virus-associated nuclear antigen in infectious mononucleosis. J. Infect. Dis. 130:231, 1974.
15. Horowtiz CA, Henle W, Henle G, Rudnick H, and Lutts E: Long-term serological follow-up of patients for Epstein-Barr virus after recovery from infectious mononucleosis. J. Infect. Dis. 151:1150, 1985.
16. Horowitz CA, Henle W, Henle G, and Schmitz H: Clinical evaluation of patients with infectious mononucleosis and development of antibodies to the R component of the Epstein-Barr virus-induced early antigen complex. Am. J. Med. 58:330, 1975.
17. Sumaya CV: Endogenous reactivation of Epstein-Barr virus infections. J. Infect. Dis. 135:374, 1977.
18. Joncas J, Lapointe N, Gervais F, Leyritz M, and Wills A: Unusual prevalence of antibodies to Epstein-Barr virus early antigen in ataxia telangiectasia. Lancet 1: 1160, 1977.
19. Akaboshi I, Jamamoto J, Katsuki T, and Matsuda I: Unique pattern of Epstein-Barr virus specific antibodies in recurrent parotitis. Lancet 2:1049, 1983.
20. Larson PD, Bloomer LC, and Brag PF: Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. Neurology 35: 435, 1985.
21. Henle W, Ho H-C, Henle G, and Kwan HC: Antibodies to Epstein-Barr virus related antigens in nasopharyngeal carcinoma. Comparison of active cases with long-term survivors, J. Natl. Cancer Inst. 51:361, 1973.
22. Henle W, and Henle G: Epstein-Barr virus-specific serology in immunologically compromised individuals. Cancer Res. 41:4222, 1981.
23. Fleisher G, and Bolognese R: Persistent Epstein-Barr virus infection and pregnancy. J. Infect. Dis. 147:982, 1983.
24. Engvall E and Perlman P: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochem. 8:871-874, 1971.
25. Engvall E and Perlman P: Enzyme-linked immunosorbent assay, ELISA. III. Quantitation of specific antibodies by enzyme-labeled anti-immunoglobulin in antigen coated tubes. J. Immunol. 109:129-135, 1972.
26. Voller A, Bartlett A, and Bidwell DE: Enzyme immunoassays with special reference to ELISA technique. J. Clin. Pathol. 31:507-520, 1978.
27. Hopkins RF, Witmer TJ, Neubauer RH, and Rabin H: Detection of antibodies to Epstein-Barr virus antigens by enzyme-linked immunosorbent assay. J. Infect. Dis. 146:734-740, 1982.
28. Procedures for the collection of diagnostic blood specimens by venipuncture. Second Edition; Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
29. Procedures for the Handling and Processing of Blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12. Approved Guideline 1990.
30. Hallee TJ, Evans AS, Neiderman JC, Brooks CM and Boegtly: Infectious mononucleosis at the United States Military Academy, a prospective study of a single class over four years. Yale J. Biol. Med. 47:182-192, 1974.
31. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens. Final Rule, Fed. Register 56:64175-64182, 1991.
32. Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guidelines – 4th Edition (2010). CLSI Document GP44-A4 (ISBN 1-56238-724-3). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, PA 19087.

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		

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