Wampole Laboratories®

EBV-VCA IgG ELISA II

REF. 424700CE

INTENDED USE

The Wampole Laboratories Epstein-Barr Virus (EBV)-Viral Capsid Antigen (VCA) IgG ELISA test system is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative detection of IgG class antibodies to Epstein-Barr Virus viral capsid antigen (EBV-VCA) 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.

SUMMARY AND EXPLANATION

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

Following seroconversion, whether symptomatic or not, EBV establishes a chronic, latent infection in B lymphocytes which probably lasts for life (6). EBV replicates in oropharyngeal epithelial cells and is present in the saliva of most patients with IM (7). 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 (10). However, 10-15% of adults and higher percentages of children and infants with primary EBV infections do not develop heterophile antibodies (11). 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, 10-12). Both IgM and IgG antibodies to the viral capsid antigen (VCA) peak 3 to 4 weeks after primary EBV infection. IgM anti-VCA decline rapidly and is usually undetectable after 12 weeks. IgG anti-VCA titers decline slowly after peaking but last indefinitely. Antibodies to EBV nuclear antigen (EBNA) develop from 1 month to 6 months after infection and, like anti-VCA, persist indefinitely (11,12). Antibodies to EBNA indicate that the infection was not recent (11).

EBV early antigens (EA) consists of two components; diffuse (D), and restricted (R). The terms D and R reflect the different patterns of immunofluorescence staining exhibited by the two components (13,14). Antibodies to EA appear transiently for up to three months during the acute phase of IM in 85% of patients (15,16). The antibody response to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children produces antibodies to the R component (5,11). A definitive diagnosis of primary EBV infection can be made with 95% of acute phase sera based on the detection of antibodies to VCA, EBNA, and EA (12).

High levels of anti-VCA together with anti-EBNA and anti-EA-R are associated with reactivation of the latent viral carrier state (16,17). High levels of IgG anti-VCA are found in sera of patients with immunodeficiencies (6, 18), recurrent parotitis (19), multiple sclerosis (20), and nasopharyngeal carcinoma (21); as well as immunosuppressed patients (8, 22), pregnant women (23), and persons of advanced age (17).

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

PRINCIPLE OF THE ELISA ASSAY

The Wampole EBV IgG ELISA test system is designed to detect IgG class antibodies to Epstein-Barr Virus in human sera. Wells of plastic microwell strips are sensitized by passive absorption with EBV 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 (γ chain specific) 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.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

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	2	Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (γ chain specific). Ready to use. One, 15 mL vial with a white cap.
CONTROL +	3	Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
CAL	4	Calibrator (Human Serum). One, 0.5 mL vial with a blue cap
CONTROL -	5	Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
DILSPE	6	SAVe Diluent™ (Sample Diluent). One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. Note: Shake Well Before Use. (Product #: 4500CC). (NOTE: This reagent may be used with any Wampole ELISA test system utilizing Product #: 4500CC) NOTE: The SAVe Diluent™ will change color in the presence of serum.
SOLN A / TMB	7	TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine(TMB). Ready to use. Contains DMSO < 15% (w).
SOLN / STOP	8	Stop solution: One 15 mL bottle (red cap) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF / 10X	9	Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) 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.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. 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, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum 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, these products should be handled 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, wash buffer, and conjugate 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.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of 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. Reagents from other sources or manufacturers should not be used.
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. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.
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. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to 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 kit.

MATERIALS REQUIRED BUT NOT PROVIDED:

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 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. (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. SAVe Diluent™: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (27, 28). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL 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 six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and returned to storage between 2° and 8°C.

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

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent™. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. 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 sample. 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 5X.
   A. Manual Wash Procedure:
   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap 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 0.5% sodium hypochlorite (bleach) at the end of the days 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 reagent blank well, at the same rate and in the same order as the specimens were added.
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 reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

	OD Range
Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

A. Calculations:

1. *Correction Factor*

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

2. *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)

3. *Index Values or OD Ratios*

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:

Mean OD of Calibrator	=	0.793
Correction Factor (CF)	=	0.25
Cut off OD	=	0.793 x 0.25 = 0.198
Unknown Specimen OD	=	0.432
Specimen Index Value or OD Ratio	=	0.432 / 0.198 = 2.18

B. Interpretations:

Index Values or OD ratios are interpreted as follows:

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

1. An OD ratio ≤ 0.90 indicates no detectable IgG antibody to EBV-VCA. A negative result indicates no current or previous infection with EBV. Such individuals are presumed to be susceptible to primary infection.
2. An OD ratio ≥ 1.10 is positive for IgG antibody to EBV-VCA. A positive test result indicates a current or previous infection with EBV.
3. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure such as the Wampole Laboratories indirect fluorescent antibody (IFA) test procedure. Additionally, specimens which remain equivocal after repeat testing should be re-evaluated by drawing another sample one to three weeks later.
4. There is no International Standard for EBV-VCA IgG, so value assignments for calibrators and controls were based on an internal reference preparation.

LIMITATION 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 VALUES

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 3 to 4 weeks (4). IgG antibodies to VCA decline slowly after peaking but persist indefinitely (15).

Primary acute EBV infection is indicated by the presence of IgG antibodies to VCA coupled with anti-EA and/or IgM anti-VCA 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 inapparent (2,4). In one study in the United States, about 50% of college freshmen were seropositive for EBV (30).

In a study conducted by the manufacturer using 135 normal samples from the Southeastern United States, 134/135 samples were positive by both the Wampole Laboratories EBV-VCA IgG ELISA and the Wampole Laboratories EBV-VCA IgG IFA. In another study consisting of 32 normal pediatric samples, 32/32 samples were negative using both the Wampole Laboratories EBV-VCA IgG ELISA, and the Wampole Laboratories EBV-VCA IgG IFA.

The number of individuals with IgG antibody to EBV-VCA varies with age and socioeconomic status. It is recommended that each laboratory establish their own expected values based upon the population type typically tested.

PERFORMANCE CHARACTERISTICS

Comparative Study:

The Wampole Laboratories EBV-VCA IgG ELISA test system was compared 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. The results of this study are summarized in Table 1:

Table 1
Wampole EBV-VCA ELISA vs. Commercial VCA IgG ELISA

		Wampole EBV-VCA IgG ELISA			
		+	-	±	Totals
Commercial VCA IgG ELISA	+	104	8**	3	115
	-	10**	46	4	60
	±	11	12	1	24
	Totals	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)

Discrepant samples were retested using the Wampole Laboratories indirect fluorescent antibody (IFA) test system for the detection of IgG antibodies against EBV-VCA. The results of this evaluation are shown in Table 2:

Table 2. Analysis of Discrepant Results

Sample ID	WAMPOLE EBV-VCA IgG ELISA	Commercial VCA-IgG ELISA	Wampole IFA
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:

- Eight samples were negative by Wampole ELISA and positive by the commercial VCA IgG ELISA. Six of these eight samples were confirmed to be negative by Wampole IFA.
- Ten samples were positive by Wampole ELISA and negative by the commercial VCA IgG ELISA. Nine of these ten samples were confirmed to be positive by Wampole IFA.
- Based upon the resolution of the discrepant samples by IFA, the relative sensitivity, relative specificity, and percent agreement were recalculated. These results are shown in Table 3:

Table 3
Recalculation of Relative Sensitivity, Specificity, and Percent Agreement based on Resolved Samples

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

Reproducibility:

To assess the intra- and inter-assay variability of the test procedure, five serum samples ranging from positive to negative were tested using two different master lots of product on three different days. Each sample was tested eight times on each master lot. The mean OD ratio and coefficient of variation (CV) were calculated for each sample. The results of this study are depicted in Table 4 (Lot A), and Table 5 (Lot B). Table 6 summarizes the overall test variability combining the data points from the lot-to-lot and day-to-day comparison.

Table 4 Summary of EBV-VCA Intra-assay and Inter-assay Variability Testing (LOT A)

Sample	Day 1			Day 2			Day 3					
	X Ratio	SD	% CV	X Ratio	SD	% CV	X Ratio	SD	% CV	X Ratio	SD	% CV
1. Equivocal	0.96	0.04	4.2	0.77	0.04	5.2	0.99	0.04	5.2	0.91	0.10	11.0
2. Low Pos.	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 Pos.	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 Pos.	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
Each sample was tested eight times on each day.												

Table 5. Summary of EBV-VCA Intra-assay and Inter-assay Variability Testing (LOT B)

Sample	Day 1			Day 2			Day 3					
	X Ratio	SD	% CV	X Ratio	SD	% CV	X Ratio	SD	% CV	X Ratio	SD	% CV
1. Equivocal	0.93	0.06	5.8	0.97	0.06	6.2	1.11	0.07	6.3	1.04	0.08	7.7
2. Low Pos.	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 Pos.	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 Pos.	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
Each sample was tested eight times on each day.												

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

SAMPLE	MEAN RATIO	SD	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.

Cross Reactivity

Studies were performed to assess interference in the Wampole EBV-VCA IgG ELISA test system 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 listed reactivities are minimal.

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ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100 µL/well
3. ⟶ *Incubate 20 to 30 minutes*
4. Wash
5. Add Conjugate – 100 µL/well
6. ⟶ *Incubate 20 to 30 minutes*
7. Wash
8. Add TMB 100 µL/well
9. ⟶ *Incubate 10 to 15 minutes*
10. Add Stop Solution 50 µL/well - Mix
11. READ

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