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 early in childhood and is asymptomatic (2). In more affluent counties, 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, 20-25% 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 serologic 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 (4,12). 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 test 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. EA restricted antibodies 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-EA 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 (6,8,18), 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 described in this report (24) may be a sensitive and reliable method for detection of antibodies to EBV antigens (25,27). 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-VCA ELISA test system is designed to detect IgG class antibodies to Epstein-Barr Virus in human sera. Wells of plastic microtiter 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 (ɣ chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase. Unreacted peroxidase conjugate is removed by washing.
3. The microtubes 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 depends upon the absorbance concentration of 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**
  Plate wells configured in twelve 1.0-ml wells coated with recombinant Epstein-Barr Virus VCA antigen. The plate is packaged in a shelf and stored in an envelope with desiccant.

- **COU**
  Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG γ chain specific. Ready to use. One 5 ml vial with a white cap.

- **CONTROL**
  Positive Control (Human Serum) One 0.25 ml vial with a red cap.

- **CAL**
  Calibration (Human Serum) One 0.5 ml vial with a blue cap.

- **ULP**
  Negative Control (Human Serum) One 0.35 ml vial with a green cap.

- **SAVIE Diluent** (Samo Dvur) One 20 ml bottle (green cap) containing Tween-20, borsal serum albumin and phosphate-buffered saline. pH 7.2 ± 0.2. Ready to use. Note: Shake Well Before Use. Product # 450CD. This reagent may be used with any Wampole ELISA test system using Product # 450CD. NOTE: The SAVIE Diluent will change color in the presence of serum.

- **SOLN 1 + TUS**
  IM. One 15 ml amber bottle (amber cap) containing 3.5% l-a-amino-2-hydroxybenzoic acid (TMB). Ready to use. Contains DMSO + 1.5% (w/v).

- **SOLN STOP**
  Stop solution. One 15 ml bottle (clear cap) containing 100 g sodium phosphate-buffered saline and Tween-20 solution (buffer). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

- **WASH**
  Wash buffer concentrate (100 X) 50 ml powder concentrate + 9 parts distilled or deionized water. One 100 ml bottle (clear cap) containing a 10X concentrated buffer-phosphate-buffered saline and Tween-20 solution (buffer). 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 and face protection. Do not breathe vapor. Dispose of waste containing 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 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 SAVIE 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. Toxins 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 blotch 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 permanently. 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 walls from condensation.
22. Wash solution should be collected in a disposable 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 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 reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED:
- ELISA microwell reader capable of reading at a wavelength of ≤600nm.
- Pipettes capable of accurately delivering 10 to 200μL.
- Multichannel pipettes 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.
- Sterilization 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. Store to room temperature.
3. 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.
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 NCCCLS document M59: Protection of Laboratory Workers from Infectious Diseases.
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 heparinized, 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 return to storage between 2° and 8°C.

PREPARE A 1:12 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 mixed with the diluent.
3. 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.
4. Add 100μL of SAVe Diluent™ to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
5. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
6. Wash the microwell strips 5X.

A. Manual Wash Procedure:
   a. Gently shake the plate to mix the sample.
   b. Fill each well 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. Drain out the wash solution from all the wells. Dip 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 container and treat with 0.5% sodium hypochlorite (bleach) included at the end of the day.

B. Automated Wash Procedure:
   If using an automated microwell wash system, set the dispensing volume to 200-350μL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
   Add 10μL of each conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
   Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
   3. Wash the microwells by following the procedure as described in step 7.
   10. 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.
   13. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
   15. 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.
   16. Set the microwell reader to 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:

<table>
<thead>
<tr>
<th>OD Range</th>
<th>Negative Control</th>
<th>Calibrator</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 0.250</td>
<td>≤ 0.300</td>
<td>≤ 0.500</td>
</tr>
<tr>
<td>a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>c. If the above conditions are not met, the test should be considered invalid and should be repeated.</td>
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<td></td>
</tr>
</tbody>
</table>
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay end point.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCCLS 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.
   1. CutOff OD Value
      To obtain the cutOff OD value, multiply the CF by the mean OD of the Calibrator determined above.
      CutOff OD Value = (CF × 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 value from step 2.
Table 2. Analysis of Discrepant Results

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Wampole EBV-VCA IgG ELISA</th>
<th>Commercial VCA IgG ELISA</th>
<th>Wampole IFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>0.251</td>
<td>1.16</td>
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</tr>
<tr>
<td>29</td>
<td>0.261</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td>0.358</td>
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<td>-</td>
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<tr>
<td>39</td>
<td>0.368</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>41</td>
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<td>-</td>
</tr>
<tr>
<td>53</td>
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<tr>
<td>94</td>
<td>0.707</td>
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<tr>
<td>81</td>
<td>1.243</td>
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<td>-</td>
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<td>89</td>
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<tr>
<td>93</td>
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<td>-</td>
</tr>
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<td>101</td>
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<td>1.57</td>
<td>-</td>
</tr>
<tr>
<td>111</td>
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<td>0.55</td>
<td>-</td>
</tr>
<tr>
<td>133</td>
<td>2.216</td>
<td>0.68</td>
<td>-</td>
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<tr>
<td>137</td>
<td>2.254</td>
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<td>139</td>
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<td>-</td>
</tr>
<tr>
<td>152</td>
<td>2.730</td>
<td>0.20</td>
<td>-</td>
</tr>
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</table>

Summary:
- Eight samples were positive 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. Nineteen of these ten samples were confirmed to be positive by Wampole IFA.
- Based on the resolution of the discrepant samples by IFA, the relative specificity, relative specificity, and percent agreement were recalculated. These results are shown in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Recalculation of Relative Sensitivity, Specificity, and Percent Agreement based on Resolved Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Sensitivity: 113/115 = 98.3%</td>
</tr>
<tr>
<td>Relative Specificity: 52/53 = 96.1%</td>
</tr>
<tr>
<td>Percent Agreement: 165/168 = 97.6%</td>
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</table>

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

Sample | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0.56</td>
<td>0.64</td>
<td>4.3</td>
<td>0.77</td>
<td>0.04</td>
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<td>0.99</td>
<td>0.04</td>
<td>5.2</td>
<td>0.91</td>
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<td>2</td>
<td>0.09</td>
<td>0.81</td>
<td>1.18</td>
<td>1.28</td>
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<td>0.07</td>
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<tr>
<td>3</td>
<td>3.11</td>
<td>0.91</td>
<td>8.3</td>
<td>1.12</td>
<td>0.19</td>
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<td>1.25</td>
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<tr>
<td>4</td>
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<td>7.9</td>
<td>1.12</td>
<td>0.69</td>
<td>8.6</td>
<td>1.43</td>
<td>0.12</td>
<td>8.4</td>
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<tr>
<td>5</td>
<td>0.16</td>
<td>0.53</td>
<td>20.1</td>
<td>0.91</td>
<td>1.82</td>
<td>0.02</td>
<td>12.3</td>
<td>0.11</td>
<td>12.3</td>
<td></td>
</tr>
</tbody>
</table>

Each sample was tested eight times on each day.

Table 5

Sample | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV | X Ratio | SD % CV |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>1</td>
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<td>0.06</td>
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<tr>
<td>2</td>
<td>1.36</td>
<td>0.50</td>
<td>6.5</td>
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<td>0.06</td>
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<td>1.35</td>
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<tr>
<td>3</td>
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<tr>
<td>4</td>
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<td>0.14</td>
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<td>1.55</td>
<td>0.12</td>
<td>7.7</td>
<td>1.49</td>
<td>0.10</td>
<td>8.7</td>
<td>1.38</td>
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<tr>
<td>5</td>
<td>0.28</td>
<td>0.52</td>
<td>7.1</td>
<td>0.22</td>
<td>0.91</td>
<td>0.24</td>
<td>12.3</td>
<td>0.03</td>
<td>12.3</td>
<td></td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>MEAN</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.97</td>
<td>0.11</td>
<td>11.9%</td>
</tr>
<tr>
<td>2</td>
<td>1.40</td>
<td>0.13</td>
<td>9.5%</td>
</tr>
<tr>
<td>3</td>
<td>1.32</td>
<td>0.14</td>
<td>10.9%</td>
</tr>
<tr>
<td>4</td>
<td>1.47</td>
<td>0.21</td>
<td>14.5%</td>
</tr>
<tr>
<td>5</td>
<td>0.70</td>
<td>0.06</td>
<td>9.2%</td>
</tr>
</tbody>
</table>

* 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:

<table>
<thead>
<tr>
<th>HSV-1 IgG</th>
<th>HSV-2 IgG</th>
<th>VZV IgG</th>
<th>CMV IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 9)</td>
<td>(n = 6)</td>
<td>(n = 10)</td>
<td>(n = 8)</td>
</tr>
</tbody>
</table>

These results indicate that interference with the test procedure by the above listed activities is minimal.

REFERENCES

ABBREVIATED TEST PROCEDURE
1. Dilute Serum 1:2

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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1-800-532-0295
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