INTENDED USE

The Wampole Laboratories EBV-ELISA test system is an enzyme-linked immunosorbent assay (ELISA) designed for the qualitative detection of EBV class antibodies in EBV-viral capsid antigen (EBV-VC) in human serum. The test system is intended to be used for the diagnosis of EBV-associated infectious mononucleosis when used in conjunction with other EBV serologies.

SUMMARY AND EXPLANATION

Epstein-Barr Virus (EBV) is a ubiquitous human virus which causes infectious mononucleosis (IM), a self-limiting lymphoproliferative disease (1). In 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 latent infection in B lymphocytes which probably lasts for life (6). EBV-infected lymphocytes can transform 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 (8). Reactivation of the latent viral 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 (10). In adults, EBV has been implicated at least as a contributing factor in the etiology of nasopharyngeal carcinoma, Burkitt’s lymphoma, and lymphomas in immunodeficient patients (11,12). The Paul-Burnell-Davidson test for heterophile antibody is highly specific for IM (10). However, 10-15% of adults and 20-40% of children with EBV infections do not develop heterophile antibodies (11). EBV-specific serological tests are used to differentiate primary EBV infections that are heterophile-negative from non-HIV-infected patients with other agents such as cytomegalovirus, adenovirus, and Toxoplasma gondii (13).

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 3-4 weeks after primary EBV infection, IgM- VCA declines rapidly and is usually undetectable after 12 weeks. IgA and anti-EBV 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 (11,13). 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 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). EBV-specific EA antibodies respond to EA in IM patients is usually to the D component, whereas silent seroconversion to EBV in children produces antibodies to the R component (15,1). A definitive diagnosis of primary EBV infection can be made with 95% of acute phase sera based on the detection of antibodies to EBV nuclear antigen (EBNA) and EA (12). High levels of anti-VCA together with anti-EBNA and anti-EA are associated with reactivation of the latent viral state (16,17). High levels of IgG anti-VCA are found in sera of patients with immunodeficiency (18,19), patients with AIDS (20), patients with 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-ELISA test system is designed to detect IgG class antibodies to EBV in human sera. Wells of plastic microtiter strips are sensitized with passive absorption with EBV antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG that precipitates and removes IgG and rheumatoid factor from the sample leaving IgG free to react with the immobilized antigen. During sample incubation any antigen specific IgG antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibodies and unbound conjugate.
2. Peroxidase Conjugated goat anti-human IgG (μ 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 plate is washed to remove unbound conjugate.
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 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: 96 wells configured in twelve 1× Well strips coated with affinity purified 125KDa capsid protein purified from induced P2-HT cells. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
- CONJ: Conjugated (horseradish peroxidase) goat anti-human IgG (μ chain specific). Ready to use. One 1.5 mL vial with a white cap.
- CONTROL: Positive Control (Human Serum). One 0.25 mL vial with a wet cap.
- CONTROL: Negative Control (Human Serum). One 0.5 mL vial with a green cap.
- Sample Diluent: One 30 mL bottle (blue cap) containing TWEEN-20, bovine serum albumin, phosphate-buffered saline, and saline anti-human IgG (μ chain specific). 1×, pH 7.2 ± 0.2. Pour solution, ready to use. Note: Store TWEEN-20. Bottle in a sealed container.
- SOLN 1: One 15 mL amber bottle (tan cap) containing 3.3/3.5% tetramethylbenzidine (TMB). Ready to use. Contains DMSO ≤ 5% (v/v).
- SOLN 2: Stop solution One 15 mL bottle (red cap) containing 1M H2SO4, 0.70 M HCl. Ready to use.
- WASHPH: 10X: 1% deionized water + 99% parts deionized water or distilled water. One 100 mL bottle (red cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (2X). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not to list number dependent and may be used interchangeably with the ELISA assay:
- TMB: Stop Solution, and Wash Buffer.

Note: Kits also contain: 1. Component list containing specific information is inside the package. 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 attention. Wash suitable gloves and laboratory clothing immediately after use. Do not breathe vapors. Dispose of waste according to all local, state, and federal laws.

3. The walls of the ELISA plate do not contain avidin or biotin. However, the strips coated with avidin may 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 antigens, 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 in a Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control and Prevention Laboratories: "Guide to Biological and Hazardous Laboratories": current edition; and OSHA’s Standard for Bloodborne Pathogens (1001).

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 suction) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.

7. The sample 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 glassware which may cause explosions on hammering. To prevent, rinse shank 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 IRITANT. Irritating to eyes, respiratory system and skin.

11. Wipe bottom of plate free of residual liquid withl/ or gritfingnats that can alter critical density (OD) readings.

12. Dilution or distortion 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 reagents 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 sample could produce erroneous results.

18. Residual glassware should be washed and thoroughly rinsed free of all contaminants.

19. Avoid splashing or generation of aerosols.

20. Do not expose reagents to strong light during storage or incubation.

21. Allow the microtiter strips and holder to equilibrate to room temperature prior to opening the protective envelope to prevent the wells from condensation.

22. Wash solutions 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 bleach solution.

24. Do not use ELISA plates if the indicator strip on the descendent puch 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 sodium azide as a preservative at a concentration of 0.1% (w/v). 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 oxidizers from bleach-containing solutions. Tense 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 microtiter reader capable of reading at a wavelength of 450mm.
- Pipettes capable of accurately delivering 10 to 200µL.
- A volumetric pipette capable of accurately delivering 50-200µL.
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microfuge washing system.
- Glass distilled water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer for incubation steps.
- Disposal basin and disinfectant, (example: 10% household bleach, 0.2% sodium hypochlorite).

STORAGE CONDITIONS
1. Store the unopened kit between 2° and 8°C. If opened, store in dark at ambient temperature. Do not store at temperatures above 40°C or below 2°C.
2. Wash with distilled water. Store between 2° and 25°C. Do not freeze.
3. Calibrator: Store between 2° and 8°C. DO NOT FREEZE.
4. Correct Factor: Positive Control: Store between 2° and 8°C.
5. MB: Store between 2° and 8°C. Do not freeze.
6. Wash Buffer concentrate (100X): 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. Sample Diluent: Store between 2° and 25°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 CLS document M48: Protection of Laboratory Workers from Infectious Disease.
2. A known test method for complete assurance that human blood samples will not transmit disease. 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. (28, 29). No anticoagulants or preservatives should be added.
4. Avoid using hemolysed, lipemic, or icteric sera.
5. Store sample at room temperature for no longer than 6 hours. If testing is not performed within 6 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delays in testing are anticipated, store 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°-30°C).
2. Determine the number of microwells needed. Allow six control/calibrator determinations (6 x blank, 6 x calibrator, 6 x 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.

EXAMPLE PLATE SETUP

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

Prepare a 1:2 dilution (e.g. 10µL of serum + 200µL of Sample Diluent. NOTE: Shale Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum.

3. To individual wells, add 10µL of each diluted control, calibrator and sample. Ensure that the sample is completely mixed. Use a different pipette tip for each sample.

4. Add 100µL of Sample 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 to 30 minutes.

6. Wash the microwell strips 5X.

- A. Manual Wash Procedure
   a. Gently shake the plate.
   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. Drain the wash solution out of the bottom of the plate.

- B. Automated Wash Procedure
   - If using an automated microwell wash system, set the dispense volume to 200-300µL/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 wells.

8. Add 10µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimen dilutions.

9. Incubate the plate at room temperature (20°-25°C) for 25 to 30 minutes.

10. Wash the microwell strips following the procedure as described in step 7.

11. Add 10µL 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 450mm and measure the optical density (OD) of each sample against the reagent blank. The plate should be read within 20 minutes after the addition of the Stop Solution.

QUALITY CONTROL
1. Each time the assay is run the Calibrator must be run in duplicate. A reagent blank, Negative Control, and Positive Control must also be included in each run to ensure that the sample is stable.
2. Calculate the mean of the three Calibrator wells. If any of the 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 range:

<table>
<thead>
<tr>
<th>OD Range</th>
<th>Negative Control</th>
<th>Calibrator</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>Positive Control</td>
<td>Positive Control</td>
<td></td>
</tr>
<tr>
<td>OD Value</td>
<td>≤ 0.500</td>
<td>0.350</td>
<td>0.500</td>
</tr>
<tr>
<td>a. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≤ 0.9.</td>
<td></td>
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<tr>
<td>b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≤ 0.9.</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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</tbody>
</table>

4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision of the assay cut-off.

5. The Calibrator controls may be used according to guidelines or requirements of local, state, and federal regulations.


INTERPRETATION OF RESULTS
- A. Calculations:
  1. Correction Factor
    - The OD value for the positive control has been determined by the manufacturer and compared 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 kits and is printed on the Component List located in the kit box.

- B. Cutoff OD Value
  - To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator above determined.

- C. Test Results Calculations
  - Correct the index value or OD value for the patient by dividing its OD value by the cutoff OD from step 2.

- Example:
  - Mean OD of Calibrator = 0.729
  - Correction Factor (CF) = 0.39
  - Cut off OD = 0.729 x 0.39 = 0.283
  - Unknown Patient OD = 0.423
  - Specimen Index Value or OD Ratio = 0.423 / 0.283 = 1.50

- B. Interpretations:
  - Index Values or OD Ratios are interpreted as follows:

<table>
<thead>
<tr>
<th>Index Value or OD Ratio</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Specimens</td>
<td>≤ 0.90</td>
</tr>
<tr>
<td>Equivocal Specimens</td>
<td>0.91 to 1.09</td>
</tr>
<tr>
<td>Positive Specimens</td>
<td>≥ 1.10</td>
</tr>
</tbody>
</table>

- LIMITATIONS OF THE ASSAY
  - Most (95%) of IF individuals have peak anti-EBV-IGM titers before they consult a physician.
  - The OD ratio ≥ 0.90 indicates no detectable IGM antibody to EBV-VCA. A negative test indicates no current infection with EBV, and should be reported as reactivity for EBV-VCA IgM antibody.
  - Such individuals are presumed to be susceptible to primary infection.
  - An OD ratio ≥ 1.10 is positive for IgM antibody to EBV-VCA. A positive test result indicates a current or reactivated infection with EBV, and should be reported as reactive for EBV-VCA IgM antibody.
  - 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 Wannigamme Laboratory indirect fluorescent antibody (IFAt) test procedure. Alternatively, specimens which remain equivocal after repeat testing should be re-evaluated by drawing another sample one to three weeks later.
  - The numeric value of the final result above the cutoff is not indicative of the amount of anti-EBV-IGM antibody present above the cutoff.

- EXPECTED VALUES
  - The presence of EBV-IGM antibodies as determined by the ELISA method is highly suggestive of acute EBV infection since such antibodies are found early on in the illness in approximately 90% of cases and are not usually present in the general population (31).
  - In 451 persons, the frequency of IgM antibody to EBV-VCA was evaluated using 74 normal blood donor specimens from southeastern United States. Of the 74 specimens, three (3) were reactive (4.0%) and seventy one (71) were non-reactive (96.0%). A frequency distribution of the actual results appears below:
Table 3. Summary of Wampole Laboratories, EBV-1gM ELISA Test System.

<table>
<thead>
<tr>
<th>Clinical Site</th>
<th>Stage/Path Activity</th>
<th>Pos.</th>
<th>Neg.</th>
<th>Equiv.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterophile-EBNA- *</td>
<td>1/170 (0.2%) * VCA IgG Negative</td>
<td>14</td>
<td>190</td>
<td>0</td>
<td>205</td>
</tr>
<tr>
<td>Heterophile-EBNA-</td>
<td>1/170 (0.2%) * VCA IgG Negative</td>
<td>14</td>
<td>190</td>
<td>0</td>
<td>205</td>
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<td>190</td>
<td>0</td>
<td>205</td>
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</table>

* Equivocal specimens were retained according to the package insert. Specimens which were repeatedly equivocal, or not reported due to insufficient volume appear in this column. These remaining equivocal specimens were not used in any calculations for sensitivity or specificity. Of the 174 specimens tested at site 2, there were 48 T1 equivalent samples. One repeated as negative, two repeated as positive, and four were not repeated due to insufficient volume.

Assay Specificity: 35/77 = 0.45 (95% CI: 0.32-0.59)

* Assayed with 0.05% equivalent calculated using the exact method.
Table 6. Effect of Dilution on ENV-HCA IgG Positive Specimens. Functional Removal of IgG Antibody

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>With Kit Diluent</th>
<th>Diluent without Anti-IgG</th>
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</thead>
<tbody>
<tr>
<td>U82-1</td>
<td>0.183</td>
<td>0.166</td>
</tr>
<tr>
<td>U78-1</td>
<td>0.211</td>
<td>0.214</td>
</tr>
<tr>
<td>U82-3</td>
<td>0.371</td>
<td>0.328</td>
</tr>
<tr>
<td>U82-5</td>
<td>0.571</td>
<td>0.587</td>
</tr>
<tr>
<td>U82-7</td>
<td>0.800</td>
<td>0.804</td>
</tr>
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Table 7. Wampole Laboratories Results of Crossreactivity Testing

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<td>0.328</td>
<td>0.361</td>
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<td>0.361</td>
<td>0.328</td>
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<tr>
<td>U82-3</td>
<td>0.571</td>
<td>0.587</td>
<td>0.587</td>
<td>0.571</td>
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<td>0.571</td>
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<td>0.571</td>
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<tr>
<td>U82-4</td>
<td>0.800</td>
<td>0.804</td>
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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