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EPSTEIN-BARR VIRUS (EBV) VIRAL CAPSID ANTIGEN (VCA) IgG

Catalog #401010-G

INTENDED USE

The Diagnostic Automation, Inc. EBV-VCA IFA Test System is designed for the qualitative and semi-quantitative detection of EBV-VCA IgG antibody in human serum and is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

The etiologic relationship of the Epstein-Barr virus (EBV) to Infectious Mononucleosis (IM) has been firmly established and is now generally accepted (1,2,3). EBV infects only lymphoid cells with B-cell characteristics resulting in the expression of four different groups of EBV related antigens to which the infected host responds with appropriate antibodies (4).

In IM, the antibodies to Viral Capsid Antigen (VCA) peak about the second week of the illness and then gradually decline to lower titers which persist for life and appear to be associated with immunity (5).

In acute phase IM, both IgM and IgG antibodies to VCA may reach peak titers before the patient sees a physician (9). Consequently, 4-fold rises of antibody in convalescent sera are observed in only 20% of the patients studied. The IgM antibodies decline and disappear rapidly; in about 4-6 weeks. The IgG antibodies decline to lower persistent levels.

Antibodies to EBV-VCA develop in all patients with Burkitt's lymphoma, nasopharyngeal carcinoma, and EBV Infectious Mononucleosis (10). In addition, high EBV antibody titers are frequently associated with Hodgkin's disease, lymphocytic leukemia (10), SLE, Sarcoidosis (11), and Izumi fever (12).

Although the heterophile antibody response, as determined by the Paul-Bunnell-Davidsohn Differential Test is relatively specific for IM (7,8), it has been observed that these antibodies fail to develop in 5 to 10% of adult patients (5). In addition, the absence of a heterophile antibody response is more pronounced in the pediatric age ranges. Therefore, the Diagnostic Automation, Inc. EBV-VCA serodiagnostic test is recommended for cases of IM-like disease which remain heterophile antibody negative. It is also useful in distinguishing IM-like illnesses caused by cytomegalovirus, *Toxoplasma gondii*, adenovirus, and other viruses (6).

PRINCIPLE OF THE IFA ASSAY

The Diagnostic Automation, Inc. fluorescent EBV-VCA IgG antibody test system is designed to detect circulating VCA antibodies in human sera. The system employs EBV-VCA infected substrate cells and goat anti-human IgG (γ chain specific) adjusted for optimum use and free of nonspecific background staining. The reaction occurs in two steps:

1. patients' sera with the EBV-VCA infected substrate cells.
2. The second is the interaction of FITC-labeled antihuman IgG with the EBV-VCA antibodies attached to the VCA localized on the infected cell membrane and in the cytoplasm.

KIT COMPONENTS

Reactive Reagents:

1. EBV-VCA Antigen Slides: Ten substrate slides containing infected cells in each well .
2. Goat anti-human IgG (γ chain specific) labeled with FITC containing 1.25% bovine albumin and Evans blue counterstain. Two, 1.5mL vials (lyophilized).
3. EBV-VCA Human Positive Control Serum: Two, 0.5mL vials composed of human sera (lyophilized).
4. EBV-VCA Human Negative Control Serum: Two, 0.5mL vials, composed of human sera (lyophilized).
5. **ZORBA-NS**[®] sample diluent formulated to reduce non-specific staining. (Product #:Z025, 25mL) or (Product #:Z125, 125mL). Contains 0.1% sodium azide as a preservative.

Non-reactive Reagents:

1. Phosphate-Buffered-Saline (PBS), pH 7.2 \pm 0.2: Sufficient to prepare 4 liters .
2. Mounting Fluid (Buffered Glycerol): 3.0mL.
3. Absorbent blotters for drying the slide after the wash procedure.

***NOTE:** These reactive reagents contain preservative: thimerosal, mercury derivative 0.04%.

PRECAUTIONS

- For *in vitro* diagnostic use.
- The thimerosal and sodium azide preservative may be toxic if ingested.
- **ZORBA-NS** contains sodium azide as a preservative. 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.
- Remove only the amount of **ZORBA-NS** needed to perform each test run to reduce the possibility of product contamination.
- Use **ZORBA-NS** for screening dilutions only. **DO NOT PREPARE SERIAL DILUTIONS FOR END-POINT TITERS IN ZORBA-NS.**
- **ZORBA-NS** should be used only as a diluent for patient specimens:
 - a. **DO NOT** use **ZORBA-NS** to reconstitute the controls or conjugate.
 - b. **DO NOT** use **ZORBA-NS** in any of the wash steps.
- The volume of **ZORBA-NS** supplied has been calculated to provide sufficient material for all the individual test wells included in this kit when used according to the instructions herein. The use of larger volumes for sample preparation will result in insufficient **ZORBA-NS** to allow each test well to be utilized.
- **NO U.S. STANDARD OF POTENCY.**
- Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, 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 Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (18).

- Do not apply pressure to slide envelope, this may damage the substrate.
- Reagents from other sources or manufacturers should not be used. Follow test procedure carefully.

- Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
- Never pipette by mouth. Avoid contact of reagents and patient samples with skin and mucous membranes.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary or automatic pipettes.
2. Small test tubes, 13 x 100mm or comparable.
3. Test tube racks.
4. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
5. Cover slips: 24 x 60mm, thickness No. 1.
6. Distilled water.
7. Properly equipped fluorescence microscope assembly.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

TRANSMITTED LIGHT			
Light Source: Mercury vapor 200 W or 50 W			
Excitation filter	Barrier filter	Red Suppression filter	
KP490	K510 or K530	BG38	
BG12	K510 or K530	BG38	
FITC	K520	BG38	
Light Source: Tungsten - Halogen 100 W			
KP490	K510 or K530	BG38	

INCIDENT LIGHT			
Light Source: Mercury vapor 200, 100, 50 W			
Excitation filter	Dichroic Mirror	Barrier filter	Red Suppression filter
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38
Light Source: Tungsten - Halogen 50 and 100 W			
KP500	TK510	K510 or K530	BG38
FITC	TK510	K530	BG38

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (16,17). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

- EBV-VCA substrate slides: Store at -20°C or lower.
- Goat anti-human IgG labeled with FITC: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- Positive and negative human EBV-VCA control serum: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C or lower.
- **ZORBA-NS**: Store at 2-8°C.
- Phosphate-buffered-saline: Store at 2-25°C or room temperature. Store reconstituted buffer at 2-8°C. Rehydrated PBS is stable for 30 days when stored at 2-8°C.
- Buffered glycerol (mounting media): Store at 2-8°C.

NOTE:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.
2. Do not freeze and thaw reagents more than once. Repeated freezing and thawing destroys antibody activity. Do not store in self-defrosting freezers.

PROCEDURE

Preparation of Reagents:

Phosphate-buffered-saline (PBS), pH 7.2 ± 0.2: Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.

EBV-VCA human positive and negative control sera: Reconstitute with 0.5mL distilled water. Represents a 1:10 screening dilution. Use as reconstituted. Do not dilute.

Goat anti-human IgG FITC-labeled conjugate: Reconstitute with 1.5mL distilled water. alternatively, aliquot in 0.5mL amounts and store at -20°C or lower in small tubes.

1. The controls are intended to be used undiluted. As an option, users may titrate the positive control(s) to endpoint. In such cases, the control(s) should be diluted two-fold in PBS. When evaluated by Diagnostic Automation, Inc. an endpoint dilution is established and printed on the positive control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own mean titer for each lot of control.
2. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected, and the use of reagents discontinued.

Test Procedure:

1. Remove substrate slides from storage and allow them to reach room temperature (20-25°C). Tear open the protective envelope and remove slides containing the EBV-VCA infected cells. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.**
2. Prepare 1:10 screening dilutions of test sera in PBS. (For example: 20µL of sample plus 180µL of PBS). (Alternatively, you may prepare 1:10 screening dilutions in **ZORBA-NS**). Positive, negative, and buffer controls should be run each time the test is performed. **DO NOT PREPARE SERIAL DILUTIONS FOR END-POINT TITERS IN ZORBA-NS.**
3. Identify each well with the appropriate patient sera and controls.
4. Spread 20µL of test and control sera over each appropriately labeled well. Be careful not to disturb the substrate cells with pipette tip.
5. Incubate slides in a sealed moist chamber at room temperature (20-25°C) for 30 minutes. **DO NOT ALLOW WELLS TO DRY.**
6. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with a stream of PBS. **DO NOT DIRECT THE STREAM OF PBS INTO THE TEST WELLS.**
7. Place slides in a staining dish and wash in PBS for two, 5 minute intervals with a change of PBS. Use a magnetic mixing set-up or other means of gentle agitation.
8. Remove slides from PBS **ONE AT A TIME**. Invert slide and key wells to holes in blotters provided. Blot slide by wiping the reverse side with an absorbent wipe. **CAUTION:** Position the blotter and slide

on a hard, flat surface. Blotting on paper towel may destroy the slide matrix. **DO NOT ALLOW THE SLIDE TO DRY DURING THE TEST PROCEDURE.**

9. Place slides in a moist chamber and add 20 μ L of conjugate to each well.
10. Incubate slides for 30 minutes at room temperature. **DO NOT ALLOW SLIDES TO DRY.**
11. Repeat steps 6, 7, and 8.
12. Add 3-4 drops of buffered glycerol to the mask area of each slide and coverslip. Slides should be examined immediately at a total magnification of 250X.

QUALITY CONTROL

1. A positive control, negative control, and a buffer should be run with each assay.
2. It is recommended that the positive and negative controls be read prior to evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described, test results are invalid.
3. The negative control is characterized by the absence of fluorescent staining and a red background staining of all the cells due to Evans blue. The reactions of the negative control may be used as a guide for interpreting patient samples.
4. The positive control will exhibit a 2+ to 4+ apple-green fluorescent staining intensity of the cell membrane, nucleus, and cytoplasm in 5-15% of the total cell population.
5. Non-specific reagent trapping may occur in cell clumps and therefore, adequate washing is important to eliminate false positives.
6. The intensity of the observed fluorescence may vary with the microscope and filter used.
7. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

- 1+ to 4+ apple-green fluorescence at the periphery (cell membrane) and in the cytoplasm of the infected cells (5-15% of the total cell population) represents a positive reaction.
- All positive test sera should be titered to endpoint. This is accomplished by preparing serial two-fold dilutions of the test sera in PBS, i.e., 1:20, 1:40, 1:80, etc. The endpoint is the last dilution that produces positive apple-green staining.
- The absence of staining in the infected cells represents a negative reaction.

LIMITATIONS OF THE ASSAY

1. Nuclear or cytoplasmic staining may be observed due to nonspecific or autoantibody reactions such as antinuclear or anti-mitochondrial antibodies associated with systemic lupus erythematosus and primary biliary cirrhosis, respectively. These reactions will occur in **all** cells.
2. Nonspecific staining of all cells may be observed in some sera at low dilutions.
3. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.

EXPECTED VALUES

In classical infectious mononucleosis EBV-VCA antibodies develop early, reaching peak titers in 2-4 weeks. Titers then gradually diminish and reach a lower level which appears to persist for life (5). A four-fold rise in titer between acute and convalescent sera is diagnostic of an acute or recent IM infection. However, since some patients may not see their physician until 7-14 days after onset, some acute sera may show EBV-VCA antibody titers from 1:10 to 1:640, or greater. Therefore, both acute and convalescent sera should be run simultaneously. A single high titer serum above 1:640 is strongly suggestive of a recent EBV infection. It should be noted that the titer of EBV-VCA antibodies may not reflect the severity of clinical symptoms (4). High titer EBV-VCA antibodies may be found in patients with a history of pneumonia or urinary tract infection (13). A subsequent rise in EBV-VCA antibodies sometimes in excess of 1:2560 may be the result of secondary disease such as Burkitt's lymphoma or nasopharyngeal carcinoma (10-14).

PERFORMANCE CHARACTERISTICS

1. Several reports have shown that the results of the IFA test for EBV-VCA antibodies paralleled the results obtained with the complement fixation test (15), but failed to correlate with the heterophile antibody test. Unlike heterophile antibody, which is transient and may fail to develop in about 10% of infected adults, and a higher

develop during most EBV infections and persist indefinitely. In addition, unlike heterophile antibody titers, EBV-VCA antibody titers are not usually affected by other febrile viruses, such as coxsackievirus, adenovirus, myxovirus, and other herpes viruses (4).

2. The performance of the Zeus Scientific, Inc. EBV-VCA IFA test system with **ZORBA-NS** as sample diluent was compared to the same test system using PBS as the sample diluent (standard methodology). Seventy-four samples from a normal plasma donor population were tested as described. All 74 samples were positive in PBS and **ZORBA-NS**.

Forty-seven samples from a pediatric population were obtained from a hospital in the Northeast. Twenty-five (25) of 47 samples were positive in PBS; the same samples were positive when diluted in **ZORBA-NS**. The remaining 22 samples were negative when tested in both sample diluents.

Ten samples, with endpoint titers ranging from 1:40 to 1:1280 in PBS were also titered in ZORBA-NS (see Test

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