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**EPSTEIN-BARR VIRUS (EBV)
VIRAL CAPSID ANTIGEN (VCA) IgM TEST SYSTEM
Catalog # 401005-M**

INTENDED USE

The Diagnostic Automation Inc. EBV-VCA IgM IFA test system is a prestandardized kit designed for the qualitative and semi-quantitative detection of EBV-VCA IgM antibody in human serum by the indirect fluorescent antibody (IFA) technique, and is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

The etiologic relationship of Epstein-Barr Virus (EBV) to Infectious Mononucleosis (IM) has been firmly established and is now generally accepted (1,2,3). EBV infects only human 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 (12).

Antibodies to EBV-VCA develop in all patients with Burkitt's lymphoma, nasopharyngeal carcinoma, and EBV infectious mononucleosis (13). In addition, high EBV antibody titers are frequently associated with Hodgkin's disease and lymphocytic leukemia (13), SLE, Sarcoidosis (14), and Izumi fever (15).

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 heterophile antibody response is more pronounced, especially in the pediatric age ranges. Therefore, the EBV-VCA serodiagnostic test is recommended for cases of IM-like diseases 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 ASSAY

The Diagnostic Automation Inc. fluorescent EBV-VCA IgM antibody test system is designed to detect circulating EBV-VCA IgM antibodies in human sera. The system employs EBV infected substrate cells and goat anti-human IgM adjusted for optimum use dilution, and free of non-specific background staining. The test procedure involves three incubation steps:

1. Test sera are first treated to remove IgG and rheumatoid factor. (See Limitations section, No. 2).
2. Test sera are added to the wells, and incubated. Antigen specific IgM will bind to the EBV infected substrate cells.
3. Fluorescein labeled anti-human IgM conjugate is added to the wells and the slides are incubated. The conjugate will react with the antigen specific IgM antibodies bound to the slides in step 2. The slides are washed to remove unbound conjugate. The slides are then mounted with a coverslip and read under a fluorescence microscope.

KIT COMPONENTS

Reactive Reagents:

- EBV-VCA Antigen Slides: Five, 10-well substrate slides containing infected cells in each well. (Product #:9152-10M).
- Goat anti-human IgM (μ chain specific) labeled with FITC: Contains 1.25% bovine albumin and counterstain. One, 1.5mL vial, lyophilized. (Product #: 9153-MB).
- Human EBV-VCA IgM Positive Control Serum: One, 0.5mL vial, lyophilized. Composed of human sera. (Product #: 9154-M).
- Human EBV-VCA IgM Negative Control Serum: One, 0.5mL vial, lyophilized. (Product #:9155-M).

Non-reactive Materials:

- Phosphate-buffered-saline (PBS): Sufficient to prepare 2 liters. (Product #:0008).
- Mounting Media (Buffered Glycerol): 3.0mL. (Product #:0009).
- Absorbent blotters for drying the slide mask after washing procedure.

NOTE: All reactive reagents, as well as buffered glycerol contain a preservative which may be toxic if ingested (thimerosal, mercury derivative 1:10,000).

PRECAUTIONS

- The preservative may be toxic if ingested.
- No U.S. Standard of Potency
- Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of the antibody to HIV-1 as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive).

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus (HIV-1), hepatitis B virus, or other infectious agents are absent, this specimen/reagent, 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 Microbiological and Biomedical Laboratories", 1988, and FDA LABELING

GUIDELINES FOR *IN VITRO* DIAGNOSTIC REAGENT MANUFACTURERS, DEC., 1985.

- Do not apply pressure to the slide envelope. This may damage the infected cell matrix of the slide.
- Reagents from other sources or manufacturers should not be used.
- Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
- For *in vitro* diagnostic use.
- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Small serological, Pasteur, capillary, or automatic pipettes.
- Small test tubes, 13 x 100mm or comparable.
- Test tube racks.

Staining Dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation

- steps.
- Cover slip, 24 x 60mm, thickness No. 1.
- Distilled water.
- Moist chamber.
- 37°C incubator.
- 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 refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (21,22). No anticoagulants or preservatives should be added. Avoid using hemolytic, lipemic, or bacterial contaminated sera. Sera should be stored at 2-8°C for no longer than 5 days. If delay in testing is anticipated, store aliquoted 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 IgM substrate slides: Store at -20°C.
- Goat anti-human IgM 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.
- Human EBV-VCA IgM positive and negative control serum: Store at 2-8°C. Stable for 90 days after reconstitution. Frozen aliquots are stable for 6 months at -20°C.
- Phosphate-buffered-saline (PBS): Store at 2-25°C. 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.
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:

1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled water. Mix until all salts are thoroughly dissolved.
2. Human EBV-VCA IgM positive and negative control sera: Reconstitute with 0.5mL of distilled water.
3. Goat anti-human IgM labeled with FITC: Reconstitute with 1.5mL of distilled water. Alternatively aliquot in 0.5mL amounts and store at -20°C or lower in small tubes.

NOTE: Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.

magnification of 250X.

Test Procedure:

1. Remove substrate slides from freezer and allow them to warm to room temperature (20-25°C). Tear open the protective envelope and remove slide(s) containing the EBV infected cells. **DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE. THIS MAY DESTROY THE INFECTED CELL MATRIX ON THE SLIDE.**
2. Pretreat the test sera to remove IgG. Precipitation with anti-human IgG is recommended because this procedure is effective in removing all subclasses of human IgG and is less cumbersome to perform than other methods. (See Limitation No. 2).
3. After the pretreatment step, test sera should be at a 1:10 screening dilution. The prediluted positive and negative serum controls, and a buffer control should be run each time the test is performed.
4. Identify each well with the appropriate patient sera and controls.
5. Spread 20µL of test and control sera over each appropriately labeled well. Be careful not to disturb the substrate cells with pipette tip.
6. Incubate slides in a sealed moist chamber at 37°C for 60 minutes. **DO NOT ALLOW WELLS TO DRY.**

7. Take slides from the moist chamber ONE AT A TIME, 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.
8. Place slides in a staining dish and wash in PBS for two, 5 minute intervals with a change of PBS. Use a magnetic mixing setup or other means of gentle agitation.
9. Remove slides ONE AT A TIME from PBS. 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 towels may destroy the slide matrix.
10. Add 20 μ L of conjugate to each well.
11. Place slides in moist chamber. Incubate slides for 30 minutes at 37°C. DO NOT ALLOW SLIDES TO DRY.
12. Repeat steps 7 to 9.
13. 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 nuclear 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 positive results.
6. The intensity of the observed fluorescence may vary with the microscope and filter used.

RESULTS

1. Conjugate Control: A small number of cells may exhibit low level fluorescence. This fluorescence is considered insignificant in the interpretation of the EBV-VCA IgM test.
2. Negative Control: Similar or slightly increased intensity of fluorescence seen in the conjugate control may be observed in the negative control.
3. Positive Control: Shows staining at a greater intensity and in more cells than that observed in the negative control. A positive reaction is expected to be \geq 1+ staining intensity.
4. A Positive Reaction: A specimen is considered positive when it shows staining at a greater intensity and in more cells than that observed in the negative control. A positive reaction is expected to be \geq 1+ staining intensity.

LIMITATIONS

1. Nuclear or cytoplasmic staining may be observed due to nonspecific or autoantibody reactions such as antinuclear or mitochondrial antibodies associated with systemic lupus erythematosus and primary biliary cirrhosis, respectively.
2. IgG antibodies to EBV-VCA, if present in the sample, may interfere with determination of IgM titers to the organism. High affinity IgG antibodies may preferentially bind to antigenic determinants leading to false negative IgM titers (16). Also, IgM

rheumatoid factor may bind to the antigen specific IgG leading to false positive IgM titers (17). Both of these problems can be eliminated by removing IgG from the samples before testing for IgM. Several different methods of separating IgG have been used. These include gel filtration, absorption with protein A (20), ion exchange chromatography (18), precipitation of IgG with anti-human IgG serum (19), or the use of **ZORBA®** IgG Removal Reagent (Diagnostic Automation Inc. Product).

3. Occasionally a test specimen will exhibit excessive nonspecific fluorescence over the total cell population. If the specimen shows a sufficiently strong positive reaction then it may be possible to interpret the specific fluorescence through the excessive background fluorescence. If the specimen cannot be interpreted at the 1:10 dilution, the result is equivocal. **NOTE:** It may be possible to detect a positive reaction by evaluating such a specimen through serial dilutions.
4. In some cases high concentration of IgM patient's sera may produce a slight nonspecific staining of all cells. This staining is distinguished from the specific staining observed in the infected cells (11).
5. A negative result does not rule out current EBV infection since the specimen may have been collected before demonstrable antibody is present or after the antibody has decreased below detectable levels. Consequently, demonstration of elevated EBV-VCA IgG titers in conjunction with specific EBV-VCA IgM increases the specificity of serological diagnosis (5).
6. 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

The presence of EBV-VCA IgM antibodies as determined by the IFA 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 usually not present in the general population (10).

PERFORMANCE CHARACTERISTICS

The Diagnostic Automation Inc. EBV-VCA IgM test system is a highly specific procedure for the determination of IgM antibodies to EBV-VCA and is not affected by other viral diseases such as coxsackievirus, adenovirus, myxovirus, and other herpes viruses (4). Proper adherence to the use of the Diagnostic Automation Inc. EBV-VCA IgM test system with recommended procedures and a properly equipped fluorescence microscope should provide reproducible results.

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