EBV-VCA IgM Test System

REF FA9151M

IVD

CE

Rx Only

INTENDED USE

The ZEUS IFA Epstein-Barr Virus Viral Capsid Antigen (EBV-VCA) IgM Test System is a pre-standardized test system designed for the qualitative and semi-quantitative detection of EBV-VCA IgM antibodies 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, and 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 - 10% of adult patients (5). In addition, the absence of heterophile antibody response is more pronounced, especially in the pediatric age ranges. Therefore, the ZEUS IFA EBV-VCA IgM Test System 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 ZEUS IFA EBV-VCA IgM Test System is designed to detect circulating EBV-VCA IgM antibodies in human sera. The assay employs EBV infected substrate cells and goat anti-human IgM adjusted for optimum use dilution, and free of non-specific background staining. The assay procedure involves three incubation steps:

1. Test sera are first treated to remove IgG and rheumatoid factor (see Limitation of Assay, 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.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. SAVe Diluent® contains Sodium Azide (<0.1% w/v) as a preservative.**

• • •		1. EBV-VCA IgM Antigen Substrate Slides: Ten, 10-well Slides containing EBV infected cells in each well. Also includes absorbent blotter and desiccant pouch.
CONJ		2. Conjugate: Anti-human IgM (µ chain specific) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5mL, amber-capped, bottle. Ready to use.
CONTROL	+	3. Positive Control (Human Serum): Will produce positive apple-green staining of the EBV infected cells. One, 0.5mL, red-capped, vial. Ready to use.
CONTROL	-	4. Negative Control (Human Serum): Will produce no detectable staining of the EBV infected cells. One, 0.5mL, green-capped, vial. Ready to use.
DIL	SPE	5. SAVe Diluent®: One, 30mL, green-capped, bottle containing phosphate-buffered-saline Ready to use. **NOTE: The SAVe Diluent® will change color when combined with serum.**
BUF	PBS	6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
MNTMED		7. Mounting Media (Buffered Glycerol): Two, 3.0mL, white-capped, dripper tipped vials.

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS IFA Test Systems, as long as the product numbers are identical: SAVe Diluent® (Product #: FA005CC), Mounting Media (Product #: FA0009S), and PBS (Product #: 0008S).**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. 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 Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous materials** and handle accordingly.
4. The Controls are **potentially bio-hazardous 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 Bio-safety 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 (20).
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 their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.

7. The SAVe Diluent®, Conjugate, and Controls 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. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
17. 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 Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened 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. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish: A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing Slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).
12. IgG Removal System (see Limitations of the Assay, 2).

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

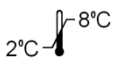
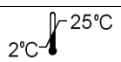
Transmitted Light		
Light Source: Mercury Vapor 200W or 50W		
Excitation Filter	Barrier Filter	Red Suppression Filter
KP490	K510 or K530	BG38
BG12	K510 or K530	BG38
FITC	K520	BG38
Light Source: Tungsten – Halogen 100W		
KP490	K510 or K530	BG38

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

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. 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.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (21). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. 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 - 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 which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (24).

STORAGE CONDITIONS

	Unopened Test System.
	Mounting Media, Conjugate, SAVe Diluent®, Slides, Positive and Negative Controls.
	Rehydrated PBS (Stable for 30 days).
	Phosphate-buffered-saline (PBS) Packets.

ASSAY PROCEDURE

1. Remove Slides from refrigerated storage and allow them to warm to room temperature (20 - 25°C). Tear open the protective envelope and remove Slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. **NOTE: The Controls are intended to be used undiluted**.
   Diluting Patient Sera:
   a. It is recommended that test sera are pre-treated 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. After the pretreatment step, test sera should be at a 1:10 screening dilution (e.g.: 10µL of serum + 90µL of SAVe Diluent® or PBS).
   b. If patient samples are to be titrated to endpoint, one should pre-treat the serum to remove IgG and then make any subsequent dilutions with SAVe Diluent® or PBS**. NOTE: The SAVe Diluent® will undergo a color change confirming the combination of specimen with Diluent.**
   c. As an option, users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in SAVe Diluent® or PBS. When evaluated by ZEUS Scientific, 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 expected end-point titer for each lot of Positive Control.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at 35 - 37°C for 60 minutes.
5. Gently rinse Slides with PBS. **Do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5 minute intervals, changing PBS between washes.
7. 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 towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure**.
8. Add 20µL of Conjugate to each well. Incubate Slide at 35 - 37°C for 30 minutes.
9. Repeat steps 5 through 7.
10. Apply 3 - 5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.

QUALITY CONTROL

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the Positive and Negative Controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If Controls do not appear as described, results are invalid.
   a. Negative Control - characterized by the absence of nuclear staining and a red, or dull green, background staining of all cells due to Evans Blue. Use the reaction of the Negative Control serum as a guide for interpretation of patient results.
   b. Positive Control - characterized by 2+ tp 4+ apple-green fluorescent staining intensity of the cell membrane, nucleus, and cytoplasm in 5 – 15% of the total cell population.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The intensity of the observed fluorescence may vary with the microscope and filter system used.

INTERPRETATION OF RESULTS

1. Buffer 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 OF THE ASSAY

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 (ZEUS Product #: FA003G).
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 RESULTS

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 ZEUS IFA 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 ZEUS IFA EBV-VCA IgM Test System with recommended procedures and a properly equipped fluorescence microscope should provide reproducible results.

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