AESKU.BION
AN AESKU.GROUP COMPANY

EBV - M (VCA)
ANTIBODY TEST SYSTEM

CE

NOTE: Changes highlighted

SYMBOL DEFINITIONS

- = Consult Directions for Use
- = Store Away From Direct Light
- = Storage Temperature
- = Expiration Date
- = Number of Tests
- = Amount
- = Single Use Only
- = Contains human blood or plasma derivatives
- IVD = *In Vitro* Diagnostic Reagent
- CONTROL + = Positive Control
- CONTROL − = Negative Control
- TITER = Endpoint Titer
- REF = Code Number
- LOT = Lot Number
- = Contains biological material of animal origin

Bion EBV-M
KITS AND REAGENTS

KITS and KIT COMPONENTS	CODE NO.
EBV-M (EBV-VCA IgM Antibody) 120 Test Kit	EBM-120
EBV-VCA Substrate Slide, twelve wells	EB-5012
EBV-VCA IgM Positive Control Serum, 0.5 ml	EBM-5030
EBV-VCA Negative Control Serum, 0.5 ml	EBN-5010
Conjugate, IgM with Counterstain, 3.5 ml	CXCM-9974
Mounting Medium, 3.5 ml	MM-9985
PBS Packet, One Liter	PBS-9990

INTENDED USE

The Bion EBV-M (VCA) ANTIBODY TEST SYSTEM (Epstein-Barr Virus Viral Capsid Antigen) is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of EBV-VCA IgM antibodies in human serum. The Bion EBV-M (VCA) ANTIBODY TEST SYSTEM is intended for use as an aid in the diagnosis of primary infection (Infectious Mononucleosis), reinfection or reactivation of the latent virus and as a determination of immunological experience with EBV.

SUMMARY AND EXPLANATION

The Epstein-Barr Virus (EBV) was first detected and described by Epstein, Barr and Achong in electron-microscopic studies of lymphoblastoid cultures obtained from patients with Burkitt's Lymphoma.[1,2] The etiologic role of EBV in Infectious Mononucleosis (IM) was first reported in 1968 and has since been well documented to be the causative agent.[3,4,5] Infection with EBV results in the expression of several viral proteins to which the host responds with appropriate antibodies. One of these viral proteins is the Viral Capsid Antigen (VCA).

Although the heterophile antibody response is relatively specific for IM, it has been observed that most children and up to 10% of adult patients fail to develop heterophile antibodies.[6] Cytomegalovirus and Toxoplasma gondii also produce infectious mononucleosis-like disease which is often clinically indistinguishable from EBV Infectious Mononucleosis. Testing for EBV specific antibodies should be performed in all cases of infectious mononucleosis-like disease and especially those that are heterophile negative.

Elevated antibody titers to EBV-VCA develop in patients with Burkitt's Lymphoma, Nasopharyngeal Carcinoma, and EBV Infectious Mononucleosis. A high incidence of elevated titers is also found in Hodgkin's Disease, Lymphocytic Leukemia, Systemic Lupus Erythematosus, Sarcoidosis, and Izumi Fever.[7,8,9]

Many symptomatic patients have already reached peak titers of IgG to EBV-VCA by the time that they consult their physician, and further increases cannot be demonstrated.[10] Testing for IgM specific EBV-VCA antibodies should be done in such cases. Methods for EBV-VCA antibody detection include Enzyme-Linked Immunosorbent Assay (ELISA) and Immunofluorescence Assay (IFA).[11] The ELISA method is not able to dependably differentiate between specific and nonspecific reactions. However, the localization of the antigen-antibody reaction can be visualized with the IFA test, thus allowing the specific antigen-antibody reaction to be recognized by the morphology of the fluorescence staining.[11]

SUMMARY OF THE IFA PROCEDURE

The Bion EBV-M VCA ANTIBODY TEST SYSTEM utilizes the indirect fluorescent antibody assay method first described by Weller and Coons[12] and further developed by Riggs, et al.[13] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum (IgM fraction) is reacted with the antigen substrate. Antibodies, if present, will bind to the antigen forming stable antigen-antibody complexes. If no antibodies are present, the complexes will not be formed and serum components will be washed away.

Step 2 - Fluorescein labeled antihuman IgM antibody is added to the reaction site which binds with the complexes formed in step one. This results in a positive reaction of bright apple-green fluorescence when viewed with a properly equipped fluorescence microscope. If no complexes are formed in step one, the fluorescein labeled antibody will be washed away, exhibiting a negative result.

REAGENTS

EBV-VCA ANTIGEN SUBSTRATE SLIDES

Ten individually foil-wrapped twelve well slides with a mixture of Epstein-Barr Virus (P3HR1 strain) infected and uninfected lymphocytic cells fixed onto each well. Each well contains an average of 10% infected cells per 200X field. Stable in sealed foil pouch at 8°C, or lower, until labeled expiration date.

POSITIVE CONTROL SERUM

One vial containing 0.5 ml EBV-VCA positive IgM human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

IgM positive controls do not have to be pretreated to remove IgG. When used undiluted as provided, specific fluorescent intensity of 3+ or greater should be seen. Optionally, the positive control can be titered to endpoint. If titered, the control should be serially diluted in PBS. When the control has been tested for the endpoint titer by Bion, an endpoint titer is printed on the positive control vial. Due to variations within each laboratory (fluorescent microscope, etc.), each laboratory should establish its own mean titer for each lot of positive control (generally ± one dilution from stated endpoint).

NEGATIVE CONTROL SERUM

One vial containing 0.5 ml EBV-VCA negative human control serum with protein stabilizer and 0.005% thimerosal. Stable at 2-8°C until labeled expiration date.

The control is intended to be used undiluted as provided. The staining should exhibit less than 1+ fluorescence.

MOUNTING MEDIUM

One dropper vial containing 3.5 ml phosphate buffered glycerol of pH 7.4 ± 0.2. Stable at 2-8°C until labeled expiration date.

FLUORESCENT ANTIBODY CONJUGATE

Two ready to use dropper vials, each containing 3.5 ml fluorescein isothiocyanate labeled goat antihuman IgM (heavy chain specific) with 0.1% Evans Blue counterstain, protein stabilizer, less than 0.1% sodium azide and 0.001% thimerosal added. Stable at 2-8°C away from direct light until labeled expiration date.

PHOSPHATE BUFFERED SALINE (PBS)

Two one-liter packets of dry PBS. Stable in sealed packet at 25°C, or lower, until labeled expiration date.

BUFFER PREPARATION

Place contents of a one-liter PBS packet in a one-liter volumetric flask, add *distilled water to the one-liter mark, mix and leave several hours or overnight to dissolve. Reconstituted buffer should have a pH of 7.4 ± 0.2. Adjust with 1N NaOH or 1N HCL if pH value is outside the stated range. Store in a clean screw capped bottle at 25°C or lower. Stable until labeled expiration date provided no gross contamination is seen. Do not use if pH changes, if the solution turns cloudy, or if a precipitate forms.

* Use deionized water with caution, as pH of this type of water may vary causing the pH of PBS to become unstable upon prolonged storage.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnostics may perform the test.
2. Substrate slides are for single use only and must not be used more than once.
3. Care should be taken when handling substrate slides due to sharp edges.
4. The antigenic substrates have been fixed in acetone and contain no detectable live Epstein-Barr Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
5. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
6. All reagents should be brought to room temperature (20-25°C) prior to use.
7. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
8. Refrigeration (2-8°C) of kit immediately upon arrival will insure stability until labeled expiration date.
9. Reagents should not be used beyond stated expiration date.
10. Substitution of components other than those provided may yield inconsistent results.
11. Do not expose conjugate to strong light during storage or use.
12. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
13. Incubation times or temperatures other than those specified may give erroneous results.
14. Reusable glassware must be washed and thoroughly rinsed free of detergents.
15. Care should be taken to avoid splashing or generation of aerosols.
16. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
17. Patient samples, as well as all materials coming into contact with them, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories", 1984 Edition. Never pipette by mouth. Avoid contact with skin and mucous membranes.

WARNINGS AND PRECAUTIONS (continued)

16. Sera used to prepare positive and negative controls have been tested by an FDA approved method and found to be negative (were not repeatedly reactive) for the presence of Hepatitis B surface Antigen (HBsAg) and antibodies to Hepatitis C (HepCAb) and HIV 1 & 2. However, because no test method can offer complete assurance of the absence to these or other infectious agents, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the CDC/NIH manual "Biosafety in Microbiological and Biomedical Laboratories," 1984 Edition.
17. The preservatives used in conjugate and controls are toxic if ingested. Azides may react with copper or lead plumbing to form explosive metal azides. When disposing, flush drains with water to minimize build-up of azide and metal compounds.

SPECIMEN COLLECTION

Blood should be collected fasting or at least one hour after meals to avoid lipemic serum, as excess lipids may produce a “film” over the substrate. Aseptically collect 5-8 ml of blood by venipuncture. Allow the blood to clot at room temperature (20-25°C) before separating serum to avoid hemolysis which could interfere with test results. Specimens should be stored refrigerated at 2-8°C and tested within one week of collection. Long term storage should be at -20°C in aliquots to avoid repeated freezing and thawing. Do not store in self-defrosting freezer.

Avoid using contaminated sera as they may contain proteolytic enzymes which will digest the substrate. It is unnecessary to heat inactivate serum specimens prior to testing; however, sera that have been heat inactivated may be used.

When testing paired samples to look for evidence of recent infection, the acute specimen should be obtained as soon as possible after onset of illness and the convalescent specimen obtained 7-14 days later. Acute and convalescent specimens must be tested simultaneously, in the same assay, looking for a significant change in antibody titer between the paired sera. If the first specimen is obtained too late during the course of the infection, a significant rise in the antibody titer may not be detected.

PROCEDURE

MATERIALS PROVIDED

1. EBV-VCA Antigen Substrate Slides
2. Fluorescent Antibody Conjugate
3. Positive Control Serum
4. Negative Control Serum
5. Phosphate Buffered Saline (PBS)
6. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

1. One liter volumetric flask or one liter graduated cylinder
2. Distilled water - CAP Type one or equivalent
3. One-liter screw capped container
4. Disposable test tubes (12 x 75 mm or comparable) and rack
5. Disposable serological pipettes
6. IgM separation system
7. Calibrated pipettes to deliver 50 µl, 100 µl and 200 µl, with disposable pipette tips
8. Pasteur pipettes and bulbs
9. Moist chambers
10. Plastic squeeze wash bottle
11. Coplin jars or staining dishes with slide racks
12. 24 x 60 mm #1 coverslips
13. Felt tip marking pen
14. Fluorescence microscope equipped with a mercury or tungsten-halogen light source, a 390-490nm excitation filter and 515-520nm barrier filter, and optics to give a total magnification of 200X or 250X. The excitation wavelength of FITC is 490nm and the emission wavelength is 520nm.

TEST PROCEDURE

The greatest source of error in IgM testing is interference by the presence of pathogen specific IgG. This can occur in one of two ways.

a. False Negative Reactions may occur due to high levels of specific IgG blocking the IgM in the competition for antigenic sites during the first step of the staining reaction. The IgG, being more avid that the IgM, will react with the antigenic sites first, blocking the IgM from participating in the reaction. The IgM will then be washed away during the first wash, leaving nothing for the anti-IgM conjugate to react with in step two of the staining reaction resulting in a false negative reaction in the presence of pathogen specific IgM.

b. False Positive Reactions may occur when Rheumatoid Factors are present along with pathogen specific IgG antibodies. Some Rheumatoid Factors can be immunoglobulin M anti-IgG. When specific IgG reacts with the antigenic sites during the first step of the staining reaction, the Rheumatoid Factor anti-IgG will then react with the bound IgG. Rheumatoid Factor being an antibody of the IgM class will then react with the anti-IgM conjugate in step two of the staining reaction, resulting in a false positive reaction in the absence of pathogen specific IgM.

It is, therefore, strongly recommended that each patient serum specimen be pre-treated to remove any IgG interference by separating the IgM from the IgG using any of the standard methodologies.[14,15,16] Two such methodologies are ion exchange chromatography[17] and IgG immunoprecipitation.[18,19]

TEST PROCEDURE (continued)

1. **SPECIMEN PREPARATION**
   Separate IgM from IgG by treating patient serum to remove IgG as suggested above.

   Screening:
   It is recommended that screening be carried out at a 1:10 and 1:40 dilution.[20]

   a. Prepare a 1:10 dilution of each patient's serum using one of the above treatments.
   b. Prepare a 1:40 dilution by adding 0.1 ml (100 µl) of the 1:10 dilution to 0.3 ml of PBS.

   Semi-quantitation:
   Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The following twofold serial titration is suggested:

   a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned above. This will be designated as tube #1.
   b. Add 0.2 ml PBS to tubes #2, #3, #4, and #5.
   c. Using a 200 µl pipette, transfer 0.2 ml (200 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.2 ml (200 µl) from the second tube to the third, from the third tube to the fourth, and from the fourth tube to the fifth, mixing after each transfer. This will give a twofold titration with the following dilutions:

Tube #1 = 1:10	Tube #4 = 1:80
Tube #2 = 1:20	Tube #5 = 1:160
Tube #3 = 1:40	

2. **SLIDE PREPARATION**
   Remove reagents and as many slides as are required from the refrigerator or freezer and allow to equilibrate to room temperature (20-25°C) for at least five minutes. Remove slides from sealed foil pouches being careful not to touch the antigen surface. Identify each slide using a felt tip marking pen.

3. **SPECIMEN APPLICATION**
   Using separate Pasteur pipettes, apply one drop (20-30 µl) of the positive control, one drop (20-30 µl) of the negative control and one drop (20-30 µl) of each patient serum dilution to individual wells of the slide. Do not touch the antigen surface with the pipette while dropping. Do not allow drops to mix, as cross contamination of samples between wells could cause erroneous results.

4. **INCUBATION 1**
   Incubate in a moist chamber at 35-37°C for 90 minutes.
   NOTE: THE ANTIGEN MUST NOT BE ALLOWED TO DRY DURING ANY OF THE FOLLOWING STEPS. Nonspecific binding may occur if the reagent is allowed to dry on the slide.

5. **RINSE 1**
   Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. Do not focus the PBS stream directly onto the wells. To prevent cross contamination tilt slide first toward wells 1-6 and, running a PBS stream along the midline of the slide, allow the PBS to run off the top edge of the slide. Then, tilt the slide toward wells 7-12 and repeat this procedure, allowing the PBS to run off the bottom edge of the slide.

6. **WASH 1**
   Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

7. **CONJUGATE APPLICATION**
   Remove slides from the wash one at a time, shake off excess PBS, dry around outside edges if necessary and return each slide to the moist chamber. Apply one drop of conjugate to each well of each slide, making sure each well is completely covered.

8. **INCUBATION 2**
   Incubate in a moist chamber at 35-37°C for 60 minutes. Protect slides from excessive light.

9. **RINSE 2**
   Remove slides from moist chamber and rinse GENTLY with PBS using a squeeze wash bottle. As suggested in step 5., do not focus PBS stream directly onto the wells.

10. **WASH 2**
    Place slides in Coplin jars or staining dishes and wash in two changes of PBS for not less than five minutes or more than ten minutes each, agitating gently at entry and prior to removal.

11. **COVERSLIP**
    Remove slides one at a time from last PBS wash, shake off excess PBS and immediately add two to four drops of mounting medium across the slide. Tilt slide and rest the edge of the coverslip against the bottom of the slide allowing the mounting medium to form a continuous bead between the coverslip and slide. Gently lower the coverslip from the bottom of the slide to the top, being careful to avoid air bubbles. Drain excess mounting medium by holding the edge of the slide against absorbent paper. Wipe off back of slide.

12. **READ**
    Examine stained slides as soon as possible using a properly equipped fluorescence microscope. It is recommended that slides be examined on the same day they are stained. If any delay is anticipated, store slides in the refrigerator (2-8°C) away from direct light and read the following day. Do not allow mounting medium to dry between slide and coverslip. If drying should occur, add additional mounting medium or recoverslip slide.

    FLUORESCENT INTENSITY GRADING
    Fluorescent intensity may be semi-quantitated by following the guidelines established by the Centers for Disease Control, Atlanta, Georgia:[21]

    4+ = Maximal fluorescence; brilliant yellow-green.
    3+ = Less brilliant yellow-green fluorescence.
    2+ = Definite but dull yellow-green fluorescence.
    1+ = Very dim subdued fluorescence.

    The degree of fluorescent intensity is not clinically relevant and has only limited value as an indicator of titer. Differences in fluorescence microscope optics, filters and light sources may result in differences of 1+ or more fluorescent intensity when observing the same slide using different microscopes.

QUALITY CONTROL

SPECIFICITY CONTROL

Both a positive and negative antibody control must be included with each run. These controls must be examined prior to reading test samples and should demonstrate the following results:

Negative Control

Using the Bion EBV-VCA NEGATIVE CONTROL SERUM as provided with the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control

Using the Bion EBV-VCA POSITIVE IgM CONTROL SERUM as provided with the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM, EBV infected cells should exhibit solid fluorescent staining of the entire cell at an intensity of 3+ or greater. Approximately 10% of the cells should exhibit this specific staining pattern with the uninfected cells staining reddish-orange due to the counterstain.

Each control must demonstrate the expected reaction in order to validate the test. If the controls fail to appear as described above, the test results should not be reported and the test should be repeated. If upon repeat testing the controls still fail to show the proper reaction, do not report test results.

SENSITIVITY CONTROL

A titered control included with each run tests substrate sensitivity, as well as, checks technique, conjugate quality and the microscope optical system. The endpoint titer of each lot of Bion EBV-VCA IgM CONTROL SERUM must be determined. There must not be more than a twofold difference (+/-) in titer from the stated endpoint. Each run should include the endpoint dilution, one twofold dilution above and one twofold dilution below the endpoint dilution. The more concentrated dilution should be positive and the less concentrated dilution negative. If the control does not behave as described, the test results are invalid and the tests should be repeated. If the control again fails to show the proper reaction upon repeat testing, do not report the test results.

READING OF TEST RESULTS

NEGATIVE

A serum dilution is considered negative for EBV-VCA IgM antibodies if the cells exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain, or if the fluorescence observed is not the specific staining pattern of EBV-VCA.

A sample is considered negative for EBV-VCA IgM antibodies if it exhibits less than 1+ fluorescence at a serum dilution of 1:10 and all greater dilutions, or if the fluorescence observed is not the specific staining pattern of EBV-VCA.

... Negative samples may exhibit fluorescent staining of the infected cells slightly greater than the Negative Control, but less than 1+.

... Nonspecific staining of all cells observed in some sera at low dilutions is most likely due to the presence of autoantibodies against cellular components in either the nucleus or cytoplasm.

... Staining of areas other than the viral infected cells should be interpreted as negative and attention should be directed to specific steps in the staining method (e.g., RINSE and WASH steps).

POSITIVE

A serum dilution is considered positive for EBV-VCA IgM antibodies if, at an intensity of 1+ or greater, there is well defined specific fluorescent staining in the EBV-VCA infected cells. The EBV-VCA fluorescent staining pattern consists of solid staining of the entire cell. The number of cells exhibiting a positive staining reaction and the type of fluorescent staining pattern should closely approximate that seen in the Positive Control.

A sample is considered positive for EBV-VCA IgM antibodies if it exhibits the characteristic EBV-VCA staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTE: Each field should contain cells that exhibit no apple-green fluorescence. Should most of the cells in the patient test wells fluoresce apple-green in the nucleus and/or cytoplasm, an autoimmune staining reaction due to the presence of autoantibodies should be considered.[22,23] It is recommended that such samples be diluted beyond the interference for better interpretation. It is possible that autoantibody staining may mask specific staining such that an interpretation cannot be made. Should this occur, test results should be reported as "Unable to interpret due to the presence of interfering antibodies."

TITRATION

If a semi-quantitative titration is performed, the result should be reported as the reciprocal of the last dilution in which 1+ apple-green fluorescent intensity of the specific staining pattern is detected.

TROUBLESHOOTING

Possible solutions to problems that may occur in immunofluorescent assays are discussed in an accompanying brochure entitled "TROUBLESHOOTING IN IMMUNOFLUORESCENCE".

INTERPRETATION OF RESULTS

RESULT	INTERPRETATION OF SINGLE SAMPLE RESULTS
Less than 10	Negative - Indicates no evidence of recent infection with EBV and susceptibility to this agent. NOTE: This may represent a primary infection with the humoral immune response not yet developed to detectable levels. If infection with EBV is still suspected, a second specimen should be obtained 7-14 days later, and the paired specimens tested simultaneously, looking for a seroconversion.
10 or Greater	Positive - Usually indicative of a recent primary EBV infection, particularly in the absence of EBNA anticomplement immunofluorescence (ACIF) positive results.

NOTE: 1. Demonstration of elevated EBV-VCA IgG titers and no antibody to Epstein-Barr Nuclear Antigen (EBNA) by anticomplement immunofluorescence (ACIF) in conjunction with a positive EBV-VCA IgM result improves the specificity of serological diagnosis.[6]

2. Low levels of EBV-VCA IgM may be present in cases of reactivated EBV infection. It is usually of low titer, especially after treatment to absorb rheumatoid factor. It is controversial whether it is virus-specific IgM or a nonspecific reaction.

ACUTE RESULT	CONVALESCENT RESULT	INTERPRETATION OF PAIRED SAMPLE RESULTS
Less than 10	Less than 10	Not likely to be an acute EBV infection. NOTE: This may represent a primary infection if time of obtaining the second specimen is too soon after the first. If this condition is suspected, obtain a third specimen 7-14 days after the second specimen and run the three simultaneously, looking for a seroconversion.
Less than 10	10 or Greater	Most likely a primary infection or reactivation with EBV unless the individual has recently acquired passive antibody.

SEROLOGICAL RESPONSES TO EPSTEIN-BARR VIRUS INFECTION[10]

PB[a]	VCA-IgM	VCA-IgG	EA/D[b]	EA/R[c]	EBNA[d]	INTERPRETATION
0	0	0	0	0	0	Susceptible
+ or 0[24]	+	+	+ or 0	+ or 0	0	Acute Primary
0	+ or 0[25,26]	+	+ or 0	+ or 0	+ or 0[10,26]	Recent Primary
0	0	+	0	0	+	Past Infection
0	0	+	+ or 0	+ or 0	+	Past Infection with Possible Reactivation
0	0	++	0	++	+	Burkitt's Lymphoma
0	0	++	++	+ or 0	++	Nasopharyngeal Carcinoma

Key: a = Paul Bunnell Heterophile Antigen
b = Early Antigen, Diffuse
c = Early Antigen Restricted
d = Epstein-Barr Nuclear Antigen

LIMITATIONS OF THE PROCEDURE

1. EBV-VCA IgM antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. The presence of Rheumatoid Factor (RF) in serum may cause a false positive reaction if pathogen specific IgG is also present. Routine RF tests may not be sensitive enough to detect small amounts of RF which exist within the normal range, but are sufficient to cause a false positive reaction in the more sensitive IFA technique.[14,15,16] Therefore, all sera should be treated by ion exchange chromatography[17] or IgG immunoprecipitation[18,19] before testing to eliminate possible RF interference.
3. High titers of viral specific IgG when present in the patient serum may compete with the pathogen specific IgM for the antigen sites, resulting in a false negative IgM reaction.[14,15,16] Therefore, all sera should be treated by ion exchange chromatography[17] or IgG immunoprecipitation[18,19] before testing to avoid this possible problem.
4. Two methods such as immunoprecipitation and ion exchange chromatography have been commonly used for neutralizing or removing possibly interfering IgG antibodies prior to testing for specific IgM antibodies in IFA tests. Immunoprecipitation neutralizes all classes of IgG while not affecting the IgM levels; however, high levels of IgG may need to be treated with proportionally increased amounts of the precipitating reagent. Ion exchange chromatography will only eliminate IgG subclasses 1,2 and 3 with subclass 4 (usually less than 5% of the total IgG) remaining in the fraction with the IgM. Also, only a portion of the IgM antibodies can be recovered.[34]
5. A negative test result does not necessarily rule out a current or recent infection.[27] The specimen may have been collected too early in the disease before demonstrable antibody is present; or, the specimen may have been collected too late in the disease after the antibody level is no longer detectable.
6. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
7. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
8. Antinuclear antibodies (ANA) present in serum may interfere with the EBV-VCA IFA test. They can be differentiated from EBV-VCA staining in that ANAs stain the nuclei in all cells; whereas, EBV-VCA antibodies exhibit staining only in the 10% infected cells.[22]
9. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[23] They can be differentiated from EBV-VCA staining in that AMA will stain the cytoplasm of all cells; whereas, EBV-VCA antibodies exhibit staining only in the 10% infected cells.
10. Endpoint reactions may vary between laboratories due to differences in type or condition of fluorescence microscope employed, diluting apparatus, IgG/IgM separation methods, as well as the experience level of personnel performing the assay.
11. If both the positive and negative control substrate cells are not visible when viewed using the fluorescence microscope, it may be necessary to replace or realign the light source and check the specific filters.
12. Cell culture substrate slides may exhibit nonspecific fluorescence due to contamination of antibodies or PBS rinse-wash solutions with bacteria or fungi. It is very important that personnel reading the staining results have experience in fluorescence microscopy.
13. Because of the possibility of contamination of cord blood with maternal IgM, it is prudent to confirm positive viral IgM antibody results on cord blood samples by testing a follow-up specimen from the infant, preferably within the first five days of life.[29]

SPECIFIC LIMITATIONS OF THE EBV ASSAY

1. Positive test results require careful interpretation since false positive reactions or heterotypic IgM responses may occur with sera from patients with Cytomegalovirus (CMV) infection.[28,29,30]
2. Heterotypic IgM antibody responses to EBV in CMV infections have been reported.[31] Reactivation of latent EBV would seem to be a possible mechanism.[29]
3. A false negative result may occur due to a prozone reaction particularly if screening at only one low dilution such as 1:10.[20,32]

EXPECTED VALUES

In the absence of antibodies specific for EBNA as determined by ACIF, the presence of EBV-VCA IgM antibodies, determined by the IFA method, is highly suggestive of acute EBV infection, since these antibodies are found early in the illness in approximately 90% of the cases, and are usually not present in the general population.[33] These antibodies are present in both symptomatic and asymptomatic acute EBV infections, and are found in both heterophile positive and heterophile negative patients. EBV-VCA IgM antibodies in IM peak 3-4 weeks after onset, and decline to undetectable levels in 8-10 weeks.[33]

One study reported that 90-97% of patients with IM developed EB virus specific antibodies. In addition, 85% of the patients studied possessed EB virus IgM antibodies one month after onset of the illness, 40% after three months, 10% after nine months, and the EB-virus IgM antibodies had disappeared by 19 months after onset of the illness.[28]

SPECIFIC PERFORMANCE CHARACTERISTICS

To investigate the relative sensitivity and specificity of the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM, one hundred serum specimens were compared qualitatively and sixteen serum specimens were compared semi-quantitatively with another commercially available indirect fluorescent EBV-VCA Antibody Test System.

As summarized in TABLE 1, there were initially three discrepant results between the BION test system and the commercial test system. Of these three specimens, the BION test system resulted in a "positive" result; the commercial test system resulted in 2 of these specimens as "negative" (by duplicate readers) and one specimen as "positive" by one reader and "negative" by the second reader. These three specimens were sent to an outside Reference Laboratory where they were determined as "positive". Therefore, screening at only one dilution on some systems may result in a false negative result due to prozone reactions. No prozone reaction problems were demonstrated using the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM.[34]

TABLE 1 - SUMMARY OF RELATIVE COMPARISON TESTING

		BION KIT		Relative Sensitivity	Relative Specificity
		Positive	Negative	100%	96%
OTHER KIT	Positive	32	0	32/32	
	Negative	2(1)*	65		65/68

* When titered, these 3 specimens were also positive by an outside Reference Laboratory.

SPECIFIC PERFORMANCE CHARACTERISTICS (continued)

In addition, sixteen serum specimens were tested semi-quantitatively on both the BION test system and the other commercial test system to investigate the relative sensitivity of the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM. As summarized in TABLE 2, all sixteen specimens ranging in tier from less than 1:10 to 1:1280 agreed with no more than one twofold difference (+/-) in titer with both systems. The tables represent results from two independent readers. If the readers differed, both results are given.[34]

TABLE 2 - SUMMARY OF RELATIVE SENSITIVITY TESTING

Spec.#	BION	Other	Spec.#	BION	Other
1	<10	<10	9	160	80/160
2	<10	<10	10	160	40/160
3	<10	<10	11	160	80/160
4	<10	<10	12	160/320	80/160
5	<10	<10	13	320/640	160/320
6	10/20	10	14	320/640	320/640
7	10/40	10/20	15	640	320/640
8	10/40	10/40	16	640	640/1280

Interlot and intralot precision of the Bion EBV-M (VCA) ANTIBODY TEST SYSTEM was evaluated by testing eleven serum specimens (4 negative and 7 positive over a range of titers) using five different lot numbers of slides, as well as, testing them four times on slides from the same lot. There was no more than a twofold difference (+/-) in titer between any of the comparison testings, which is within the confidence limits of this methodology. None of the tests vacillated between a positive or a negative result.[34]

In addition, a cross-reactivity study of IgM tests done by IFA was conducted to insure that IgM antibodies to one virus would exhibit no specificity for other antigen substrates. A total of 40 serum specimens were treated to separate IgG and IgM and then tested for IgM. Ten specimens had IgM antibodies to EBV; ten specimens had IgM antibodies to HSV; ten specimens had IgM antibodies to CMV, and ten specimens had IgM antibodies to VZV. These specimens were then tested against all five antigen substrates.

As shown in TABLE 3, there did not appear to be any specific pattern of cross-reactions between antibodies of any one virus against the other four antigens except between HSV types 1 and 2. There were some multiple positive results. Some with comparatively low titers (*) may be of little or questionable significance while others had higher titers (**) which may be of importance.[34]

TABLE 3 - SUMMARY OF HERPES GROUP SPECIFICITY STUDY

No. of Spec.	EBV	HSV1	HSV2	CMV	VZV
9	160-1280	<10	<10	<10	<10
1	1280	<10	<10	10*	<10
10	<10	80-320	20-160	<10	<10
9	<10	<10	<10	10-160	<10
1	80**	<10	<10	640	<10
7	<10	<10	<10	<10	40-320
1	>80**	20*	20*	<10	640
1	<10	20*	20*	<10	80
1	20*	<10	<10	<10	80

Several limitations must be kept in mind when interpreting IgM serology tests. This is true even after separation treatment of IgG and IgM eliminates the problems of Rheumatoid Factors and competitive IgG binding. IgM specific antibody responses can occur not only with primary infection but can be attributed to reactivation or reinfection particularly in CMV, HSV, EBV and VZV infections.[15,30] It is also well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[32,35,36,37] In addition, with some viruses such as CMV and EBV, IgM specific antibodies may continue for many months. Thus, a person may have residual IgM from one infection and become infected with a different agent.[15,30] It is not uncommon for one childhood infection to follow closely after another. Also, an infection with a new agent may cause an anamnestic response in IgM from the recent previous infection.[38] Therefore, requesting a convalescent specimen looking for a change in antibody levels between paired specimens may help to clarify the actual current infection from the previous past infection.

IgM serology performed by IFA is very attractive since it combines specificity with sensitivity and in most cases only a single serum sample is required. However, the need for careful interpretation of the significance of positive IgM tests in relationship to patients' clinical situations must be emphasized. It is essential to have an awareness of understanding of the many problems associated with IgM testing to avoid the many pitfalls that can trap the most experienced of workers. IgM results must always be interpreted with caution.[34]

BIBLIOGRAPHY

1. Epstein, M.A., B.G. Achong, Y.M. Barr, Virus Particles in Cultured Lymphoblasts from Burkitt's Lymphoma, Lancet, 1:702-703, 1964.
2. Epstein, M.A., Y.M. Barr, B.G. Achong, Studies with Burkitt's Lymphoma, Wistar Inst. Sympos. Monogr., 4:69-82, 1965.
3. Henle, G., W. Henle, V. Diehl, Relation of Burkitt's Tumor-Associated Herpes-type Virus to Infectious Mononucleosis, Proc. Nat. Acad. Sci., 59:94-101, 1968.
4. Henle, W., G. Henle, Epstein-Barr Virus: The Cause of Infectious Mononucleosis, A Review, In: Oncogenesis and Herpesviruses, Biggs, I.M., G. de The, L.N. Payne (eds), IARC, Sci, Publ. No. 2, Lyon, 269-274, 1972.
5. Miller, D., Epstein-Barr Herpes Virus and Infectious Mononucleosis, Prog. Med. Virol., 20:84-112, 1975.
6. Henle, W., G. Henle, C.A. Horwitz, Epstein-Barr Virus Specific Diagnostic Tests in Infectious Mononucleosis, Human Path., 5:551-564, 1974.
7. Henle, W., G. Henle, Epstein-Barr Virus-Related Serology in Hodgkin's Disease, Natl. Cancer Inst. Monogr., 36:79-84, 1973.
8. Evans, A.S., The Spectrum of Infections with EB Virus: A Hypothesis, J. Infect. Dis., 124:330-335, 1971.
9. Takada, M., et al., The Establishment of Cultured Cell Lines from the Patients with Izumi Fever and Infectious Mononucleosis Like Diseases, Japan J. Exp. Med., 43:209-214, 1971.
10. Lennette, E.T., Diagnosis of Epstein-Barr Virus Infections, In: Laboratory Diagnosis of Viral Infections, Lennette, E.H. (ed), Marcel Dekker, New York, 257-271, 1985.
11. Henle, G., W. Henle, The Diagnosis of Epstein-Barr Virus Infections, in: The Human Herpesviruses, Nahamias, A.J., W.R. Dowdle, R.F. Schinazi (eds), Elsevier, New York, 374-378, 1981.
12. Weller, T.H., A.H. Coons, Fluorescent Antibody Studies with Agents of Varicella and Herpes Zoster Propagated In Vitro, Proc. Soc. Exp. Biol. Med., 86:789-794, 1954.
13. Riggs, J.L., R.J. Seiwald, J.H. Burckhalter, C.M. Downs, T.G. Metcalf, Isothiocyanate Compounds as Fluorescent Labeling Agents for Immune Serum, Am. J. Pathol., 34:1081-1097, 1958.
14. Herrmann, K.L., IgM Determinations, In: Clinical Virology Manual, Specter, S., G.J. Lancz (eds), Elsevier, New York, 219-228, 1980.
15. Gardner, P.S., J. McQuillin, Rapid Virus Diagnosis: Application of Immunofluorescence, In: Detection of Virus-Specific IgM by Immunofluorescence, Butterworths, Boston, 259-287, 1980.
16. Chantler, S., J.A. Diment, Current Status of Specific IgM Antibody Assays, In: Immunoassays for the 80's, Voller, A., A. Bartlett, D. Bidwell (eds), University Park Press, Baltimore, 417-430, 1981.
17. Johnson, R.B., R. Libby, Separation of Immunoglobulin M (IgM) Essentially Free of IgG From Serum for Use in Systems Requiring Assay of IgM-Type Antibodies Without Interference From Rheumatoid Factor, J. Clin. Micro., 12:451-454, 1980.
18. Gispen, R., J. Nagel, B. Brand-Saathof, S. DeGraff, Immunofluorescence Test for IgM Rubella Antibodies in Whole Serum After Absorption with Specific Anti-gamma Fc. Clin. Exp. Immunol., 22:431-437, 1975.
19. Joassin, L., M. Reginster, Elimination of Nonspecific Cytomegalovirus Immunoglobulin M Activities in the Enzyme Linked Immunosorbent Assay by Using Antihuman Immunoglobulin G., J. Clin. Microbiol. 23:576-581, 1986.
20. Ascher, M., Supervisor, Immunology/Virology Laboratory, Elmhurst Memorial Hospital, Elmhurst, Illinois, Personal Communication.
21. Lyerla, H.C., F.T. Forrester, The Immunofluorescence (IF) test, in: Immunofluorescence Methods in Virology, USDHHS, Georgia, 71-81, 1979.
22. Holborow, E.J., D.M. Weir, G.D. Johnson, A Serum Factor in Lupus Erythematosus With Affinity for Tissue Nuclei, Br. Med. J., 11:732-734, 1957.
23. Berg, P.A., I. Roitt, D. Doniach, H.M. Cooper, Mitochondrial Antibodies in Primary Biliary Cirrhosis, Immunol., 17:281-293, 1969.
24. McCormack, J.G., E. Nowakowski, B.B. Fernandez, The Place of Epstein-Barr Virus Testing in a Clinical Laboratory, Lab Med, Vol 8, No 8:12-15, 1977.
25. Smith, T., Director, Dept.of Virology, Section of Clinical Microbiology, Mayo Clinic, Personal Communication.
26. Goldman, J.M., M.L. Goodman, D. Miller, Antibody to EB Virus in American Patients with Carcinoma of the Nasopharynx, J.A.M.A., 216:1618-1622, 1971.
27. Epstein, M.A., B.G. Achong, The EB Virus, Annual Rev. Microbiol., 27:413-436, 1973.
28. Sutton, R.N.P., The Diagnosis of Infections with the Epstein-Barr Virus. J. Infect. 1:301-322, 1979.
29. Schmidt, N.J., Update on Class-Specific Viral Antibody Assays, Clin. Immunology Newsletter 5(6):81-85, 1984.
30. Chernesky, M.A., C.G. Ray, and T.F. Smith, Laboratory Diagnosis of Viral Infections, Cumitech 15. Cumulative Techniques and Procedures in Clinical Microbiology, ASM, Washington, D.C., 1982.
31. Forsgreen, M. and A. Demissie, IgM Responses to EBV/CMV in Cytomegalovirus and Epstein-Barr Infections in The Human Herpesviruses, Nahmias, A.J., W.R. Dowdle, and R.F. Schinazi (Eds), Elsevier, New York, 662, 1981.
32. Paxton, H., Director, Immunology, Maryland Medical Lab., Baltimore, MD, Personal Communication.
33. Nickoskelainen, J., P. Hanninen, Antibody Response to Epstein-Barr Virus in Infectious Mononucleosis, Infec. & Immun., 11:42-51, 1975.
34. Data on file, Bion, Des Plaines, Illinois.
35. Landry, M.L. and G.D. Hsiung, Diagnosis of Dual Herpesvirus Infection: Varicella Zoster and Herpes Simplex Viruses in: The Human Herpesviruses, Nahmias, A.J., W.R. Dowdle and R.F. Schinazi (Eds), Elsevier, New York, 652-653, 1981.
36. Joncar, J., et al., Dual Congenital Infections with the Epstein-Barr Virus (EBV) and the Cytomegalovirus (CMV) in: The Human Herpesviruses, Nahmias, A.J., W.R. Dowdle, and R.F. Schinazi (Eds), Elsevier, New York, 614, 1981.
37. Ho, M., Cytomegalovirus, Biology and Infection, Plenum, New York, pp44, 133, 169, 1982.
38. Rossier, E., H.R. Miller, P.H. Phipps, Rapid Viral Diagnosis by Immunofluorescence, An Atlas and Practical Guide, Univ. of Ottawa Press, Ottawa, 127-137, 1989.

AESKU.BION
AN AESKU.GROUP COMPANY

MANUFACTURER

Bion Enterprises, Ltd.
455 State Street, Suite 100
Des Plaines, IL 60016 USA
Phone: +1-847-544-5044
Fax: +1-847-544-5051

EC REP

Qarad, Cipalstraat 3,
B-2440 Geel, Belgium