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EBV-VCA

ANTIGEN SUBSTRATE SLIDE

CE

NOTE: Changes highlighted

PRODUCT AVAILABILITY

The following Epstein-Barr Viris (VCA) Antigen Substrate Slides are available individually from Bion Enterprises, Ltd. (Bion):

Antigen Substrate Slides	Code No. REF
EBV-VCA (Epstein-Barr Virus Viral Capsid Antigen)	EB-5012
Σ Number of Tests	12-Well

INTENDED USE

The Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES may be used as the antigenic substrate in indirect fluorescent antibody assays for the qualitative and/or semi-quantitative determination of IgG or IgM EBV-VCA antibodies in human serum. Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES are 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 they consult their physicians, and further increases cannot be demonstrated.[10] Testing for IgM specific EBV-VCA antibodies should be done in these cases. Methods for EBV-VCA antibody detection include the Radioimmunoassay (RIA), Enzyme-Linked Immunosorbent Assay (ELISA), and Immunofluorescence Assay (IFA).[11] The RIA and ELISA are 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]

PRINCIPLE OF THE IFA PROCEDURE

The Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES may be utilized in the indirect fluorescent antibody assay method first described by Weller and Coons[13] and further developed by Riggs, et al.[14] The procedure is carried out in two basic reaction steps:

Step 1 - Human serum 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 IgG (or 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

Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES are 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.

STORAGE AND STABILITY

The Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES are stable in sealed foil pouches at 8°C or lower until labeled expiration date.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use. Thus, only staff trained in methods of in vitro diagnsotics may perform the test. IVD
2. Substrate slides are for single use only and must not be used more than once.
3. Use with non-Bion reagents could result in erroneous results.
4. Care should be taken when handling substrate slides due to sharp edges.
5. The antigenic substrates have been fixed and contain no detectable live Epstein-Barr Virus. However, they should be handled and disposed of as any potentially biohazardous laboratory material.
6. Do not remove slides from pouches until ready for testing. Do not use if pouch has been punctured, as indicated by a flat pouch.
7. Antigen substrate slides should be brought to room temperature (20-25°C) prior to use.
8. Abnormal test results may be seen if the antigen substrate slides are allowed to dry during the staining procedure.
9. Refrigeration (2-8°C) of antigen substrate slides immediately upon arrival will insure stability until labeled expiration date.
10. Antigen substrate slides should not be used beyond stated expiration date.
11. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
12. Incubation times or temperatures other than those specified may give erroneous results.
13. Reusable glassware must be washed and thoroughly rinsed free of detergents.
14. Care should be taken to avoid splashing or generation of aerosols.
15. Previously frozen specimens after thawing should be thoroughly mixed prior to testing. It is recommended that sera is freeze thawed no more than one time. If repeated testing is required, it is suggested that specimen be aliquoted.
16. 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.

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

Detailed descriptions of indirect immunofluorescence techniques may be found in the references listed in the bibliography.[14,15,16]

MATERIALS PROVIDED

Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES.
Lot Number provided on label. LOT

MATERIALS AVAILABLE FROM Bion

1. Fluorescent Antibody Conjugate with 0.1% Evans Blue counterstain
2. Epstein-Barr Virus (VCA) Positive Human Control Serum
3. Epstein-Barr Virus (VCA) Negative Human Control Serum
4. Phosphate Buffered Saline (PBS)
5. Mounting Medium

MATERIALS REQUIRED BUT NOT PROVIDED

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

TEST PROCEDURE

1. **SPECIMEN PREPARATION**

Screening:
Each laboratory should establish its own protocol for the preparation of serum screening dilutions. Most indirect fluorescent antibody staining procedures utilize a 1:10 dilution of each patient's serum which is prepared by adding 0.05 ml (50 µl) of the patient's serum to 0.45 ml of PBS.

NOTE: If testing for IgM specific antibodies using an IgM specific fluorochrome conjugate, each patient serum specimen must be pre-treated to remove any IgG interference by separating the IgM from the IgG, and then running the screening test on the IgM eluate. Suggested methodologies are ion exchange chromatography[17] or IgG immunoprecipitation.[18,19]

Semi-quantitation:
Serum dilutions are utilized to measure antibody titer. Each laboratory should establish its own titering protocol. The selection of either twofold or fourfold dilution procedures depends upon the experience level and training of the individual(s) reading the fluorescent antibody assay.

The following fourfold serial titration is suggested for IgG testing:

a. Prepare a 1:10 dilution of each patient's serum by adding 0.05 ml (50 µl) of patient's serum to 0.45 ml of PBS in tube #1.

b. Add 0.3 ml PBS to tubes #2, #3, #4, and #5.

c. Using a 100 µl pipette, transfer 0.1 ml (100 µl) from tube #1 to tube #2. Mix. Using a new tip for each dilution, transfer 0.1 ml (100 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.

The following twofold titration is suggested for IgM testing:

a. Prepare a 1:10 dilution of each patient's serum using one of the treatment methodologies mentioned in the "Screening NOTE" 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.

These titrations will have the following dilutions:

Fourfold	Twofold
Tube #1 = 1:10	Tube #1 = 1:10
Tube #2 = 1:40	Tube #2 = 1:20
Tube #3 = 1:160	Tube #3 = 1:40
Tube #4 = 1:640	Tube #4 = 1:80
Tube #5 = 1:2560	Tube #5 = 1:160

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 room temperature (20-25°C) for 30 minutes. 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.

NOTE: For IgM testing, incubate the substrate slides in a moist chamber at 35-37°C for 90 minutes.

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 an appropriate fluorescent antibody (IgG or IgM) conjugate with counterstain (diluted to its predetermined proper working dilution) to each well of each slide, making sure that each well is completely covered.

8. **INCUBATION 2**
Incubate in a moist chamber at room temperature (20-25°C) for 30 minutes. Protect slides from excessive light.

NOTE: For IgM testing, incubate in a moist chamber at 35-37°C for 60 minutes.

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 the 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:[20]

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 a negative control serum on Bion EBV-VCA SUBSTRATE SLIDES, the infected cells should exhibit less than 1+ fluorescence and appear reddish-orange due to the counterstain.

Positive Control:
Using a positive control serum on Bion EBV-VCA SUBSTRATE SLIDES, the 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 this control must be determined and there must not be more than a twofold difference (+/-) in titer from this determined endpoint. Each run should include the endpoint dilution, one twofold or fourfold dilution above and one twofold or fourfold 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 to be negative for EBV 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.

A sample is considered negative for EBV 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.

... 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 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 antibodies if it exhibits the characteristic EBV staining pattern with a fluorescent intensity of 1+ or greater at a serum dilution of 1:10 or greater.

NOTES:
1. 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.[21,22] 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.”
2. In a few sera with high concentrations of IgM, a nonspecific fluorescence of all cells may be noticed and mask specific IgG staining.[23] Samples may be diluted beyond this interference for better interpretation.

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. When reading fourfold serial dilutions, endpoints can be extrapolated where necessary.

EXAMPLE OF ENDPOINT EXTRAPOLATION:
1:10 = 4+
1:40 = 3+
1:160 = 2+
1:640 = +/-
The extrapolated endpoint is reported as 320.

TROUBLESHOOTING

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

INTERPRETATION OF RESULTS

Detection of the presence of EBV-VCA antibodies indicates a current or previous infection with EBV. A significant (fourfold or greater) increase in titer between acute and convalescent serum samples and/or a positive test for IgM specific antibodies usually indicates recent or active infection be it a primary infection, reinfection or reactivation of latent virus.

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	++	NPC

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

NOTES:
1. Testing for Early Antigen (Diffuse and Restricted) and IgM specific EBV-VCA antibodies may help confirm a diagnosis of primary infection when only one specimen is available.
2. Testing for antibodies to EB Nuclear Antigen may help to differentiate a primary infection from either a previous infection or a reactivation of latent virus.
3. 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]

LIMITATIONS OF THE PROCEDURE

1. EBV antibody test results should be used in conjunction with information available from clinical evaluation and other diagnostic information.
2. A single serological IgG antibody titer to EBV should not be used as the only criterion for diagnosis. Paired serum samples (acute and convalescent), testing for IgM specific EBV, EBNA and Early Antigen antibodies may provide more meaningful data.
3. A negative test result does not necessarily rule out current or recent infection. The specimen may have been collected too early in the disease before demonstrable antibody is present.
4. Lack of significant rise in titer does not exclude the possibility of recent infection but may indicate an acute phase specimen was obtained too late.
5. Test results on specimens from immunosuppressed patients and pregnant women may be difficult to interpret.
6. Positive test results may not be valid in persons who have received blood transfusions or various blood products within the past several months.
7. Antinuclear antibodies (ANA) present in serum may interfere with the EBV IFA test. They can be differentiated from EBV staining in that ANAs stain the nuclei in all cells; whereas, EBV antibodies exhibit nuclear inclusion staining in an average of only five to fifteen cells per 200X field.[21]
8. Cytoplasmic fluorescence in the majority of the cells may be due to the presence of antimitochondrial antibodies (AMA) often seen in primary biliary cirrhosis.[22] They can be differentiated from the specific antigen staining in that AMA will stain the cytoplasm of all cells; whereas, EBV antibodies exhibit staining in only an average of five to fifteen cells per 200X field.
9. Positive test results from cord blood or neonates should be interpreted with caution. The presence of EBV IgG antibodies in cord blood is usually the result of passive transfer from mother to the fetus. A negative test, however, may be useful in excluding possible infection.[27] 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.[28]
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. If testing for IgM specific antibodies, 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.[29] Therefore, all sera should be treated by ion exchange chromatography[17] or IgG immunoprecipitation[18,19] before testing to eliminate possible RF interference.
14. If testing for IgM specific antibodies, high titers of 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.[29] Therefore, all sera should be treated by ion exchange chromatography[17] or IgG immunoprecipitation[18,19] before testing to avoid this possible problem.
15. 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.[37]

SPECIFIC LIMITATIONS OF THE EBV ASSAY

1. A false negative result may occur due to a prozone reaction particularly if screening at only one low dilution such as 1:10.[30,31]
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.
3. Positive test results require careful interpretation since false positive reactions or heterotypic IgM responses may occur with sera from patients with Cytomegalovirus (CMV) infection.[27,28,35]
4. Heterotypic IgM antibody responses to EBV in CMV infections have been reported.[32] Reactivation of latent EBV would seem to be a possible mechanism.[28]

EXPECTED VALUES

EBV-VCA IgG antibodies develop early, reach peak titers within two to four weeks and then decline to lower levels which persist indefinitely.[33] A fourfold increase in titer between acute and convalescent sera is diagnostic of an acute or recent IM infection. A single high titer serum above 1:640 is strongly suggestive of recent IM. The titer of EBV-VCA IgG antibodies may not reflect the severity of clinical symptoms in IM.[10]

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.[34] 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.[34]

One study reported that 90-97% of patients with IM developed EB virus specific antibodies. In addition, 85% of the patients studied possessed EBV-VCA IgM antibodies one month after onset of the illness, 40% after three months, 10% after nine months, and no EBV-IgM antibodies by 19 months.[35]

Approximately 80-90% of the U.S. adult population is positive for IgG antibodies to EBV-VCA.[36] A subsequent rise in IgG EBV-VCA antibodies, sometimes in excess of 1:2560 may be the result of secondary disease such as Burkitt's Lymphoma or Nasopharyngeal Carcinoma.[8,26]

SPECIFIC PERFORMANCE CHARACTERISTICS

Interlot and intralot precision of the Bion EBV-VCAANTIGEN SUBSTRATE SLIDES were evaluated by testing for both IgG and IgM antibodies. For IgG, 10 serum specimens (3 negative and 7 positive ranging in titer from 1:80 to 1:640) were tested on four different lots of slides, and three times on the same lot. For IgM, 11 serum specimens (4 negative and 7 positive ranging in titer from 1:10 to 1:640) were tested on five different lost of slides, and four times on the same lot. In each instance 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 sera vacillated between a positive or negative result.[37]

As Epstein-Barr Virus is a member of the Herpesvirus Group, a study was performed to insure there were no cross-reactions between IgG antibodies to the other members of this group and the Epstein-Barr antigen on the Bion EBV-VCAANTIGEN SUBSTRATE SLIDES. Eight serum specimens with IgG antibodies to Cytomegalovirus (CMV), Herpes Simplex Virus Type 1 (HSV 1), Herpes Simplex Virus Type 2 (HSV 2) and/or Varicella Zoster Virus (VZV) were tested. All eight specimens were negative on the BION EBV-VCA antigen substrate; thus, there was no cross-reactivity with the EBV substrate and antibodies to other members of this group. Summary of this data is presented in TABLE 1.[37]

In addition, a cross-reactivity study was performed to insure there were no cross-reactions between IgM antibodies to the other members of this group and the Epstein-Barr antigen on the Bion EBV-VCA ANTIGEN SUBSTRATE SLIDES. 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 to HSV, ten to CMV, and to VZV. These specimens were then tested against all five antigen substrates.

As shown in TABLE 2, 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.[37]

TABLE 1
HERPES GROUP IgG SPECIFICITY STUDY

Spec.#	EBV	CMV	HSV 1	HSV 2	VZV
1	<10	20/40	<10	<10	<10
2	<10	<10	160/320	80/160	20
3	<10	<10	160/320	80	20/40
4	<10	40	<10	<10	<10
5	<10	<10	640	320	20/40
6	<10	<10	320	160	40
7	<10	10	<10	<10	20
8	<10	<10	<10	<10	40/80

TABLE 2
HERPES GROUP IgM SPECIFICITY STUDY

# of Spec.	EBV	HSV 1	HSV 2	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.[27,29] It is also well known that dual infections can occur and have been reported between several members of the Herpesvirus Group.[31,38,39,41] 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.[27,29] 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.[40] 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.[40]

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