INSTRUCTIONS FOR USE

EBV Viral Capsid Antigens
IgM Antibody Kit

Catalog Number: VCM-120
Size: 120
Storage: 2-8˚C

An Indirect Immunofluorescence assay for the detection of IgM class antibody against EBV Viral Capsid Antigens in human serum or plasma

For in-vitro diagnostic use only

INTENDED USE

The Epstein-Barr virus VCA IgM Antibody kit is intended for the detection and semi-quantitation of IgM class human antibody to the capsid antigens (VCA) of Epstein-Barr virus.

SUMMARY AND EXPLANATION OF TEST

Epstein-Barr virus (EBV) is a widely-disseminated human pathogen. Its most common manifestation is infectious mononucleosis, which occurs predominantly in adolescents and young adults. More often, infections are silent or subclinical, occurring in early childhood. After the initial infection, which generally resolves within a 2-3 week period, a chronic, usually asymptomatic infection is maintained by the virus. This chronic state involves oropharyngeal excretion of virus and is the main source of case-to-case spread.

Complications of EBV infection can involve the neurologic, cardiac, ocular, respiratory, hematologic, digestive, and renal systems. Neurologic manifestations include meningitis, encephalitis, Guillain-Barre' syndrome, Bell’s palsy, myelitis, cranial nerve neuritis, and psychotic disorders. Bulbar involvement with ensuing respiratory paralysis can be fatal. EBV is also associated with Burkitt’s lymphoma, nasopharyngeal carcinoma and neoplasias of the thymus, parotid gland, and supraglottic larynx.

Antibody response to EBV infection can be determined by indirect immunofluorescence tests utilizing three different groups of antigens. The test for viral capsid (VCA) antibody utilizes lymphoblastoid cells with a productive infection and, therefore, detects a wide range of antibody specificities, including capsid antigens, early antigens, and membrane antigens. The test for early antigens (EA) primarily recognizes reactivity to enzymes produced by EBV prior to viral DNA synthesis (2-3). The third major group of antigens consists of nuclear antigens (EBNA), which are expressed in both productively and latently infected cells. This antibody specificity is classically detected by anti-complement immunofluorescence (ACIF), due to the very low density of these antigens in lymphoblastoid cell nuclei.

Principle of the Test

The indirect fluorescent antibody (IFA) test for VCA was originally described by Henle and Henle in 1966 (1). This procedure utilizes productively infected human lymphoblastoid cells as an IFA substrate. Reagents are included in this kit to pretreat the patient serum, removing the IgG class antibody that is often a source of false reactions due to rheumatoid factor. Removal of IgG also reduces competition for substrate binding sites, enabling detection of IgM class antibody in sera with relatively high IgG titers to the same antigens. Dilutions of the treated patient sera are then allowed to react with the EBV substrate. After the unbound serum proteins are removed by washing with PBS, the bound VCA-specific antibodies are then tagged with an anti-human IgM monoclonal antibody. Following another wash step a fluorescent conjugate is added to amplify the double-sandwich reaction, labeling the positive cells with an apple-green fluorescence. Using fluorescence microscopy, this label appears as fluorescence in the 5-15% positive cells and contrasts with the red counter stain of the negative control cells. Serial serum dilutions may be utilized to arrive at an endpoint titer.

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A positive reaction appears as apple-green fluorescent cells, approximately 5-15% of the total cell monolayer. These cells should contrast distinctly with the red counterstain and 200X magnification.

### INTERPRETATION OF RESULTS

1. Titer = 1:80.
2. Titer = 1:160.
3. Titer = 1:400.
4. Titer = 1:1600.

For each serum dilution add 10 µL to one slide well. For each assay run include the Negative Control (untreated) and dilutions of the Positive Control prepared above.

1. Place slides in a humid chamber and incubate for 60 minutes at 37±0.5°C.
2. Remove humidity chamber from incubator and add 10 µL of IgM Sample Diluent to each slide well.
3. Mix serum with Diluent and allow 20 minutes for precipitin reaction to proceed. Sample may be clarified by centrifugation, although this is usually not necessary.
4. Prepare dilutions of the Positive Control at 1:4, 1:8, and 1:16 in PBS. As the bottled control starts at 1:10, the final dilutions will actually be 1:40, 1:80 and 1:160.

### ASSAY PROCEDURE

1. Prepare 1:10 screening dilutions (1 part patient serum with 9 parts IgM Sample Diluent) for all patient sera. Mix serum with Diluent and add 20 minutes for precipitin reaction to proceed. Sample may be clarified by centrifugation, although this is usually not necessary.
2. Prepare dilutions of the Positive Control at 1:4, 1:8, and 1:16 in PBS. As the bottled control starts at 1:10, the final dilutions will actually be 1:40, 1:80 and 1:160.
3. For each serum dilution add 10 µL to one slide well. For each assay run include the Negative Control (untreated) and dilutions of the Positive Control prepared above.
4. Place slides in a humid chamber and incubate for 60 minutes at 37±0.5°C.
5. Remove humidity chamber from incubator and add 10 µL of IgM Sample Diluent to each slide well.
6. Mix serum with diluent and allow 20 minutes for precipitin reaction to proceed. Sample may be clarified by centrifugation, although this is usually not necessary.
7. For each slide well add 10 µL of IgM Label, then return slides to humid chamber for 30 minutes incubation at 37±0.5°C.
8. Wash slides as in step 5, above.
9. Add 2-3 drops of Mounting Medium to each slide and apply coverglass.

### Materials Required But Not Supplied

- **Conjugate**: Mouse monoclonal antibody specific for human IgM heavy chain, with bovine serum albumin.
- **IgM Sample Diluent**: Goat antiserum to human IgM heavy chain at working dilution.
- **Mounting Medium**: 1 mL

### Positive Control, 0.5 mL

1. Prepare 1:10 screening dilutions (1 part patient serum with 9 parts IgM Sample Diluent) for all patient sera. Mix serum with Diluent and add 20 minutes for precipitin reaction to proceed. Sample may be clarified by centrifugation, although this is usually not necessary.
2. Prepare dilutions of the Positive Control at 1:4, 1:8, and 1:16 in PBS. As the bottled control starts at 1:10, the final dilutions will actually be 1:40, 1:80 and 1:160.
3. For each serum dilution add 10 µL to one slide well. For each assay run include the Negative Control (untreated) and dilutions of the Positive Control prepared above.
4. Place slides in a humid chamber and incubate for 60 minutes at 37±0.5°C.
5. Remove humidity chamber from incubator and add 10 µL of IgM Sample Diluent to each slide well.
6. Mix serum with diluent and allow 20 minutes for precipitin reaction to proceed. Sample may be clarified by centrifugation, although this is usually not necessary.
7. For each slide well add 10 µL of IgM Label, then return slides to humid chamber for 30 minutes incubation at 37±0.5°C.
8. Wash slides as in step 5, above.
9. Add 2-3 drops of Mounting Medium to each slide and apply coverglass.
10. Read the reactions at 200X and/or 400X magnification, comparing each well to the visual intensity and appearance of the Positive and Negative Control wells. Slides may be stored at 2-8°C in the dark for up to 24 hours.

### LIMITATIONS

- All results from this test must be related to the patient's clinical presentation, results of other EBV-specific assays (EA, VCA-IgG, and EBNA), and tests for other possible diseases and etiological agents. Elevated EBV titers and four fold titer increases in patients that are negative for VCA-IgM antibody are occasionally reported. It is not apparent whether such titers implicate EBV as an etiological agent or simply give evidence of EBV activation, secondary to malignancy or other source of immune suppression.
- Fluorescence intensity will be affected by the type of microscope, light source, filter system, and age of bulb used to read this test. Positive and Negative Controls are to be compared with the reactions of test sera as a means of standardizing the test results from day-to-day and between laboratories with different types of equipment.
- Non-specific antibody (Non-EBV-specific) may on occasion react with specific intensity to obscure accurate reading. Such reactivity is distinguished by an unusually high percentage of fluorescent cells and, often, by the pattern of reactivity. This type of reaction is most readily noted by comparison with the appearance of Positive and Negative Control wells if higher serum dilutions do not remove the non-specific staining and reveal appropriate EBV-specific staining. The VCA titer cannot be reported and 'non-specific reactivity' may be listed as the reason for non-reporting.

### EXPECTED VALUES

The prevalence of VCA-IgM class antibody is limited to those currently or recently infected with EBV for the first time. Of 110 sera submitted to a western U.S. laboratory for premarital testing (presumed healthy), there were 10 VCA-IgM positive sera.

### SPECIFIC PERFORMANCE CHARACTERISTICS

Seroepidemiological studies with paired sera in early sera from an acute EBV infection. A healthy population submitted for premarital testing (N=110) were also IgM positive by this method. When compared with other commercial procedures, all three agreed on 30/30 VCA-IgM positives and 151/151 negatives.

No cross reactivity was seen when testing sera with elevated titers to other members of the herpesvirus family (CMV, HSV, VZV). Although not strictly cross-reactive, acute EBV is often accompanied by the reappearance of CMV-IgM antibody. The reverse situation does not hold, as VCA-IgM does not reappear following convalescence from primary infection.

### BIBLIOGRAPHY


### REAGENTS

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>1 liter</td>
</tr>
<tr>
<td>Wash and PBS</td>
<td>0.5 mL</td>
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<tr>
<td>Positive Control</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>0.5 mL</td>
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<tr>
<td>IgM Sample Diluent</td>
<td>12 µL</td>
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### SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer serum aseptically to tightly closing screw cap vials or tubes at 2-8°C before opening sealed envelopes or using.

### STORAGE AND HANDLING

Store vials at 2-8°C. Reconstituted conjugates and controls are stable under these storage conditions for at least 3 weeks. Bring all reagents to ambient temperature (20-25°C) before opening sealed envelopes or using.

### SPECIMEN COLLECTION

Allow blood samples to clot and separate sera by centrifugation. Transfer sera aseptically to tightly closing screw cap vials or tubes at 2-8°C. Acute specimens should be drawn within 48 hours of the onset of illness and convalescent sera at 2 and 4 weeks intervals to check for titer changes.

### PROCEDURE

This reagent supplies sufficient materials for 120 determinations.