Platelia™ EBV-VCA IgM

1 plate - 96

Enzyme immunoassay for qualitative determination of IgM antibodies to epstein-barr virus (viral capsid antigen) in human serum

IVD  CE

2015/04
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1 - INTENDED USE
This immunoassay kit is for the qualitative determination of IgM antibodies to Epstein-Barr Virus Viral Capsid Antigen (VCA) in human serum. The Platelia™ EBV-VCA IgM assay may be used in conjunction with other Epstein-Barr serology’s (Platelia™ EBV-VCA IgG, Platelia™ EB-NA-1 IgG and Platelia™ EBV-EA-D IgG) as an aid in the diagnosis of infectious mononucleosis.

2 - CLINICAL VALUE
Epstein-Barr virus (EBV) is a common human pathogen, affecting 80% of adults in the US. Since the discovery of Epstein-Barr virus in 1964, EBV has been etiologically implicated in an increasing number of human diseases, such as infectious mononucleosis. EBV has also been associated with B cell lymphomas in immunosuppressed individuals, including both transplant patients and patients with AIDS. EBV is classified as a member of the herpes virus family based upon its characteristic morphology. All herpes viruses share the ability to establish a latent infection in their hosts.

Infectious mononucleosis (IM) is an acute, self-limited lympho-proliferative disease caused by EBV. Although primary infection with EBV during childhood is usually asymptomatic, nearly one-half to two-thirds of primary infections with the virus in older adolescents and young adults result in overt clinical disease such as infectious mononucleosis, showing the following symptoms: fever, pharyngitis, tonsilitis, lymphadenopathy, malaise, headache, myalgia, spleno- and hepatomegaly, rash and leucocytosis.

Infection by EBV results in the production of antibodies to four distinct complexes: EBV induced Nuclear Antigen (EBNA), EBV induced Early Antigen (EA), Viral Capsid Antigen (VCA) and EBV induced Membrane Antigen (MA). The EA complex is divided into two components which include EA-D (diffused component) and EA-R (restricted component).

IgM antibodies to VCA are detectable in the early course of disease. The levels of antibody rise early and peak after 3-4 weeks, then decline to non-detectable levels.

3 - PRINCIPLE OF THE PROCEDURE
The Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials, (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). The Platelia™ EBV-VCA IgM kit utilizes the ELISA technology in which an affinity purified VCA antigen is bound to the wells of a microplate.
When antigens bound to the solid phase are brought into contact with a patient’s serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Serum pre-treatment solution is used to remove the interference potentially caused by IgG and IgM-RF. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgM globulin conjugated with horseradish peroxidase, which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Substrate, tetramethylbenzidine (TMB). If specific IgM antibody to the antigen EBV-VCA is present in the patient’s serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is proportional to the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader. The sensitivity, specificity and reproducibility of ELISA can be comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radioimmunoassays.

4 - CONTENTS OF THE KIT
All reagents are exclusively for in vitro diagnostic use

<table>
<thead>
<tr>
<th>Label</th>
<th>Nature of reagents</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 Microplate</td>
<td>Microplate: 12 Strips (8 wells each) coated with inactivated affinity purified EBV-Viral Capsid Antigen (gp125), stored in a foil pouch with desiccant/humidity indicator.</td>
<td>1</td>
</tr>
<tr>
<td>R2 Concentrated Washing Solution (20x)</td>
<td>Concentrated Washing Solution (20x): Tris buffer (pH 7.2 ± 0.2) with 1% Tween® 20. Preservatives: ProClin™ 300 (0.1%).</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>R3 Non reactive control</td>
<td>Non reactive control (human) for anti-EBV-VCA IgM antibodies. Negative for HBs Ag and HIV-1, HIV-2 and HCV antibodies ; Preservatives: sodium azide (&lt; 0.1%) and pen/strep (0.01%)</td>
<td>1 x 0.4 ml</td>
</tr>
<tr>
<td>R4a Positive Control I</td>
<td>Positive control I (human) for anti-EBV-VCA IgM antibodies. Negative for HBs Ag and HIV-1, HIV-2 and HCV antibodies ; Preservatives: sodium azide (&lt; 0.1%) and pen/strep (0.01%)</td>
<td>1 x 0.4 ml</td>
</tr>
<tr>
<td>Label</td>
<td>Nature of reagents</td>
<td>Presentation</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>R4b</td>
<td><strong>Positive control II</strong> (human) for anti-EBV-VCA IgM antibodies. Negative for HBs Ag and HIV-1, HIV-2 and HCV antibodies; Preservatives: sodium azide (&lt; 0.1%) and pen/strep (0.01%)</td>
<td>1 x 0.4 ml</td>
</tr>
<tr>
<td>R5</td>
<td><strong>Calibrator</strong> (human) for anti-EBV-VCA IgM antibodies, with kit specific factor printed on the outer box label. Negative for HBs Ag and HIV-1, HIV-2 and HCV antibodies; Preservatives: sodium azide (&lt; 0.1%) and pen/strep (0.01%)</td>
<td>1 x 0.4 ml</td>
</tr>
<tr>
<td>R6</td>
<td><strong>Conjugate</strong> (ready-to-use): goat antibodies to human IgM coupled with horseradish peroxidase. Preservatives: ProClin™ 300 (0.1%) and gentamycin</td>
<td>1 x 16 ml</td>
</tr>
<tr>
<td>R7</td>
<td><strong>Diluent II</strong>: ready-to-use buffer (pH 7.5) with protein stabilizers. Contains goat/sheep anti-human IgG for serum adsorption to remove competing IgG. Preservatives: ProClin™ 300 (0.1%)</td>
<td>2 x 45 ml</td>
</tr>
<tr>
<td>R9</td>
<td><strong>Chromogen/Substrate solution</strong> (ready-to-use): Tetramethylbenzidine (TMB). The reagent should remain closed when not in use; otherwise, a precipitate may form in the reaction wells.</td>
<td>1 x 15 ml</td>
</tr>
<tr>
<td>R10</td>
<td><strong>Stopping solution</strong> (ready-to-use): 1N Sulfuric acid</td>
<td>1 x 15 ml</td>
</tr>
</tbody>
</table>

5 - **WARNING AND PRECAUTIONS**

**Precautions**
The reliability of the results depends on correct implementation of the following Good Laboratory Practices:
- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.

REMARK: It is possible to use other lots than those contained in the kit, provided the same lot is used within a given test run: washing solution (R2), Chromogen/Substrate solution (R9) and stopping solution (R10).
These reagents can be used with Platelia™ EBV-VCA IgG, Platelia™ EBV-EA-D IgG and Platelia™ EB-NA-1 IgG of our catalogue. Contact our technical service for detailed information.

- Before use, it is necessary to wait 30 minutes for the reagents to stabilize to room temperature.
- Carefully reconstitute the reagents avoiding any contamination.
- Do not carry out the test in the presence of reactive vapors (acid alkaline, aldehyde vapors) or dust that could alter the enzyme activity of the conjugate.
- Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solution.
- The Chromogen/Substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. Use a new pipette tip for each sample.
- Washing the microplate is a critical step in the procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- Never use the same container to distribute conjugate and development solution.
- Check the pipettes and other equipment for accuracy and correct operation.
- Do not change the assay procedure

Health and safety instructions
All the reagents provided are intended for in vitro diagnostic use.

- Wear disposable gloves when handling reagents.
- Do not pipette by mouth.
- Human origin material used in the preparation of the reagents has been tested and found non-reactive for hepatitis B surface antigen (HBs Ag), antibodies to hepatitis C (HCV) and to human immunodeficiency virus (anti-HIV1 and anti-HIV2). Because no method can absolutely guarantee the absence of infectious agents, handle reagents of human origin and patient samples as if capable of transmitting infectious disease.
• Consider any material directly in contact with samples and reagents of human origin, as well as washing solution, as infectious material.
• Avoid spilling samples or solutions containing samples.
• Spills must be rinsed with bleach diluted to 10 %. If the contaminating fluid is an acid, spills must be initially neutralized with sodium bicarbonate, then cleaned with bleach and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
• Samples, reagents of human origin, as well as contaminated material and products should be discarded after decontamination.
  - either by immersion in bleach at the final concentration of 5 % of sodium hypochloride for 30 minutes,
  - or by autoclaving at 121°C for 2 hours minimum.
Autoclaving for at least one hour at 121°C, is the best method to inactivate the HIV viruses and the HB virus.

CAUTION: DO NOT INTRODUCE SOLUTIONS CONTAINING SODIUM HYPOCHLORIDE INTO THE AUTOCLAVE.

• Chemicals should be handled and disposed of in accordance with Good Laboratory Practices.
• Avoid any contact of the substrate buffer, the chromogen and the stopping solution with the skin or mucosa (risk of toxicity, irritation or burn).
• For hazard and precaution recommendations related to some chemical components in this test kit, please refer to the pictogram(s) mentioned on the labels and the information supplied at the end of instruction for use. The Safety Data Sheet is available on www.bio-rad.com.

6 - MATERIAL REQUIRED BUT NOT PROVIDED
• Vortex mixer.
• Microplate reader equipped with 450 nm filters (*).
• Container for biohazard waste.
• Sodium hypochloride (bleach) and sodium bicarbonate.
• Distilled or deionized water.
• Graduated cylinders.
• Disposable latex gloves.
• Goggles or safety glasses.
• Absorbent paper.
• Automatic or semi-automatic, adjustable or preset, pipettes or multipipettes to measure and dispense 10, 100 and 1000 µl.
• Manual, semi-automatic or automatic microplate washer (*).
• Disposable tubes.

(*) Consult us for detailed information about the equipment recommended by our technical department.

7 - RECONSTITUTION AND STORAGE OF REAGENTS
The kit should be stored at +2-8°C until the expiry date mentioned on the package (except for specific instructions). Before use, allow reagents to reach room temperature (+21-25°C). Return all reagents to refrigerator promptly after use.

1) Ready-to-use reagents
• Reagent 1 (R1): microplate
  Each tray containing 12 strips is wrapped in a sealed foil lined bag. Cut the bag with scissors or a scalpel 0.5 - 1 cm above the line. Replace unused strips into the bag immediately. Reseal the bag carefully and replace it at +2-8°C. Then, the strips are stable for 1 month.
• Reagent 3 (R3): non-reactive control
• Reagent 4a (R4a): positive control I
• Reagent 4b (R4b): positive control II
• Reagent 5 (R5): calibrator
• Reagent 6 (R6): conjugate
• Reagent 7 (R7): diluent II
• Reagent 9 (R9): Chromogen/Substrate solution
• Reagent 10 (R10): stopping solution

2) Reagents to be reconstituted
• Reagent 2(R2): washing solution concentrated 20x
  Dilute 50 ml of the 20X washing solution to 1.0 l with distilled and/or deionized water to obtain the ready-for-use solution. Mix well. After dilution, store at +2-8°C for 5 days.

8 - SPECIMENS
Collect a blood sample according to the current practices. Test will be performed with non diluted samples. Separate the serum from the clot or red cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with aggregates should be clarified by centrifugation prior testing.
Suspended fibrin particles or aggregates may yield falsely positive results. The specimens can be stored at +2-8°C if screening is performed within 5 days or they may be deep-frozen at -20°C for several months. Avoid repeated freeze/thaw cycles. If the specimens are to be shipped, they must be packaged in accordance with the regulations in force regarding the transport of etiological agents.

DO NOT USE CONTAMINATED, HYPERLIPAEMIC, ICTERIC HYPERHAEMOLYSED OR HEAT-INACTIVATED SERA.

9 - ASSAY PROCEDURE
Strictly follow the proposed procedure.

Use the calibrator and the controls for each series of determinations to validate the test results.

Follow the following Good Laboratory Practice:
1. Carefully establish the sample distribution and identification plan.
2. Prepare the diluted washing solution (R2) (refer to section 7).
3. Take the carrier tray and the strips (R1) out of the protective pouch. Place the desired number of antigen-coated strips into a microwell frame. Allow 1 well for the reagent blank, 3 wells for Calibrator, 1 well for each Negative, Positive I and Positive II Controls.

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<thead>
<tr>
<th>1</th>
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<tbody>
<tr>
<td>A</td>
<td>B1</td>
<td>S2</td>
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<td>C</td>
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<tr>
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<tr>
<td>H</td>
<td>S1</td>
<td>S9</td>
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</tr>
</tbody>
</table>

4. All samples, controls and calibrator should be vortexed before use. Dilute test samples, Calibrator, Negative and Positive Controls 1:81 (e.g. 10 µl + 800 µl) with sample Diluent II (R7) within dilution tubes or dilution plate.
5. Pipette 100 µl of each diluted Calibrator, Control or patient sample into each well. Pipette 100 µl of Sample Diluent II (R7) into the first well for the reagent blank.

6. Incubate the microplate for 30 minutes ± 2 minutes at room temperature (21-25°C).

7. Aspirate the contents of all wells into a container for biohazards waste (containing sodium hypochloride). Add immediately into each well, a minimum of 300 µl of washing solution. Aspirate again. Repeat this procedure at least 4 times (a total of a minimum of 5 washes). If necessary, dry the plate by turning it upside down on absorbent paper. If an automatic washer is used, follow the same procedure.

8. Distribute 100 µl of the ready-to-use Conjugate (R6) into all wells.

9. Incubate 30 minutes ± 2 minutes at room temperature (21-25°C).

10. Empty all wells by aspiration and wash 5 times as described above.

11. Quickly dispense 100 µl of Chromogen/Substrate Solution (R9) into each well.

12. Allow the reaction to develop in the dark for 10 minutes ± 2 minutes at room temperature (21-25°C). Do not use adhesive film during this incubation.

   The Chromogen/Substrate Solution will turn blue in wells containing detectable levels of IgM antibodies.

13. Add 100 µl of Stopping Solution (R10) to each well. Mix by gently tapping the plate. Addition of the Stop Solution (R10) will result in a color change from blue to yellow.

14. Wait for a minimum of 5 minutes and read. Carefully wipe the plate bottom. Using a wavelength of 450 nm, blank the reader on the reagent blank well and measure the optical density of each well. The plate should be read within 30 minutes of adding the Stop Solution (the strips must always be kept away from light before reading).

15. Check all results for agreement between the reading and the plate and sample distribution and identification plan.

**10- CALCULATION AND INTERPRETATION OF THE RESULTS**

1) **Calculate the mean absorbance**

   1. Mean Cut-off Calibrator O.D. (Optical Density) – Calculate the mean O.D. value for the Cut-off Calibrator from the three Calibrator (R5) determinations. If any of the three calibrators Values differ by more than 15 % from the mean, discard that value and calculate the average of the two remaining values.
2. Correction Factor - To account for day-to-day fluctuations in assay activities due to room temperature and timing, a Correction Factor is determined for each lot of kits. The Correction Factor is printed on the Calibrator vial.

3. Cut-off Calibrator Value - The Cut-off Calibrator value for each assay is determined by multiplying the Correction Factor by the mean Cut-off Calibrator O.D. determined in step 1.

4. ISR Value - Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cut-off Calibrator Value determined in Step 3.

Example: O.D.’s obtained for Calibrator (R5) = 0.380, 0.400, 0.420
Mean O.D. for Calibrator (R5) = 0.400
Correction factor = 0.5
Cut-off Calibrator Value = 0.5 x 0.400 = 0.200
O.D. obtained for patient sera = 0.600
ISR Value = 0.600/0.200 = 3.00

2) Assay validation
1. Reagent Blank (when read against blank air) must be < 0.150 at 450 nm: OD (RB) < 0.150
2. Negative control (R3) must be ≤ 0.250 at 450 nm (when read against reagent blank): OD (R3) ≤ 0.250
3. Each calibrator (R5) must be ≥ 0.250 at 450 nm (when read against reagent blank): OD (R5) ≥ 0.250
4. Positive control (R4b) must be ≥ 0.500 at 450 nm (when read against reagent blank): OD (R4b) ≥ 0.500
5. The ISR (Immune Status Ratio) Values for negative, positive I and positive II controls (R3, R4a, R4b) should be in their respective ranges printed on the vials.

If those criteria are not within their respective ranges, the test should be considered invalid and should be repeated.
3) Interpretation of the results
The patients’ ISR (Immune Status Ratio) Values are interpreted as follows:

<table>
<thead>
<tr>
<th>ISR Value</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 0.90</td>
<td>Negative</td>
<td>No significant level of detectable EBV-VCA IgM antibody</td>
</tr>
<tr>
<td>0.91-1.09</td>
<td>Equivocal</td>
<td>Samples should be retested</td>
</tr>
<tr>
<td>≥ 1.10</td>
<td>Positive</td>
<td>Significant level of detectable EBV-VCA IgM antibody. Indicative of current or recent infection.</td>
</tr>
</tbody>
</table>

1. The following is a recommended method for reporting the results obtained: «The following results were obtained with the Platelia™ EBV-VCA IgM test. Values obtained with different methods may not be used interchangeably. The magnitude of the reported IgM level cannot be correlated to an endpoint titer”.

2. Accurate interpretation of EBV infection is based on the results of the four distinctive EBV antibodies are used to provide a comprehensive picture of EBV infection: IgG EBV-induced Nuclear Antigen (EB-NA), IgG EBV-induced Early Antigen (EBV-EA), IgG and IgM Viral Capsid Antigen (EBV-VCA). Accurate interpretation of EBV infection is based on the results from all these antibodies, and usually should not rely on single test result for a diagnosis.

3. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken.

4) Expected values

- **Acute Phase**
  EBV-VCA IgM increases rapidly in early acute phase and is detectable before or currently with EBV-VCA IgG and EB-NA-1 IgM and heterophile antibodies. EBV-VCA IgM decreases during the late phase along with EB-NA-1 IgM, but EBV-VCA IgG persists.

- **Transitional Phase**
  EBV-VCA IgM has decreased to low and approximately similar levels as EB-NA-1 IgG which is beginning to increase. EBV-VCA IgG persists.
**Convalescent Phase**

EBV-VCA IgM very low level to negative with EB-NA-1 IgG increasing to high levels.

**5) Prevalence**

A group of 158 sera from a normal population from various ages, genders and geographical areas of the U.S. were tested on the Platelia™ EBV-VCA IgM test. The positive rate for the Platelia™ EBV-VCA IgM assay was found to be 2.5 % and the equivocal rate was 1.9 %. The distribution of ISR values from this study is presented in the Chart below.

![Chart showing the distribution of ISR values in a normal population (n=158)](chart.png)
The following is a summary of the normal population stratified by age groups:

<table>
<thead>
<tr>
<th>Age</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 20</td>
<td>8</td>
<td>3</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>21 - 30</td>
<td>47</td>
<td>0</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td>31 - 40</td>
<td>48</td>
<td>0</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td>41 - 50</td>
<td>29</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>51 - 60</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>3</td>
<td>4</td>
<td>158</td>
</tr>
</tbody>
</table>

6) Limitations of use

1. Kit procedures or practices outside those in this package insert may yield questionable results.
   - Non repeatable reactions are often caused by:
     - Inadequate microplate washing,
     - Inadequate timing of the incubation steps,
     - Contamination of negative samples by serum or plasma with a high antibody titre,
     - Contamination of the development solution by oxidizing agents (bleach, metal ions...),
     - Contamination of the stopping solution.
   - Contaminated, icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and should be avoided.
   - The performance characteristics have not been established for any matrices other than serum.

2. The physician in the light of other clinical findings and diagnostic procedures should interpret results of this test.
   - This assay is intended for the determination of immune response to indicate primary infection or virus reactivation. The performance characteristics have not been established for patients with nasopharyngeal carcinoma, Burkitt’s lymphoma, other EBV associated lymphadenopathies, and other EBV associated diseases other than EBV related mononucleosis. The results of Platelia™ EBV-VCA IgM performed on serum from immunosuppressed patients must be interpreted with caution. The performance characteristics for this assay have not been established for pediatric specimens.
   - The absence of detectable EBV-VCA IgM antibody does not rule out the possibility of recent or current infection. The sample may have been collected before development of demonstrable antibody or after antibody still detectable.
If EBV infection is still suspected, obtain a second specimen 5-7 days later and repeat the testing. Often, however, at the time of presentation, IgM antibodies are in decreasing concentrations.

- Specific IgG may compete with the IgM for sites and may result in a false negative. Conversely, rheumatoid factor in the presence of specific IgG may result in a false positive reaction.

The goat/sheep anti-human IgG in the Serum Diluent Plus diminishes competing specific IgG and minimizes rheumatoid factor interference in samples.

- Some antinuclear antibodies have been found to cause a false positive reaction on some ELISA tests.

### 11- PERFORMANCES

#### 1 - Relative Sensitivity and Specificity Based on Serum Characterization

One hundred and sixty-six selected serums were tested at a clinical lab. The serum from the study were characterized as seronegative (no serological evidence of past or present EBV infection), acute (EBV-VCA IgM and heterophile antibody present, EB-NA IgG absent), or seropositive (presence of EBV-VCA IgG antibodies and EB-NA IgG, no evidence of EBV-VCA IgM or heterophile antibody, indicative of past infection). The assays utilized for the serum characterization were commercially available ELISA assays. The sensitivity, specificity and agreement of the assay were determined based on this characterization. It was assumed that the EBV-VCA IgM response should be negative for seronegative, and convalescent serum, and positive for acute serum. The results are summarized in the table below.

<table>
<thead>
<tr>
<th>Platelium™ EBV-VCA IgM</th>
<th>Acute EBV-VCA IgM + EB-NA IgG - Heterophile +</th>
<th>Seropositive EBV-VCA IgG + EB-NA IgG + EBV-VCA IgM - Heterophile -</th>
<th>Seronegative EBV-VCA IgG - EB-NA IgG - EBV-VCA IgM - Heterophile -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>37</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Equivocal</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>98</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>99</td>
<td>28</td>
</tr>
</tbody>
</table>
Relative Sensitivity (Acute) = 37/38 = 97.4 %
• 95 % Confidence Interval = 92.2 % - 100 %
Relative Specificity (Seronegative) = 27/28 = 96.4 %
• 95 % Confidence Interval = 89.4 % - 100 %
Relative Specificity (Seropositive) = 98/99 = 99.0 %
• 95 % Confidence Interval = 97.0 % - 100 %
Relative Agreement = 162/165 = 98.2 %
• 95 % Confidence Interval = 96.1 % - 100 %

Equivocal results were not included in the calculations.
Equivocal results were not retested. They were reported as equivocal.
The 95 % confidence intervals were calculated using the normal method.
Please be advised that «relative» refers to the comparison of this assay’s results to that of a similar assay. There was not an attempt to correlate the assay’s results with the disease presence or absence. No judgement can be made on the comparison assay’s accuracy to predict disease.

2 – Repeatability (Intra-Assay Precision)
The Platelia™ EBV-VCA IgM test was evaluated for precision by testing three sera ten times each on the same plate. The results are summarized in the Table below.

<table>
<thead>
<tr>
<th>Serum</th>
<th>n</th>
<th>X</th>
<th>S.D.</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>10</td>
<td>0.06</td>
<td>0.03</td>
<td>54</td>
</tr>
<tr>
<td>Low positive</td>
<td>10</td>
<td>1.71</td>
<td>0.16</td>
<td>9.2</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>4.15</td>
<td>0.21</td>
<td>5.1</td>
</tr>
</tbody>
</table>

X = Mean ISR Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation

3 – Reproducibility (Inter-Assay Precision)
The Platelia™ EBV-VCA IgM test was evaluated for precision by testing three sera ten times each on three different days. The results are summarized in the Table below.
4 - Cross-reactivity

Sera containing IgM antibody detectable by ELISA to Herpes Simplex Virus I & II, Cytomegalovirus, and Varicella-Zoster Virus were assayed. Sera containing rheumatoid factor (RF) were also assayed. All of the alternate assays were commercially available ELISA assays.

The data summarized in the table below indicate that antibodies to Herpes Viruses and sera containing RF do not cross-react with the Platelia™ EBV-VCA IgM.

### Cross-Reactivity Study with the Platelia™ EBV-VCA IgM test

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Platelia™ EBV-VCA IgM</th>
<th>Alternate Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF+</td>
<td>0.04 -</td>
<td>1.87 +</td>
</tr>
<tr>
<td>RF+</td>
<td>0.03 -</td>
<td>1.82 +</td>
</tr>
<tr>
<td>RF+</td>
<td>0.01 -</td>
<td>1.73 +</td>
</tr>
<tr>
<td>RF+</td>
<td>0.01 -</td>
<td>1.80 +</td>
</tr>
<tr>
<td>RF+</td>
<td>0.02 -</td>
<td>1.85 +</td>
</tr>
<tr>
<td>VZV IgM+</td>
<td>0.33 -</td>
<td>3.28 +</td>
</tr>
<tr>
<td>VZV IgM+</td>
<td>0.10 -</td>
<td>5.46 +</td>
</tr>
<tr>
<td>VZV IgM+</td>
<td>0.04 -</td>
<td>4.98 +</td>
</tr>
<tr>
<td>VZV IgM+</td>
<td>0.08 -</td>
<td>2.34 +</td>
</tr>
<tr>
<td>VZV IgM+</td>
<td>0.03 -</td>
<td>2.18 +</td>
</tr>
<tr>
<td>HSV 1 IgM+</td>
<td>0.02 -</td>
<td>2.53 +</td>
</tr>
<tr>
<td>HSV 1 IgM+</td>
<td>0.02 -</td>
<td>1.65 +</td>
</tr>
<tr>
<td>HSV 1 IgM+</td>
<td>0.01 -</td>
<td>1.34 +</td>
</tr>
<tr>
<td>HSV 1 IgM+</td>
<td>0.01 -</td>
<td>1.32 +</td>
</tr>
<tr>
<td>HSV 2 IgM+</td>
<td>0.06 -</td>
<td>1.76 +</td>
</tr>
<tr>
<td>HSV 2 IgM+</td>
<td>0.05 -</td>
<td>1.60 +</td>
</tr>
<tr>
<td>HSV 2 IgM+</td>
<td>0.03 -</td>
<td>2.09 +</td>
</tr>
<tr>
<td>HSV 2 IgM+</td>
<td>0.04 -</td>
<td>1.96 +</td>
</tr>
<tr>
<td>CMV IgM+</td>
<td>0.07 -</td>
<td>1.23 +</td>
</tr>
<tr>
<td>CMV IgM+</td>
<td>0.04 -</td>
<td>1.92 +</td>
</tr>
<tr>
<td>CMV IgM+</td>
<td>0.04 -</td>
<td>3.83 +</td>
</tr>
<tr>
<td>CMV IgM+</td>
<td>0.06 -</td>
<td>1.32 +</td>
</tr>
</tbody>
</table>


• This product contains human or animal components. Handle with care.
Danger
Causes severe skin burns and eye damage. May cause an allergic skin reaction.
Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. If skin irritation or rash occurs: Get medical advice/attention. Dispose of contents/container in accordance with local/regional/national/international regulations.

Peligro
Provoca quemaduras graves en la piel y lesiones oculares graves. Puede provocar una reacción alérgica en la piel. Llevar guantes/prendas/gafas/máscara de...
protección. EN CASO DE INGESTIÓN: Enjuagarse la boca. NO provocar el vómito. EN CASO DE CONTACTO CON LOS OJOS: Aclarar cuidadosamente con agua durante varios minutos. Quitar las lentes de contacto, si lleva y resulta fácil. Seguir aclarando. EN CASO DE CONTACTO CON LA PIEL (o el pelo): Quitarse inmediatamente las prendas contaminadas. Aclararse la piel con agua o ducharse. En caso de irritación o erupción cutánea: Consultar a un médico. Eliminar el contenido o el recipiente conforme a la reglamentación local/regional/nacional/internacional.

(VI) Vaara

(HR) Opasnost

(HU) Veszély

(IT) Pericolo
Pavojinga

Smarkiai nudegina odą ir pažeidžia akis. Gali sukelti alerginę odos reakciją.

Mūvėti apsaugines pirštines/dėvėti apsauginius drabužius/naudoti akių (veido) apsaugos priemones.

PRARIJUS: išskalauti burną. NESKATINTI vėmimo.

PATEKUS Į AKIS: Kelias minutes atsargiai plauti vandeniu. Išimti kontaktinius lęšius, jeigu jie yra ir jeigu lengvai galima tai padaryti. Toliau plauti akis.


**NL**

Gevaar

Veroorzaakt ernstige brandwonden en oogletsels. Kan een allergische huidreactie veroorzaken.


**NO**

Fare

Forårsaker alvorlige hudforbrenninger og øyeskader. Kan forårsake allergiske hudreaksjoner.


**PL**

Niebezpieczeństwo

Powoduje poważne oparzenia skóry oraz uszkodzenia oczu. Może powodować reakcję alergiczną skóry.


**PT**

Perigo

Provoca queimaduras na pele e lesões oculares graves. Pode provocar uma reacção alérgica cutânea.


**RO**

Pericol

Provoacă arsuri grave ale pielei și lezarea ochilor. Poate provoca o reacție alergică a pielei.


**SE**

Fara

Orsakar allvarliga frätskador på hud och ögon. Kan orsaka allergisk hudreaktion.

Nevarno
Povzroča hude opekline kože in poškodbe oči.
Lahko povzroči alergijski odziv kože.

SK)
Nebezpečenstvo
Provoacă arsuri grave ale pielii și lezarea ochilor.
Môže vyvoláť alergickú kožnú reakciu.