Anti-EBV VCA IgM ELISA

Enzyme-Immunoassay for in-vitro detection of IgM antibodies against virus capsid antigen (VCA) p23/p18 of Epstein-Barr-Virus (EBV) in serum or plasma

**Intend of use**
The Anti-EBV VCA IgM ELISA is an in-vitro diagnostic device for the detection of IgM-antibodies against the VCA antigens p23 and p18 of EBV. Results obtained with this test, in conjunction with other clinical and patient data obtained in assays for other Epstein-Barr-virus-specific antibod-
ies such as anti-Epstein-Barr-Virus-antibodies (anti-EBNA IgG and anti-EBNA1 IgG), assist in serological diagnosis of EBV infection. Primary infection with EBV can result in infectious mononucleosis (IM = mons Pfeiffer, 1.2), the illness predomin-
antly occurs among older adolescents and young adults. The acute disease can show the following symptoms: Fever, pharyngitis, tonsillitis, lymphaden-
opathy, malaise, headache, myalgia, spleno- and hepatomegaly, rash, and leucocytosis (2). Other pathogenic infectious agents such as cytomegalovi-
rus, Toxoplasma gondii, rubella virus, hepatitis viruses, human immunodefi-
ciency virus (HIV) may cause similar symptoms. The Anti-EBV VCA IgM ELISA can be used for the identification of an EBV infection.

**Principle of the assay**
The Anti-EBV VCA IgM ELISA is a highly sensitive IgM (µ-chain)-specific capture enzyme immunoassay sorbent assay (ELISA) for the detection of EBV-specific antibodies in serum or plasma (4). During the first incubation step IgM antibodies of the sample will bind to the MTP*. Other immunoglobulin-
types will be removed by washing. During a second incubation, VCA-p23-
18-specific, immobilized IgM antibodies will be detected. This is performed by the addition of an antigen-enzyme conjugate. The recombinant (rec) p23-18 is directly iodinated and covalently linked with horse radish peroxidase (HRP). Non-
specifically bound conjugate is removed by another washing step. For the last incubation the substrate solution (TMB, 3,3',5,5'-Tetramethylbenzidine) is filled into the wells. The enzyme reaction is stopped by adding sulphuric acid (colour change from blue to yellow) and the optical density is measured with a spectrophotometer at 450 nm and a reference wavelength of 615-690 nm.

**Kit contains**
- **MTP**: 1 Microplate 12 single strips with 8 wells each, coated with polyclonal anti-human IgM antibody (concentration: > 0.5 µg/ml).
- **NC**: 1.2 ml EBV IgM negative control (ready for use, human) preservative: 0.005% gentamycin, 0.05%, streptomycin, 0.05 % penicillin V.
- **PC**: 1.2 ml EBV IgM positive control (ready for use, human) preservative: 0.005% gentamycin, 0.05%, streptomycin, 0.05% penicillin V.
- **DIL**: 45 ml Sample diluent (ready for use) preservative: 0.01% neomycinsulfat, 0.03% chlorampheni-
- **CONJ**: 15 ml Rec. p23-18 fusionprotein conjugated (ready for use) preservative: 0.025% penicillin V, 0.025% streptomycinsulf-
- **SUB**: 13 ml Substrate solution (ready for use) 3,3',5-tetramethylbenzidine (TMB) solution < 0,05 % in
- **STOP**: 15 ml Stop solution (ready for use) Sulfuric acid < 1N H2SO4.
- **SB**: 1 Storage bag Polyethylene bag for storing remaining microplate strips.
- **FCL**: 3 Self-adhesive transparent foils Self-adhesive transparent foils for sealing the microplate wells during incubation.
- **L**: 1 Package insert information Preservatives: total concentration < 0,11%

**Materials required but not supplied with the kit**
- micropipettes, spectral photometer (450 nm, reference wavelength 615–690 nm), microplate washer (with bottom wash) and incubator (37°C) for MTP.

**Warning and precautions**
Do not incorporate reagents. Avoid contact with eyes and skin. All samples and materials used for the test must be treated as being potentially infectious and appropriate safety precautions taken. The controls are negative for anti-HIV 1/2, anti-HCV, HBsAg, anti-lues and elevated transaminases. Do not pipet with mouth. According to good laboratory practice wear gloves, laboratory coat and safety glasses. Liquids and non-irritable materials should be decon-
aminated with sodium hypochlorite (final concentration: 3 %, activity time at least 30 minutes). Liquid waste which contains acids must be neutralised before disposal. Used MTP and all materials that are to be re-used must be autoclaved for 1 hour at 221°C. The **SUB** is sensitive of light and has to be protected from light. The test must be performed by well-trained and author-
ised laboratory technicians. Testing is performed under aseptic and microbiologi-

cally controlled conditions. Inform the manufacturer if the original test kit is damaged.

**Storage**
The reagents are stable up to the stated expiry dates on the individual labels when stored at 2-8°C. After opening reagents have to be used within 30 days. For repeatedly testing store the reagents immediately after usage at 2-8°C. The **MTP** sealed in an aluminium bag with a desiccant and must be at room temperature before opening. Return unused strips with the desiccant to the zip-lock bag and store in this way at 2-8°C. Do not touch the upper rim or the bottom of the wells with fingers.

**Preparation of reagents**
Dilute the wash buffer with demineralised or deionised water (1:501). The prepared buffer is stable for 1 week when stored at 2-8°C. All other test components are prepared ready for use. All reagents are lot specific and can not be used with kits of other lots. Do not use reagents of other manufactur-
ers.

**Sample preparation**
Fresh serum or plasma samples, free from haemolysis should be used. Highly Iaipemic, icteric or microbially contaminated sera or plasma samples and concentrated immunoglobulin preparations can lead to unreliable test results. Avoid repeated freezing and thawing of the samples. If samples are to be transported, they must be packed in accordance with legal requirements for the transportation of infectious materials. The samples should not be inactivated, as unspecific reactions may otherwise occur.

**Performance of test**
The protocol (see pipetting procedure) has to be followed strictly.

- **Sample dilution 1:21 with predilution in tubes**: Dilute [PC], [NC] and samples 1:21 in a tube (e. g. 25 µl control or sample + 500 µl [DIL]). Mix well.
- **Sample dilution 1:21 with dilution directly in plate**: Pipet 200 µl [DIL] into every well. The dilution directly in the microtost plate is particularly suitable for the use of automatic pipetting devices. If the dilution directly in the plate is performed manually it is important to avoid non-specific protein binding by observing the following steps: Pipet first 200 µl of [DIL] into the well and add 10 µl of sample or controls subsequently. Mix 5 up to 7 times when adding 10 µl sample or control.

**Pipetting procedure of qualitative IgM determination (dilution tube)**
Allow all reagents to reach room temperature before use.

- **step 1**
  - **well [µl]**
    - A1/B1 C1/D1 E1/F1 G1... 200 [DIL]
    - NC double test -- -- 200 [NC] -- --
    - PC double test -- -- 200 [PC] --
    - Sample 1/21 -- -- 200 [MTP]
  - **step 2**
    - **well [µl]**
      - Processor*: 60 ± 2 min., 37 ± 1°C Processor*: 30 ± 1 min., 37 ± 1°C
      - 5 x wash (s. W1) 550 550 550 550
      - [WB] 550 550 550 550
      - [SB] 100 100 100 100
      - Processor*: 30 ± 1 min., 37 ± 1°C processor*: 15 ± 1 min., at room temperature in the dark Processor*: 15 ± 1 min., at room temperature in the dark
    - **step 3**
      - **well [µl]**
        - [SB] 100 100 100 100
        - Processor*: 30 ± 1 min., at room temperature in the dark Processor*: 30 ± 1 min., at room temperature in the dark

**Measure the extinction immediately or within 15 min. after stop at 450 nm using a spectral photometer (reference wavelength: 615 – 690 nm).**
Calculation of qualitative determination
After measuring the extinction values at 450 nm in all wells (reference filter: 615-690 nm), the mean value of the blanks is subtracted from the extinction values of the controls and samples. Mean extinction of blanks \(\leq 0.150\) OD. After subtraction of the blank, the control values must meet the following criteria of validity:

- Mean OD-value of \(\text{NC} \leq 0.200\)
- Mean OD-value of \(\text{PC} \geq 0.400\)

Calculation of the Cut-off-value and grey area
The cut-off value is calculated from the mean OD value of the negative control (\(\text{NC} \)) plus 0.200. Cut-off value = \(\text{NC} + 0.200\). The grey area extends between cut-off-value and cut-off-value minus 10%.

Interpretation of the result
Samples with an extinction value below the grey zone are considered to be negative. If a sample has an extinction value equal to or greater than the grey zone, it is considered to be positive for EBV VCA-specific IgM antibodies. If the OD-value of the retested sample is within the grey area (questionable result), we recommend to request for a follow up sample.

Interpretation of Anti EBV VCA IgM positive results.

<table>
<thead>
<tr>
<th>State of infection</th>
<th>VCA IgM</th>
<th>VCA IgG</th>
<th>EBNA IgG</th>
<th>information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seronegative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Primary infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early phase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acute phase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Late phase</td>
<td>-</td>
<td>+</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Past Infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past Infection</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Persisting IgM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>Reactivation</td>
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<td>+</td>
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</tr>
<tr>
<td>Implausibel</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>Implausibel</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
</tbody>
</table>

1. In the very early phase of a primary infection, the serology can still be negative. It is recommended that in the case of a VCA IgM result within the grey zone, the sample be treated as a “possible early phase of a primary infection” and to confirm or rule out this result by analysis of a follow up sample.
2. There is an exception in the case of an immunodeficient or immune suppressed patient, where this can be a secondary pattern caused by the loss of EBNA IgG. In this situation, it is recommended that EBNA IgG values within the grey zone be evaluated as positive and the sample reported as a “past infection”.
3. IgM persists in many cases for longer than 6 months and can at this point in time coincide with a positive EBNA IgG result. However, a “late phase of a primary infection” should be reported only in the case of very weak EBNA IgG (OD < 0.500). The diagnosis is otherwise “past infection”.
4. A reactivation cannot be defined using VCA serology. Quantification of the VCA IgG can help. Values which extend well above the normal range (> 2,500 RU/ml) are suspect. The classic marker for the serological definition of EBV reactivation are IgG antibodies against early antigen (EA) (e.g. anti-EBV EA IgG ELISA, Art. No. 807 016). Anti-EA IgG should be determined in addition if an EBV reactivation is suspected.
5. The implausibly reactivy patterns are extremely unlikely constellations which have not been found in clinical studies. VCA IgG values within the grey zone should be defined as positive. The interpretation in this case is “past infection”. For VCA IgG values below the grey zone, the EBV serology should be repeated completely to rule out any errors in the test procedure. If the implausible result is obtained again, it is recommended that a new sample be requested. If the result is reconfirmed, “a past infection” must be reported.

Limitations of the method
A negative test result in the Anti-EBV VCA IgM ELISA does not completely exclude an EBV infection. The test results should be used in conjunction with information available from the patient clinical evaluation and other available diagnostic procedures. Test results of specimens from immunosuppressed patients may be difficult to interpret. Positive test results may not be valid in patients who have received blood transfusions or other blood products within the past several months. The Anti-EBV VCA IgM ELISA was analysed with the following potential cross reactive samples: anti-rhumatoid sera (23), Anti-Hepatitis B Virus-positive, acute (6), Anti-Hepatitis C Virus-positive acute (6), Anti-Variella Virus-positive, acute (3), Anti-Cytomegalievirus-positive acute (3) and Anti Toxoplasmose positive, acute (4). One HCV positive sample gave a positive result in the Anti-EBV VCA IgM ELISA.

Performance data
Results obtained with the Anti EBV VCA IgM test in conjunction with further assays such as anti-VCA IgG and anti-EBNA-1 IgG assist in serological diagnosis of EBV infection. From 69 sera of patients which were primary infected with EBV 67 (97,1%) were positive (5). All 46 seronegative samples were tested negative (Specificity 100%) with the Anti EBV VCA IgM ELISA (5).

Precision study
The intra-assay variability of the Anti-EBV VCA IgM ELISA was evaluated by pipetting a negative, a weak reactive and a strong reactive sample within one test run repeatedly in 6 wells each. We obtained the following coefficient of variation respectively (CV): 11,7%, 2,3% and 4,4%. The inter-assay variability was evaluated by testing 3 samples of negative, weak and strong reactivity in 6 wells each and in 3 subsequent runs. We obtained the following coefficient (CV) 7,4%, 3,5% and 4,8%.

References

Trouble Shooting Guide
1) Unexpected high rate of reactive results: Samples and controls were pipetted prior to pipetting of [DIL] or mixing was insufficient.
2) Mean blank value higher than criteria of validity, \(\geq 0.150\) OD:
   a) [SUB] turned blue due to oxidation or contamination.
   c) Incubation fault: Temperature too high, incubation time was exceeded or plate was not incubated directly after finishing of pipetting.
   d) Wavelength fault: Measurement without reference filter will increase OD values approximately \(\geq 0.120\) OD.
3) Yellow coloration in all wells: (see 2a, 2b)
   a) [WB] contamination; Prepare new [WB]
   b) [DIL] or [CON] contamination; Repeat test with reagents from unopened vials. Use reagents under less microbial conditions.
4) Mean value of [PC] below \(\leq 0.400\) OD:
   a) Exceed of expire date.
   b) Temperature too low or fall below incubation time.
   c) Washing fault: Too intensive washing or mechanic contact of manifold and solid phase of the well.
   d) Contamination of [PC] or 3b.
5) Mean value of [NC] higher than \(\geq 0.200\) OD: (see 1 and 2 a-d)
   a) [NC] was not pipetted subsequent to pipetting of samples; Pipet all samples prior to pipetting of blanks and controls.
   b) Contamination with the lid of the [PC]