Anti-EBV EA IgM ELISA

Enzyme-Immunoassay for in-vitro detection of IgM antibodies against virus early antigen (EA) p54/138 of Epstein-Barr-Virus (EBV) in serum or plasma

Materials required but not supplied with the kit

- Microplate (12 single strips with 8 wells each, coated with rec. EBV- EA p54/138 antigens: concentration: > 0.5 µg/ml).
- EBV EA IgM negative control (ready for use, human): preservative: 0.005% gentamyccin, 0.05%, streptomycycin, 0.05% penicillin V.
- EBV EA IgM positive control (ready for use, human): Preservative: 0.005% gentamyccin, 0.05%, streptomycycin, 0.05% penicillin V.
- Sample diluent (ready for use): preservative:0.01% neomycinsulfat, 0.03% chloramphenicol.
- Anti human IgM Conjugate, monoclonal antibody: preservative: 0.005% gentamyccin, 0.025% streptomycinsulfat, 0.2% proclin-300.
- Wash buffer concentrate (500fold): preservative:0.01% 2-bromo-2-nitro-1,3-propanediol.
- Substrate solution (ready for use): 3.3, 5,5-tetramethylbenzidine (TMB) solution: < 0.05% in H2O.
- Storage bag Polyethylene bag for storing remaining microplate strips.
- Self-adhesive transparent foils
- Polyethylene bag for storing remaining microplate strips.

Preservatives: total concentration < 0.11%

Warnings 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, HBsAg, anti-HCV, anti-HBc, anti-HBc and all the extracts. Do not pipet with mouth. According to good laboratory practice wear gloves, laboratory coat and safety glasses. Liquids and non-combustible materials should be contaminated 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 121°C. The SUB is sensitive of light and has to be protected from light. The test must be performed by well-trained and authorised laboratory technicians. Testing is performed under aseptic and microbiologically 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 stored in a dry place 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 manufacturers.

Sample preparation

- Fresh serum or plasma samples, free from haemolysis should be used. Highly lipaemic, 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 500 µl into every well. The dilution directly in the microplate 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)

- All reagents and the blank should be pipetted last. After pipetting the controls and samples immediately begin with incubation of the plate.

Kit contains

- MTP 1
- NC 1,2 ml
- PC 1,2 ml
- DIL 45 ml
- CONJ 15 ml
- WB 6 ml
- SUB 13 ml
- STOP 15 ml
- SB 1
- FOL 3
- LB 1

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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 \( \leq 0.100 \text{ OD} \). After subtraction of the blank, the control values must meet the following criteria of validity:

\[
\text{Mean OD-value of } [\text{NC}] \leq 0.200; \quad \text{Mean OD-value of } [\text{PC}] \geq 0.400
\]

Calculation of the Cut-off-value and grey area
The cut-off value is calculated from the mean OD value of the negative control (NC) plus 0.200. Cut-off value = 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 EA-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. For further interpretations of reactivity pattern (6) a complete interpretation scheme is available on request from Biotest.

Limitations of the method
A negative test result in the Anti-EBV EA 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 persons who have received blood transfusions or other blood products within the past several months. The Anti-EBV EA IgM ELISA was analysed with the following potential cross reactive antisera: Anti-Varians Virus-positive, acute (6), Anti-Cytomegalivirus-positive acute (6), Anti-Herpes simplex type 1 and 2 (5) and Anti-Toxoplasmosis (5). None of the samples was positive with the Anti-EBV EA IgM ELISA.

Performance data
Results obtained with the Anti-EBV EA IgM test in conjunction with further assays such as anti-EA IgG and anti-EBNA-1 IgG assist in serological diagnosis of EBV infection (4). The sensitivity of the ELISA test system of these three assays was defined in IM patients by 99,2%. The specificity was determined with 98,8% (5).

Precision study
A testpanel of 10 sera representing low, low-reactive and high reactive samples was tested on 11 different days. The inter-assay variability of these sera were 10-16.9%.

Expected values
Ninety-nine serum specimens obtained from healthy, asymptomatic blood donors were tested with the Biotest EA IgM ELISA. Of the 99 specimens, 5 were found to be positive (5.05%) and 94 were found to be negative (94.95%). This is consistent with published rates for the prevalence of exposure to EBV in the adult population. Prevalence may vary depending on a variety of factors such as geographical location, age, socioeconomic status, race, type of test employed, specimen collection and handling procedures, clinical and epidemiological history (6, 7). Sera from patients after CMV- (20), Toxoplasma- (20) and rheumatic diseases (20) were analysed. Using the EA IgM, EA IgG and EBNA IgG ELISA system 7, 17 and 29 samples could be identified with primary- reactivated- and past EBV infection.

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, \( \geq 0.100 \text{ OD} \):
   a) SUB turned blue due to oxidation or contamination.
   b) Washing fault: Perform 5x wash cycles/washing step. If using a manual washing device, perform 7x wash cycles/washing step. Use Biotest WB as contained in the kit.
   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 \( \geq 0.120 \text{ OD} \).
3) Yellow coloration in all wells: (see 2a, 2b)
   a) WB contamination; Prepare new WB
   b) DIL or CONJ contamination; Repeat test with reagents from unopened vials. Use reagents under less microbial conditions.
   c) Washing fault: Too intensive washing or mechanic contact of manifold and solid phase of the well.
   d) Contamination of PC or 3b.
4) Mean value of PC below \( \leq 0.400 \text{ OD} \):
   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.
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5) Mean value of NC higher than \( \geq 0.200 \text{ OD} \): (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.

Interpretation schema of the EA IgM test

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