DRG® Epstein Barr Virus (EIA-2519)

Revised 7 May 2007 **RUO in the USA**

Introduction

The **Epstein Barr Virus (EBNA-1)** provides materials for the quantitative determination of human IgA/IgG/IgM antibodies against EBNA-1 of Epstein Barr Virus (EBV) in serum and plasma. This assay is intended for in-vitro use only.

In 1961 an infectious disease was identified in Uganda, which was correlated with the appearance of a defined type of tumor with children. The illness, which is found predominantly in Africa and Papua-New Guinea, was named Burkitt lymphoma from ist discoverer. In 1964, Epstein, Barr and Achong characterized by electron microscopy as the causing agent a hitherto unknown virus, which belongs to the family of herpes viruses.

The Epstein Barr virus is made responsible for a variety of diseases like infectious mononucleosis, Burkitt lymphoma, as well as nasopharyngeal carcinoma. In addition, a role of the virus is discussed in connection with Hodgkin´s disease. Especially with teenagers there appears a glandular fever syndrome, which is called “kissing disease”.

Diseases which are caused by the Epstein Barr virus are found mainly in persons with reduced immunity. For example, the virus is associated with a lymphoproliferative disease which occurs after transplantation. The immune system of such patients is usually impaired by drug therapy. Also in immune-deficient AIDS patients, there appears frequently a state where cells at the rim of the tongue are infected (oral hairy leukoplakia).

Infected persons keep the Epstein-Barr virus forever in their body, they are however mostly not ill. In the developing countries practically all the people are infected, in the western world the incidence is between 80% and 90%. The transmittance occurs already during childhood, perhaps by transfer from the mother, mainly via the saliva.

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During the active phase of the viral cycle, the Epstein-Barr virus produces about 100 different antigens, in the inactive phase around 10. The latter comprises among others the EBV nuclear antigen EBNA-1, which is closely correlated with a past infection and an immunity. The early antigen (EA) as well as the virus capsid antigen (VCA) from the active phase are also used as diagnostic markers.

In a fresh infection, IgM antibodies against VCA and EA are determined by immunofluo-rescence or ELISA. Later VCA IgG and afterwards EBNA-1 IgG antibodies appear. The simultaneous activation of VCA IgM and EBNA-1 IgG indicates correspondingly a reactivation of a latent EBV infection.

The present EBNA-1 IgG ELISA monitors a past infection satisfactorily and can above all be utilized for the assessment of blood samples before transfusions or transplatations and the exclusion of transmittance between children.

Principle of the Test

The Epstein Barr Virus (EBNA-1) IgA/IgG/IgM ELISA is based on the principle of the enzyme immunoassay (EIA). Affinity-purified, recombinant EBNA-1 (Sf-9/ Baculovirus) is bound on the surface of the microtiter strips. Diluted patient serum or ready to use standards and controls are pipetted into the wells of the microtiter plate. A binding between the IgA/IgG/IgM antibodies of the serum and the immobilized antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready to use anti human IgA/IgG/IgM peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The colour development is terminated by the addition of a stop solution, which changes the colour from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgA/IgG/IgM antibodies is directly proportional to the intensity of the colour.

Precautions

- The assay calibrators and controls are of human origin and have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All standards, however, should be treated as potential biohazards in use and for disposal.
- The assay reagents contain sodium azide or thimerosal, which may be toxic if ingested. Sodium azide may react with copper and lead piping to form highly explosive salts. On disposal, flush with large quantities of water.
- The stop solution contains H_2SO_4. If it comes into contact with skin, wash thoroughly with water and seek medical attention. Since the H_2SO_4 used to terminate the colour reaction is corrosive, the instrumentation employed to dispense it should be thoroughly cleaned after use.
- This kit is for in vitro use only.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with germicidical soap and copious amounts of water.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents, and wash hands thoroughly afterwards. Microbial contamination of reagents or specimens may give false results.

Storage and Stability

Store all reagents at 2 - 8 °C and use before expiry date. When stored at 2 - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Once the foilbag of the coated microtiter strips has been broken, care should be taken to close it tightly again. The immunoreactivity of the coated microtiter strips are stable for approx. 6 weeks in the broken, but tightly closed bag. Allow all reagents and required number of strips to reach room temperature prior to use.

Contents of the Test Kit

Microtiter Strips break apart strips coated with EBNA-1	12 x 8 wells
Negative Control 2 ml, ready to use, human serum diluted by PBS, contains no IgA/IgG/IgM antibodies against EBNA-1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial
Cut-Off Standard 2 ml, ready to use, human serum diluted by PBS, contains a low concentration of IgA/IgG/IgM antibodies against EBNA-1, addition of 0.01 % potassium tetraiodomercurate, refer to label for exact concentration.	1 vial

Weakly Positive Control 1 vial
2 ml, ready to use, human serum
diluted by PBS, contains a medium concentration of
IgA/IgG/IgM antibodies against EBNA-1,
addition of 0.01 % potassium tetraiodomercurate,
refer to label for exact concentration.

Positive Control 1 vial
2 ml, ready to use, human serum
diluted by PBS, contains a high concentration of
IgA/IgG/IgM antibodies against EBNA-1,
addition of 0.01 % potassium tetraiodomercurate,
refer to label for exact concentration.

Enzyme Conjugate 1 vial
11 ml, ready to use, anti human IgA/IgG/IgM
conjugated to POD,
in protein-containing buffer solution.

TMB Substrate Solution 1 vial
11 ml, ready to use, containing a solution of
tetramethylbenzidine (TMB).

TMB Stop Solution 1 vial
11 ml, ready to use, 1 M sulphuric acid (H_2SO_4)
Avoid contact with stop-solution
it may cause skin irritations and burns.

Sample Diluent 1 vial
60 ml, ready to use, PBS/BSA buffer, addition of
0.01 % potassium tetraiodomercurate.

Wash Buffer, concentrate (10x) 1 vial
60 ml, concentrate, containing PBS buffer with Tween 20,
dilute 1 : 10 (1 + 9) with distilled water
prior to use (e. g. 10 ml concentrate + 90 ml distilled water).

Plastic Foils 2 pieces
2 pieces to cover the microtiter strips during the incubation.

Plastic Bag
1 piece

Resealable, for the dry storage of non-used strips.

Material required but not provided

- Automatic pipettes to dispense 5, 50, 100 and 500 µl (a multichannel pipetting device such as Titertek is suitable for adding reagents to the wells)
- Distilled water
- Microtiter plate spectrophotometer (ELISA reader) with 450 nm filter
- Microtiter plate washer

Specimen Collection and Storage

- Serum or plasma (EDTA, heparin) should be used, and the usual precautions for venipuncture should be observed. No special sample pretreatment is necessary. The specimen may be stored at 2 - 8 °C for up to 48 hours, and should be frozen at - 20 °C or lower for longer periods
- Repeated freeze - thawing should be avoided.
- Thawed samples should be inverted several times prior to testing.
- Do not use grossly hemolyzed or grossly lipemic specimens.

Preparation of Samples and Reagents

Samples

Dilute patient sample 1 to 101 with ready to use sample diluent (e. g. 5 µl sample + 500 µl buffer).
For IgM:
In order to avoid interference of rheumatoid factors, patient sera should be treated with RF absorbent (IBL Cat. No. RE 590 59). Alternatively, positive results can be confirmed in a second test run. Do not treat the controls!

Wash Buffer

Dilute the wash buffer concentrate with distilled water 1 to 10 (1 + 9)

(e. g. 10 ml concentrate + 90 ml distilled water.). If during the cold storage crystals precipitate, the concentrate should be warmed up at 37 °C for

15 minutes.

Assay Procedure
General Remarks:

It is recommended to use control samples according to state and federal regulations. The use of control sera or plasma is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.

Use new disposable plastic pipette tips for each reagent, standard or specimen in order to avoid cross contamination. For the dispensing of the TMB substrate solution and the TMB stop solution avoid pipettes with metal parts. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without

interruption. Leave sufficient microtiter strips in the strip holder to enable the running of standards, controls, and samples, plus one well for the substrate blank. Secure the desired number of microtiter strips in the holder.

Pipet **100 µl of standards, controls and diluted samples** into the appropriate wells of the strips. Leave one well empty for the substrate blank.

Cover plate with the enclosed foil and **incubate for 60 minutes** at room temperature (18 - 24 °C).

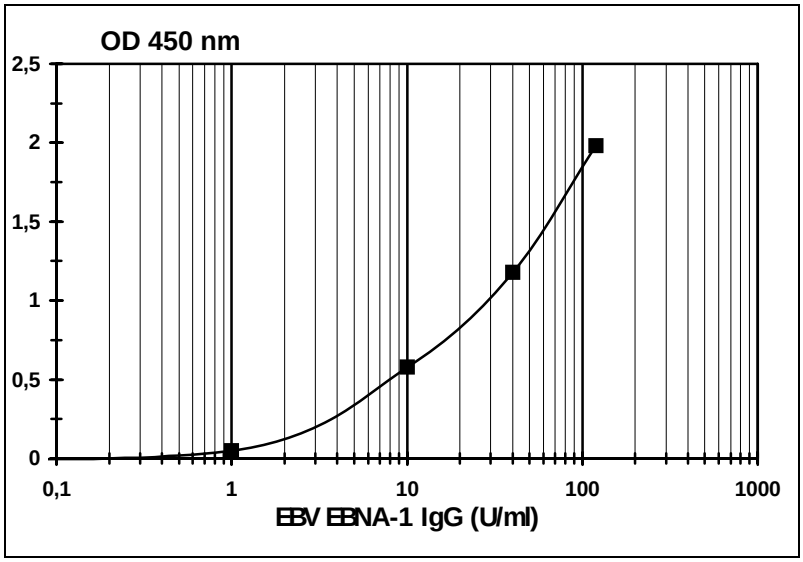
Washing: discard the incubation solution, rinse the wells 3x with 300 µl diluted wash buffer (dilute concentrate 1 to 10 with distilled water) and remove any residual. Add **100 µl of enzyme conjugate** to each well in sequence. Leave one well empty for the substrate blank. Cover plate with the enclosed foil and **incubate for 30 minutes** at room temperature (18 - 24 °C). **Washing**: discard the incubation solution, rinse the wells 3 x with 300 µl diluted wash buffer (dilute concentrate 1 to 10 with distilled water) and remove any residual. Promptly pipet **100 µl of the TMB substrate solution** into the rinsed wells. This time also the substrate blank is pipetted. Cover plate with the enclosed foil and **incubate for 20 minutes** at room temperature (18 - 24 °C) in the dark. Stop the reaction by adding **100 µl** of **TMB stop solution** to each well. **Shake gently** the Microtiter Strips being careful not to let the content come from the wells and **read at 450 nm** within 60 minutes from the stopping.

Calculation of Results - Quantitative Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10 %. The ready to use standards and controls of the Epstein Barr Virus (EBNA-1) IgA/IgG/IgM ELISA are defined and expressed in Units (U). This results in an exact and reproducible quantitative evaluation. Consequently for a given patient follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials. For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each patient sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs, i.e. 4 parameter, spline, logit-log.

Standards	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.015		
Negative Control	0.036/0.039	0.021/0.024	0.023
Cut-Off Standard	0.605/0.587	0.590/0.572	0.581
Weakly Positive Control	1.177/1.210	1.162/1.195	1.179
Positive Control	1.975/2.017	1.960/2.002	1.981

Typical Standard Curve
Epstein Barr Virus (EBNA-1) IgG ELISA



Interpretation
The results of each patient sample can be assessed as follows:

> 12 U/ml	positive
8 - 12 U/ml	borderline
< 8 U/ml	negative

Qualitative Evaluation
The calculated absorptions for the patient sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result. For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/- 20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, should be recommended. Both samples should be measured in parallel in the same run. The positive control must show at least the double absorption compared with the cut-off standard.

Assay Characteristics
Precision
The intra-assay coefficient of variation of the Epstein Barr Virus (EBNA-1) IgA/IgG/IgM ELISA was assessed by a ten-fold determination in a positive serum sample to less than 10 %.

Limitations of use
Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbances.

Warranty
Any modification of this test as well as exchange or mixture of any components from different lots might influence the results. In such cases there is no claim for a replacement.

References

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