ANTI-EBV EA IgM ELISA

Σ 96 REF 807015

Enzyme-Immunoassay for *in-vitro* detection of IgM antibodies against virus early antigen (EA) p54/p138 of Epstein-Barr Virus (EBV) in human serum or plasma

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1. INTENTED USE

The Anti-EBV EA IgM ELISA is an *in vitro* diagnostic device for the detection of IgM-antibodies against the early antigens (EA) p54 and p138 of EBV. Results obtained with this test, in conjunction with other clinical and patient data obtained in assays for other Epstein-Barr virus-specific antibodies such as anti-EA IgG, anti-VCA IgG/IgM and anti-EBNA-1 IgG, assist in serological diagnosis of EBV infection. Primary infection with EBV can result in infectious mononucleosis (IM = morbus Pfeiffer) (1,2). The illness predominantly occurs among older adolescents and young adults. The acute disease can show the following symptoms: fever, pharyngitis, tonsillitis, lymphadenopathy, malaise, headache, myalgia, spleno- and hepatomegaly, rash, and leucocytosis (2). Other pathogenic infectious agents such as cytomegalovirus, *Toxoplasma gondii*, rubella virus, hepatitis viruses, human immunodeficiency virus (HIV) may cause similar symptoms. The Anti-EBV EA IgM ELISA can be used for the identification of an EBV infection.

2. PRINCIPLES OF THE PROCEDURE

The Anti-EBV EA IgM ELISA is a highly sensitive indirect enzyme immunosorbent assay (ELISA) for the detection of EBV-specific antibodies in serum or plasma. During the first incubation step IgM antibodies of the sample will bind to the recombinant (rec) antigens p54 and p138 (3-5) coated to the microplate. Unspecific material will be removed by washing. The resulting antibody-antigen complex is detected using a specific, enzyme-labelled monoclonal antibody directed against human IgM. 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.

3. REAGENTS

Supplied quantities of reagents have been calculated to allow 96 tests. All reagents are exclusively for *in vitro* diagnostic use.

Label		Description	Presentation
R1	**Microplate**	**Microplate:** 12 single strips with 8 wells each, coated with rec. EBV EA p54/138 antigens (concentration: ≥ 0.4 µg/ml).	1 **Ready-to-use**
R2	**EBV Concentrated Washing Solution (500x)**	**Concentrated Washing Solution (500x):** Preservative: 0.01% 2-bromo-2-nitro-1,3-propanediol	1 x 6 ml **To be diluted**
R3	**EBV EA IgM Negative Control**	**EBV EA IgM Negative Control:** Negative human serum for EBV-EA IgM antibodies, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: 0.005 % gentamycin, 0.05 % streptomycin, 0.05 % penicillin V	1 x 1.2 ml **Ready-to-use**
R5	**EBV EA IgM Positive Control**	**EBV EA IgM Positive Control:** Human serum reactive for EBV-EA IgM antibodies, and negative for HBs antigen, anti-HIV1, anti-HIV2 and anti-HCV Preservative: 0.005 % gentamycin, 0.05 % streptomycin, 0.05 % penicillin V	1 x 1.2 ml **Ready-to-use**
R6	**Conjugate**	**Anti-Human IgM Conjugate:** Monoclonal antibody peroxidase labeled Preservative: 0.025% penicillin V, 0.025% streptomycinsulfat, < 1.5% ProClin™ 300	1 x 15 ml **Ready-to-use**
R7	**EBV Sample Diluent**	**Sample Diluent:** Preservative: 0.01% neomycinsulfat, 0.03% chloramphenicol	1 x 45 ml **Ready-to-use**
R9	**EBV Chromogen TMB**	**Chromogen TMB:** 3, 3´, 5, 5´ tetramethylbenzidine (TMB) solution < 0.05 % in H_2O. See Warnings and Precautions	1 x 13 ml **Ready-to-use**
R10	**EBV Stopping Solution**	**Stopping Solution:** Sulfuric acid < 1N H_2SO_4	1 x 15 ml **Ready-to-use**

Label		Description	Presentation
	Storage Bag	**Storage Bag:** Polyethylene bag for storing remaining microplate strips.	1
	Self-adhesive transparent foils	**Self-adhesive transparent foils:** Self-adhesive transparent foils for sealing the microplate wells during incubation.	4

Preservatives: total concentration < 0.11%

Storage and handling requirements

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 microplate sealed in an aluminum bag with a desiccant must be at room temperature before opening. Return unused strips with the desiccant to the storage bag and store in this way at 2-8°C. Do not touch the upper rim or the bottom of the wells with fingers.

4. WARNING AND PRECAUTIONS

Do not ingest 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-combustible materials should be decontaminated with sodium hypochlorite (final concentration: 3%, activity time at least 30 minutes). Liquid waste which contains acids must be neutralized before disposal. Used microplates and all materials that are to be re-used must be autoclaved for 1 hour at 121°C. The Chromogen TMB (R9) is sensitive of light and has to be protected from light. The test must be performed by well-trained and authorized laboratory technicians. Testing is performed under aseptic and microbiologically controlled conditions. Inform the manufacturer if the original test kit is damaged.

CAUTION: Some of the reagents contain ProClin™ 300 < 1.5%

For risks and security recommendations refer to the table at the end of the package insert.

5. SPECIMENS

Fresh serum or plasma samples, free from haemolysis should be used. Highly lipaemic, icteric or microbiologically 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.

6. MATERIALS REQUIRED NOT PROVIDED

Micropipettes, spectral photometer (450 nm, reference wavelength 615-690 nm), microplate washer (with bottom wash) and incubator (37°C) for microplates.

7. INSTRUCTIONS FOR USE

Reagents preparation

Dilute the Concentrated Washing Solution (R2) with demineralized or deionized water (1:501). The Washing Solution prepared is stable for 1 week when stored at 2-8°C. All other test components are 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.

Specimen preparation

The protocol (see Pipetting Procedure) has to be followed strictly.

- Sample dilution 1:21 with pre-dilution in tubes:
  Dilute Negative Control (R3), Positive Control (R5) and samples 1:21 in a tube (e.g. 25 µl control or sample + 500 µl of Diluent (R7)). Mix well.
- Sample dilution 1:21 with dilution directly in plate:
  Pipet 200 µl of Diluent (R7) into every well. The dilution directly in the microtest 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 Diluent (R7) 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.

Washing procedure

The wash procedure is critical. Insufficient washing will result in poor precision and unspecific reactions.

Wash five times with wash buffer. For that remove the liquid in the well and dispense with 300µl washing buffer.

This washing procedure is repeated 5 times. Tap out the plate briefly after washing. Do not allow the plate to dry out.

Pipetting procedure of qualitative IgM determination (dilution tube)

Allow all reagents to reach room temperature before use.				
The controls and the blank should be pipetted last. After pipetting the controls and samples immediately begin with incubation of the plate.				
Step 1	**Well [µl]**			
	A1/B1	C1/D1	E1/F1	G1…
Blank	200 µl R7	-	-	-
Negative Control (R3) in duplicate	-	200 µl R3	-	-
Positive Control (R5) in duplicate	-	-	200 µl R5	-
Sample 1:21	-	-	-	200 µl Sample
Seal microplate using self-adhesive foils (not required in an ELISA processor).*				
Incubation 60 ± 2 min., 37 ± 1°C	**Processor*: 60 ± 2 min., 37 ± 1°C**			
5 washes				
Diluted Washing Solution (R2)	300 µl	300 µl	300 µl	300 µl
Step 2	**Well [µl]**			
Conjugate (R6)	100 µl	100 µl	100 µl	100 µl
Seal microplate using self-adhesive foils (not required in an ELISA processor).*				
Incubation 30 ± 1 min., 37 ± 1°C	**Processor*: 30 ± 1 min., 37 ± 1°C**			
5 washes				
Diluted Washing Solution (R2)	300 µl	300 µl	300 µl	300 µl
Step 3	**Well [µl]**			
Chromogen TMB (R9)	100 µl	100 µl	100 µl	100 µl
Incubation 30 ± 1 min., at room temperature in the dark	**Processor*: 15 ± 1 min.**, at room temperature in the dark			
Stopping Solution (R10)	100 µl	100 µl	100 µl	100 µl
*Measure the extinction immediately or **within 15 min.** after stop at **450 nm** using a spectral photometer (reference wavelength: 615 - 690 nm).*				

* If an ELISA processor is used the operator has to validate the test under his own reliability.

8. QUALITY CONTROL

All manufactured reagents are prepared according to our Quality System, starting from reception of raw material to commercialization of the final product. Each lot is submitted to quality control assessments and is released to the market only after conforming to pre-defined acceptance criteria. The records related to production and controls of each single lot are kept within Bio-Rad.

9. INTERPRETATION OF RESULTS

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.

Calculation of the cut-off-value and grey area

The cut-off value is calculated from the mean OD value of the Negative Control ($R3_X$) plus 0.200:

- Cut-off value = $R3_X + 0.200$

The grey area extends between cut-off-value and cut-off-value minus 10%.

Test validation criteria for 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 ≤ 0.100 OD

After subtraction of the blank, the control values must meet the following criteria of validity:

- Mean OD-value of R3 ≤ 0.200
- Mean OD-value of R5 ≥ 0.400

Interpretation schema of the EA IgM test

Status of EBV Infection	Reaction pattern Anti-EBV EA + EBNA ELISA				
	EA IgM	EA IgG	EBNA IgG	EA IgM threshold (OD=0.5)	EBNA threshold (OD=0.5)
Acute					
Early phase	+	–	–	≥ 0.5	–
Primary	+	+	–	–	–
Primary	+	+	(+)	–	< 0.5
Late Phase	–	+	–	–	–
Questionable Acute					
Seronegative	–	–	–	–	–
Questionable Early	(+)	–	–	< 0.5	–
Questionable Late	+	–	(+)	–	< 0.5
Reactivation					
Secondary (weak)	+	–	+	≥ 0.5	≥ 0.5
Reactivation	+	+	+	–	≥ 0.5
Past-infection					
Past-infection	–	+	+	–	–
Past-infection	–	–	+	–	–
Past-infection	(+)	–	+	< 0.5	–

10. TEST LIMITATIONS

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 analyzed with the following potential cross reactive samples: Anti-Varizella Virus-positive acute (6), Anti-Cytomegalovirus-positive acute (6), Anti-Herpes simplex type 1 and 2 (5), and Anti-*Toxoplasma* (5). None of the samples was positive with the Anti-EBV EA IgM ELISA.

11. EXPECTED VALUES

Ninety-nine serum specimens obtained from healthy, asymptomatic blood donors were tested with the Bio-Rad EBV EA IgM ELISA assay. 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 (5,6). Sera from patients after CMV- (20), *Toxoplasma*- (20) and rheumatoid diseases (20) were analyzed. Using the EA IgM, EA IgG and EBNA IgG ELISA systems, 7, 17 and 29 samples could be identified with primary- reactivated- and past EBV infection (4).

12. PERFORMANCES CHARACTERISTICS

Sensitivity and specificity

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 (3). 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% (4).

Precision study

A test panel of 10 sera representing low, low-reactive and high reactive samples was tested on 11 different days. The inter-assay variability of these sera was 10.0% - 16.9%.

The samples of the same panel were tested 8 times within one test run. The intra-assay variability of these sera was 2.8% - 6.3%.

13. REFERENCES

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14. TROUBLE SHOOTING GUIDE

1. Unexpected high rate of reactive results:
   a. Samples and controls were pipetted prior to pipetting of Diluent (R7).
   b. Mixing was insufficient.
2. Mean blank value higher than criteria of validity (≥ 0.100 OD):
   a. Chromogen TMB (R9) 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 Bio-Rad Washing Solution (R2) 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 + 0.120 OD.
3. Yellow coloration in all wells (see 2a, 2b):
   a. Washing Solution (R2) contamination. Prepare a new Washing Solution (R2).
   b. Diluent (R7) or Conjugate (R6) contamination; Repeat test with reagents from unopened vials. Use reagents under less microbial conditions.
4. Mean value of Positive Control (R5) ≤ 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 Control (R5) or 3b.
5. Mean value of Negative Control (R3) ≥ 0.200 OD (see 1 and 2a-d):
   a. Negative Control (R3) 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 Positive Control (R5).

(GB) Irritant
(FR) Irritant
(ES) Irritante
(IT) Irritante
(DE) Reizend
(PT) Irritante
(SE) Irriterande
(DK) Lokalirriterende
(GR) Ερεθιστικό
(PL) Produkt drażniący
(LT) Dirginanti
(HU) Irritatív
(EE) Ärritav
(SK) Dráždivý
(CZ) Dráždivý
(RO) Iritant
(BG) Дразнещ
(LV) Kairinošs
(MT) Irritanti
(NL) Irriterend
(SI) Dražilno
(FI) Ärsyttävä

R43:

(GB) • May cause sensitisation by skin contact.
(FR) • Peut entraîner une sensibilisation par contact avec la peau.
(ES) • Posibilidad de sensibilización en contacto con la piel.
(IT) • Può provocare sensibilizzazione per contatto con la pelle.
(DE) • Sensibilisierung durch Hautkontakt möglich.
(PT) • Pode causar sensibilização em contacto com a pele.
(SE) • Kan ge allergi vid hudkontakt.
(DK) • Kan give overfølsomhed ved kontakt med huden.
(GR) • Μπορεί να προκαλέσει ευαισθητοποίηση σε επαφήμε το δέρμα.
(PL) • Może powodować uczulenie w kontakcie ze skórą.
(LT) • Gali sukelti alergiją susilietus su oda.
(HU) • Bőrrel érintkezve túlérzékenységet okozhat (szenzibilizáló hatású lehet).
(EE) • Kokkupuutel nahaga võib põhjustada ülitundlikkust.
(SK) • Môže spôsobiť senzibilizáciu pri kontakte s pokožkou.
(CZ) • Může vyvolat senzibilizaci při styku s kůží.
(RO) • Poate provoca o sensibilizare în contact cu pielea.
(BG) • Възможна е сенсибилизация при контакт с кожата.
(LV) • Saskaroties ar ādu, var izraisīt paaugstinātu jutīgumu.
(MT) • Jista' jikkaġuna sensitizzazzjoni meta jmiss il-ġilda.
(NL) • Kan overgevoeligheid veroorzaken bij contact met de huid.
(SI) • Stik s kožo lahko povzroči preobčutljivost.
(FI) • Ihokosketus voi aiheuttaa herkistymistä.

S24-37-60:

(GB) • Avoid contact with skin. Wear suitable gloves. This material and its container must be disposed of as hazardous waste.
(FR) • Éviter le contact avec la peau. Porter des gants appropriés. Élminer le produit et son récipient comme un déchet dangereux.
(ES) • Evítese el contacto con la piel. Úsense guantes adecuados. Elimínense el producto y su recipiente como residuos peligrosos.
(IT) • Evitare il contatto con la pelle. Usare guanti adatti. Questo materiale e il suo contenitore devono essere smaltiti come rifiuti pericolosi.
(DE) • Berührung mit der Haut vermeiden. Geeignete Schutzhandschuhe tragen. Dieses Produkt und sein Behälter sind als gefährlicher Abfall zu entsorgen.
(PT) • Evitar o contacto com a pele. Usar luvas adequadas. Este produto e o seu recipiente devem ser eliminados como resíduos perigosos.
(SE) • Undvik kontakt med huden. Använd lämpliga skyddshandskar. Detta material och dess behållare skall tas om hand som farligt avfall.
(DK) • Undgå kontakt med huden. Brug egnede beskyttelseshandsker under arbejdet. Dette materiale og dets beholder skal bortskaffes som farligt affald.
(GR) • Να φοράτε κατάλληλα γάντια. Το υλικό και ο περιέκτης του να θεωρηθούν κατά τη διάθεσή τους επικίνδυνα απόβλητα.
(PL) • Unikać zanieczyszczenia skóry. Nosić odpowiednie rękawice ochronne. Produkt i opakowanie usuwać jako odpad niebezpieczny.
(LT) • Vengti patekimo ant odos. Mūvėti tinkamas pirštines. Šios medžiagos atliekos ir jos pakuotė turi būti šalinamos kaip pavojingos atliekos.
(HU) • A bőrrel való érintkezés kerülendő. Megfelelő védőkesztyűt kell viselni. Az anyagot és/vagy edényzetét veszélyes hulladékként kell ártalmatlanítani.
(EE) • Vältida kokkupuudet nahaga. Kanda sobivaid kaitsekindaid. Kemikaal ja tema pakend kõrvaldada kui ohtlikud jäätmed.
(SK) • Zabráňte kontaktu s pokožkou. Noste vhodné rukavice. Tento materiál a príslušná nádoba musia byť zlikvidované ako nebezpečný odpad. Tento materiál a príslušná nádoba musia byť zlikvidované ako nebezpečný odpad.
(CZ) • Zamezte styku s kůží. Používejte vhodné ochranné rukavice. Tento materiál a jeho obal musí být zneškodněny jako nebezpečný odpad.
(RO) • A se evita contactul cu pielea. A se purta mănuşi corespunzătoare. Acest produs şi ambalajul său se vor depozita ca un deşeu periculos.
(BG) • Да се избягва контакт с кожата. Да се носят подходящи ръкавици. Този материал и неговата опаковка да се третират като опасен отпадък.

(LV) • Nepieļaut noklūšanu uz ādas. Strādāt aizsargcimdos. Apglabāt šo produktu un tās iepakojumu kā bīstamos atkritumus.

(MT) • Evita l-kuntatt mal-ġilda. Ilbes ingwanti adatt. Dan il-materjal u l-kontenitur tiegħu għandhom jintremew ma'skart perikoluż.

(NL) • Aanraking met de huid vermijden. Draag geschikte handschoenen. Deze stof en de verpakking als gevaarlijk afval afvoeren.

(SI) • Preprečiti stik s kožo. Nositi primerne zaščitne rokavice. Snov/pripravek in embalažo odstraniti kot nevarni odpadek.

(FI) • Varottava kemikaalin joutumista iholle. Käytettävä sopivia suojakäsineitä. Tämä aine ja sen pakkaus on käsiteltävä ongelmajätteenä.

Printed in France

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08/2011
881073