ELISA-Malaria antibody test

2 x 96 tests 750001

PLASMODIUM SPP. ANTIBODY DETECTION

Product-Identification: 46460

IVD For *In Vitro* Diagnostic Use

Manufacturer Quality Control

All manufactured and commercialised reagents are under complete quality system starting from reception of raw material to the final commercialisation of the product.
Each lot is submitted to a quality control and only is released on the market when conforming to the acceptance criteria.
The records relating to production and control of each single lot are kept within our company.

BIO-RAD

1 - INTRODUCTION

Malaria is a life-threatening disease caused by blood-borne protozoans, *Plasmodium spp.*, transmitted by Anopheles mosquitoes. However, it also could be acquired from a blood transfusion [1-3]. Four parasitic species infect humans: *Plasmodium falciparum*, *P. vivax, P. ovale* and *P. malariae*. *P. falciparum* is the deadly type of malaria infection and is the most frequent, along with *P. vivax*.

Malarial infection leads to the production of specific antibodies that can generally be detected a few days after the appearance of parasites in the blood. Specific antibody levels are proportional to the intensity and the duration of the infection. Antibody detection is more sensitive than direct detection of the parasite as it is independent of fluctuations in the level of parasitaemia over the short term and stays positive in cases of sub-patent parasitaemia. Specific antibody rapidly declines in primo-infected subjects following recovery from infection or slowly declines, during 2-3 years, for initially positive subjects moving to non-endemic regions.

There are four indications for performing serological investigation:
1 - screening of blood donors returning from malarial endemic areas,
2 - patients returning from the tropics with fever of unknown origin,
3 - patients with a suspicion of malarial “big spleen” disease,
4 - epidemiological field studies.

2 - PRINCIPLE OF THE ELISA PROCEDURE

ELISA-Malaria antibody test is a rapid and highly sensitive immuno-enzymatic assay for the detection of specific antibodies, IgG and IgM, to *Plasmodium spp.* [4]

Specific antibodies in sample material bind to antigens immobilized on a 96-well ELISA plate. Antigen used is a total extract from *P. falciparum* cultivation enriched with recombinant antigens from *P. vivax*, allowing detection of antibodies to *P. falciparum* and *P. vivax*. Due to antigenic similarity between *Plasmodium* species, *P. ovale* and *P. malariae* antibodies can be detected as well. Unbound material is removed during a washing step and peroxydase (HRP)-linked antibodies directed against human IgG and IgM are used in a second incubation step. Excess HRP-conjugate is washed off and final incubation with an HRP substrate solution based on tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) leads to a blue coloration directly correlated to the antibody concentration in the patient’s specimen. The enzymatic reaction is stopped by addition of diluted sulfuric acid and absorbance values are measured at 450 nm.

3 - REAGENTS

IVD **For professional use only!**

- **ELISA-Malaria antibody** test Microplate:
  2 x 96 wells microplates (12 breakable-8-wells-strips) coated with *Plasmodium* antigens.

- **ELISA-Malaria antibody test** Washing solution 10X:
  2 x 100 ml concentrated (10X) phosphate buffered washing solution.
  Preservative: ProClin™ 300 (<1.5%)

- **ELISA-Malaria antibody test** Diluent buffer:
  1 x 30 ml diluent buffer.
  Preservative: ProClin™ 200 (0.2%)

- **ELISA-Malaria antibody test** Conjugate solution:
  1 x 25 ml ready to-use yellow solution of horseradish peroxydase-conjugated monoclonal antibodies, anti-human IgG and IgM.

- **ELISA-Malaria antibody test** Substrate solution:
  1 x 25 ml tetramethylbenzidine substrate buffer and hydrogen peroxide combined in a safe, ready-to-use, one step, chromogen solution. Avoid exposure to light and heat.

- **ELISA-Malaria antibody test** Stopping solution:
  1 x 15 ml sulfuric acid 0,5 M solution.

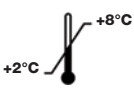
- **ELISA-Malaria antibody test** Positive/Negative controls:
  1 x 1 ml Positive and 2 x 1 ml Negative control vials containing human serum positive and negative for malaria antibodies, respectively.
  Preservative: ProClin™ 300 (<1.5%)

Caution: *the source materials from which these controls were manufactured were found non reactive for HBsAg, HCV and HIV1/2 when tested with licenced reagents. However, no known test method can assure that infectious agents are absent. Blood derivative products should be considered potentially infectious.*

- *Some reagents contain ProClin™ 200 (0.2%) or ProClin™ 300 (<1.5%)*

Xi Irritant
R43: may cause sensitisation by skin contact.
S23-24-37-60: Do not breathe vapour/spray. Avoid contact with skin. Wear suitable gloves. This material and its container must be disposed of as hazardous waste.

- *The Safety Data Sheet is available upon request.*



Stability: see expiry date on labels.

4 - FURTHER MATERIALS REQUIRED

- Micropipettes with disposable tips
- Clean standard laboratory volumetric glassware
- Microplate incubator
- Microplate reader with dual wavelength 450-620/630 nm spectrophotometer

5 - SAMPLE MATERIAL

Draw blood samples using acceptable phlebotomy techniques. Blood samples can be drawn into Citrate, EDTA, Heparin, CPD-A or plain tubes.
Either serum or plasma samples can be used.

6 - TEST PROCEDURE

Use a separate disposable tip for each transfer to avoid cross-contamination.
Allow samples and reagents to reach room temperature for 15 minutes before use.
Once the assay has been started, all steps should be completed without interruption.

Reconstitution of the Washing solution
Dilute 100 ml of concentrated Washing solution (10X) to 1000 ml with distilled water.
Caution: crystals may form when concentrated Washing solution is stored at 2-8°C. The crystals can easily be dissolved when bringing the vials to room temperature or placing them at 37°C for few minutes. The reconstituted washing solution can be stored at 2-8°C for one month.

Assay procedure

1. Once the microplate has reached room temperature, open the sealed bag and place the necessary numbers of wells and strips (including 4 wells for the controls) into the plate frame. Identify the position of the positive and negative control wells and the sample wells. Unused wells, replaced in the original bag and properly closed, may be stored at 2-8°C for one month.

2. Dispense **125 µl** Diluent buffer into each well.

3. Add **25 µl** of the Positive control to the Positive control well, **25 µl** each of the Negative control to the 3 Negative control wells. Return the controls to the refrigerator (2-8°C).

4. Add **25 µl** each of serum/plasma samples to the other wells.

5. Cover the wells and incubate for **60 minutes** at **37°C**.

6. Wash all wells with the reconstituted Washing solution with a suitable automated microplate washer or manually by briskly shaking out the contents of the wells over a suitable container and immediately refilling each well with **350-400 µl** Washing solution (for automated washers, use excess Washing solution and continuous overflow programme). For the first cycle, leave the Washing solution in the wells for **2 minutes** (soaking time). Repeat the wash cycle 5 times. No soaking time needed between the further wash cycles. After the last cycle, empty the wells and, especially for a manual procedure, remove excess fluid by gently tapping the inverted wells on a clean absorbent paper.

7. Add **100 µl** of Conjugate solution (HRP) to each well.

8. Cover the wells and incubate for **30 minutes at 37°C**.

9. Wash the wells 5 times as decribed in “Test procedure 6.”.

10. Add **100 µl** of Substrate solution into each well.

11. Cover the wells and incubate in the dark for **15 minutes at 37°C**.

12. Add **50 µl** of Stop solution into each well.

13. Within **15 minutes**, read absorbance of each well with a spectrophotometer at **450 nm** with reference wavelength at **620/630 nm**.

14. Record the results.

7 - INTERPRETATION OF THE RESULTS

Test validation
The individual value of the absorbance (optical density or OD) of the Positive control, ODpos, has to be above 0,500, and the average OD value of Negative controls, ODneg, below 0,200.

ODneg < 0,200 **and** ODpos > 0,500

If one of this specifications is not met, the results should not be considered valid and tests series should be repeated.

Results interpretation
ODneg is used to calculate the cut-off by multiplying this value by 4.

cut-off value = ODneg x 4

Calculate the Ab (antibody) Index of each determination by dividing the OD value of each sample by cutoff value.

Ab Index = ODsample/cut-off

A positive reaction corresponds to an Ab Index above or equal to 1,0 and negative reaction to an Ab Index below or equal to 0,8.
A “grey-zone” between 0,8 and 1,0 of Ab Index has to be considered because some samples can produce higher backgrounds than others.
This can mimic or mask a low reactivity. Sample inside this “grey zone” should be considered as doubtful and retested, best using a new fresh sample, in order to follow a possible ongoing generation of antibodies.

negative result: Ab Index < or = 0,8 **positive result:** Ab Index > or = 1,0 **doubtful result:** 0,8 < Ab Index < 1,0

A negative result indicates the absence of specific antibody to *Plasmodium spp.*, bearing in mind that a negative result does not exclude a starting primary infection. Where malarial infection is strongly suspected, a new sample may be retested one or two weeks later due to the serological window and appropriate measures for the detection of parasites (examination of blood films, detection of malarial antigen by OptiMAL-IT or ELISA-Malaria antigen test) should be undertaken without delay.

A Positive result indicates the presence of specific antibody to *Plasmodium spp.* The assay does not distinguish between *Plasmodium* species, nor IgG and IgM and between an acute and chronic infection.
Based on the clinical context, appropriate microscopic blood film examination and detection of specific plasmodial antigens should be considered.

Doubtful results should be retested. If the result remains doubtful, a new sample should be retested in order to clarify the serological status of the subject.

Important remark: where the assay is used for blood screening, positive and doubtful results should lead to the destruction of the blood bag and the definitive elimination of the blood donor has to be considered.

8 - PERFORMANCE

ELISA-Malaria antibody test exhibited a clinical sensitivity of 84,2% and a clinical specificity of 99,6%, compared with 70,5% and 99,6% respectively for Indirect Fluorescence Antibody test (IFAT) [4].

Positive results of ELISA-Malaria antibody test compared to IFAT for malaria-infected patients (95) and non-exposed healthy individuals (2152).

	ELISA-Malaria antibody test	IFAT
P. falciparum (66)	63	63
P. vivax (24)	18	6
P. ovale (2)	2	2
P. malariae (2)	2	2
P. falciparum + *P. malariae* (1)	1	1
Non-exposed blood donors (2152)	7	7

9 - LIMITATIONS

a) Contamination of the materials used can cause aberrant results.
b) Old, lipemic or hemolyzed samples may lead to erroneous results.
c) Strict adherence to the procedures and the prescribed equipment and material is essential. The equipment should be checked regularly according to GLP procedure.

10 - BIBLIOGRAPHY

1. Bruce-Chwatt LJ (1982). Transfusion malaria revisited. Trop Dis Bull 79:827-840.

2. Chiodini PL, Hartley S, Hewitt P, Barbara J, Lalloo K, Bligh J, Voller A (1997). Evaluation of a malaria antibody ELISA and its value in reducing potential wastage of red cell donations from blood donors exposed to malaria, with a note on a case of transfusion transmitted malaria. Vox Sang 73:143-148.

3. Dodd RY (1998). Transmission of parasites by blood transfusion. Vox Sanguinis 74:161-163.

4. Doderer C, Heschung A, Guntz P, Cazenave JP, Hansmann Y, Senegas A, Pfaff AW, Abdelrahman T, Candolfi E (2007). A new ELISA kit which uses a combination of *Plasmodium falciparum* extract and recombinant *Plasmodium vivax* antigens as an alternative to IFAT for detection of malaria antibodies. Malar J. 6:19.

11 - PRODUCTS

- **ELISA-Malaria antibody test** Kit
  2 x 96 tests . . . . . REF 750001
  (Id-n°: 46460)

- **ELISA-Malaria antibody test** Washing solution 10X
  10 x 100 ml . . . . . REF 750005
  (Id-n°: 43530)

These products are guaranteed to perform as described on the label and in the instruction sheet. The manufacturer declines all responsibility arising out of the use or sale of these products in any way or for any purpose other than those described therein.

(GB) - CE marking (European directive 98/79/CE on *in vitro* diagnostic medical devices)
(FR) - Marquage CE (Directive européenne 98/79/CE relative aux dispositifs médicaux de diagnostic *in vitro*)
(ES) - Marcado CE (Directiva europea 98/79/CE sobre productos sanitarios para diagnóstico *in vitro*)
(IT) - Marchiatura CE (Direttiva europea 98/79/CE relativa ai dispositivi medico-diagnostici *in vitro*)
(DE) - CE Konformitätskennzeichnung (Europäische Richtlinie 98/79/EG über *In-vitro*-Diagnostika)
(PT) - Marcação CE (Directiva europeia 98/79/CE relativa aos dispositivos médicos de diagnóstico *in vitro*)
(SE) - CE-märkning (Europeiskt direktiv 98/79/EG om medicintekniska produkter för *in vitro*-diagnostik)
(DK) - CE-mærkningen (Europa direktiv 98/79/EF om medicinsk udstyr til *in vitro*-diagnostik)
(GR) - Χαρακτηρισμος CE (ευρωπαικη οδηγια 98/79/CE περι *in vitro* διαγνωστικες ιατρικες συσκευες)
(PL) - CE oznaczenie (Dyrektywa unijna 98/79/CE dotycząca produktów medycznych do badań *in vitro*)
(LT) - CE ženklas (Europos sąjungos direktyva 98/79/CE dėl *in vitro* diagnostikos medicinos prietaisų)
(HU) - CE jelzés (98/79/CE Európai Irányelv az *in vitro* orvosi diagnosztikai eszközökről)
(EE) - CE märgistus (Euroopa direktiiv 98/79/CE *in vitro* diagnostikameditsiiniseadmete kohta)
(SK) - CE označenie o zhode (Európska direktíva 98/79/CE pre *in vitro* diagnostické zdravotnícke postupy)
(CZ) - CE značka (Evropská direktiva 98/79/CE o diagnostických zdravotnických prostředcích *in vitro*)
(NO) - CE-merking (EU-direktiv 98/79/CE om medisinsk utstyr til *in vitro*-diagnostikk)
(RO) - Marca CE (Directiva europeana 98/79/CE pentru dispozitive medicale de diagnostic *in vitro*)
(BG) - CE маркировка (Европейска директива 98/79/CE за *ин витро* диагностичните медицински изделия)

IVD

(GB) - For *in vitro* diagnostic use
(FR) - Pour diagnostic *in vitro*
(ES) - Para diagnóstico *in vitro*
(IT) - Per uso diagnostico *in vitro*
(DE) - *In-vitro*-Diagnostikum
(PT) - Para uso em diagnóstico *in vitro*
(SE) - *In vitro*-diagnostik
(DK) - *In vitro* diagnose
(GR) - Για *in vitro* διαγνωστικη χρηση
(PL) - Do stosowania *in vitro*
(LT) - *in vitro* diagnostikai
(HU) - Csak *in vitro* diagnosztikai alkalmazásra
(EE) - *In vitro* diagnostiliseks kasutamiseks
(SK) - Na diagnostiku *in vitro*
(CZ) - Pro diagnostiku *in vitro*
(NO) - Til *in vitro*-diagnostikk
(RO) - Pentru diagnostic *in vitro*
(BG) - За *ин витро* диагностика

REF

(GB) - Catalogue number
(FR) - Référence catalogue
(ES) - Número de catálogo
(IT) - Numero di catalogo
(DE) - Bestellnummer
(PT) - Número de catálogo
(SE) - Katalognummer
(DK) - Katalognummer
(GR) - Αριθμος καταλογου
(PL) - Numer katalogu
(LT) - Katalogo numeris
(HU) - Cikkszám
(EE) - Katalooginumber
(SK) - Katalógové číslo
(CZ) - Katalogové číslo
(NO) - Katalognummer
(RO) - Număr de catalog
(BG) - Каталожен номер

(GB) - Manufacturer
(FR) - Fabricant
(ES) - Fabricante
(IT) - Produttore
(DE) - Hersteller
(PT) - Fabricante
(SE) - Tillverkad av
(DK) - Fremstillet af
(GR) - Κατασκευαστης
(PL) - Producent
(LT) - Gamintojas
(HU) - Gyártó
(EE) - Tootja
(SK) - Výrobca
(CZ) - Výrobce
(NO) - Produsent
(RO) - Producător
(BG) - Производител

EC REP

(GB) - Authorised Representative
(FR) - Représentant agréé
(ES) - Representante autorizado
(IT) - Distributore autorizzato
(DE) - Bevollmächtigter
(PT) - Representante Autorizado
(SE) - Auktoriserad representant
(DK) - Autoriseret repræsentant
(GR) - Εξουσιοδοτημενος αντιπροσωπος
(PL) - Upoważniony Przedstawiciel
(LT) - Įgaliotasis atstovas
(HU) - Meghatalmazott Képviselő
(EE) - Volitatud esindaja
(SK) - Autorizovaný zástupca
(CZ) - Zplnomocněný zástupce
(NO) - Autorisert representant
(RO) - Reprezentant autorizat
(BG) - Упълномощен представител

LOT

(GB) - Batch code
(FR) - Code du lot
(ES) - Código de lote
(IT) - Codice del lotto
(DE) - Chargen-Bezeichnung
(PT) - Código do lote
(SE) - Batchnr
(DK) - Batchkoden
(GR) - Κωδικας παρτιδας
(PL) - Numer serii
(LT) - Serijos numeris
(HU) - Gyártási szám
(EE) - Partii kood
(SK) - Číslo šarže
(CZ) - Číslo šarže
(NO) - Partikode
(RO) - Număr de lot
(BG) - Партиден номер

(GB) - Expiry date YYYY/MM/DD
(FR) - Date de peremption AAAA/MM/JJ
(ES) - Estable hasta AAAA/MM/DD
(IT) - Da utilizzare prima del AAAA/MM/GG
(DE) - Verwendbar bis JJJJ/MM/TT
(PT) - Data de expiração AAAA/MM/DD
(SE) - Utgångsdatum ÅÅÅÅ/MM/DD
(DK) - Anvendes før ÅÅÅÅ/MM/DD
(GR) - Ημερομηνια ληξης YYYY/MM/DD
(PL) - Data ważności YYYY/MM/DD
(LT) - Galioja iki YYYY/MM/DD
(HU) - Szavatossági idő ÉÉÉÉ/HH/NN
(EE) - Aegumistähtaeg AAAA/KK/PP
(SK) - Použiteľné do RRRR/MM/DD
(CZ) - Datum exspirace RRRR/MM/DD
(NO) - Utløpsdato ÅÅÅÅ/MM/DD
(RO) - Data expirarii AAAA/LL/ZZ
(BG) - Срок на годност година/месец/ден

(GB) - Storage temperature limitation
(FR) - Limites de températures de stockage
(ES) - Temperatura límite
(IT) - Limiti di temperatura di conservazione
(DE) - Lagertemperatur
(PT) - Limites de temperatura de armazenamento
(SE) - Temperaturbegränsning
(DK) - Temperaturbegrænsning
(GR) - Περιορισμος θερμοκρασιας αποθηκευσης
(PL) - Temperatura przechowywania
(LT) - Saugojimo temperatūriniai apribojimai
(HU) - Tárolási hőmérsékleti határok
(EE) - Piirangud säilitustemperatuurile
(SK) - Skladovacia teplota od do
(CZ) - Teplotní rozmezí od do
(NO) - Oppbevaringstemperatur
(RO) - Limitele de temperatură la stocare
(BG) - Температурни граници на съхранение

(GB) - Consult Instruction for use
(FR) - Consulter le mode d'emploi
(ES) - Consulte las instrucciones de uso
(IT) - Consultare le istruzioni per uso
(DE) - Siehe Gebrauchsanweisung
(PT) - Consulte o folheto informativo
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(DK) - Se instruktion før brug
(GR) - Συμβουλευθειτε τις οδηγιες χρησης
(PL) - Sprawdź instrukcję
(LT) - Ieškokite informacijos vartojimo instrukcijoje
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