DRG® Malaria ELISA (EIA-4263)

Revised 29 July 2008 (Vers. 3.0) **RUO in the USA**

1 CLINICAL BACKGROUND

Malaria is one of the most common diseases in the world. More than half the world population lives in malaria-infected areas. Over 200 million cases annually result in up to 3 million deaths each year; a majority of which are in young children. In non-endemic areas, it is one of the most important imported diseases, resulting in a number of deaths in late-diagnosed or unsuspected cases each year.

The disease is caused by protozoa of the genus *Plasmodium*, transmitted by the bite of the female *Anopheles* mosquito. There are four species causing human malaria: *P. falciparum, P. vivax, P. malariae,* and *P. ovale*. The disease may also be transmitted by transfusion of infected blood. Once in the blood the sporozoite makes its way to the liver where for the next 2 weeks merozoites are produced. These are released into the blood where they invade the red cells and produce more merozoites, causing the cells to rupture. It is this rupturing that is responsible for the clinical symptoms.

Of the four species, *P. falciparum* is the most common and the most virulent, causing most malaria-related deaths. *P. vivax* is the next most common cause of malaria. Although rarely fatal, this form of malaria can be accompanied by severe clinical symptoms. It is a common cause of malaria in S.E. Asia and S. America.

People infected with *Plasmodium* spp. form antibodies in response.

This Malaria ELISA kit is designed to detect antibodies occurring in subjects infected with *P.falciparum, P.vivax, P.ovale* and *P.malariae*

2 INTENDED USE

These kits are intended for use by appropriately trained and qualified personnel for the detection of antibodies to *P.falciparum, P.vivax, P.ovale* and *P.malariae* in human serum and plasma.

3 PRINCIPLE OF THE TEST

The Malaria ELISA use four recombinant antigens in a sandwich test to produce a test that is both highly specific and sensitive. The antigens will detect *P.falciparum, P.vivax, P.ovale* and *P.malariae* -specific IgG, IgM, and IgA; enabling the test to detect antibodies during all stages of infection. All reagents except the Conjugate and Wash solution are supplied ready to use and colour-coded, and the procedure uses undiluted samples and standard volumes for ease of both manual and automated use. The assay can be used with both serum and plasma.

The plastic wells are coated with a mixture of *P. falciparum* and *P. vivax* recombinant antigens. The antigenic similarity between *Plasmodium* species means that antibodies to all species can be detected. Specific antibodies in serum or plasma specimens combine with these antigens and with the same antigens conjugated to horseradish peroxidase, when conjugate is added to a well in which the specimen has been incubated. After unreacted material has been removed by washing, the presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a colour change in the substrate/chromogen mixture. The intensity of the colour is compared to that in control wells to determine the presence or absence of specific antibody.

4 KIT CONTENTS

R1	**Plate** (96 wells in 12 strips of 8),	Polystyrene coated with recombinant antigens		1 (96 tests)
R2	**Positive control**	Human serum	Red	1.5 mL
R3	**Negative control**	Human serum	Yellow	2.0 mL
R4	**Conjugate**	Recombinant antigens conjugated to horseradish peroxidase	Purple	0.8 mL
R5	**Conjugate dilution buffer**	Buffered saline containing surfactant and stabilisers	Green	8 mL
R6	**Substrate**	Urea peroxide and tetramethyl benzidine	Pink	7 mL
R7	**Wash,** (20 x concentrated)	Saline containing surfactant	Colourless	125 mL
R8	**Stop**	0.5M H_2SO_4	Colourless	7 mL
	Bag for storing unused wells.			
	Directions for use			

5 WARNINGS AND PRECAUTIONS

For *in - vitro* use only.

The control materials supplied are derived from human serum. They have been tested at donor level and found negative for Hepatitis B and C, and for HIV 1 and 2. **However, they should be treated as if capable of transmitting disease**.

Specimens of human serum and plasma should be treated as microbiologically hazardous, and handled in accordance with the applicable regulations.

Do not use the kit after its expiry date.

Do not combine or interchange reagents from kits with different lot numbers.

6 STORAGE

Store at 2 – 8 °C when not in use. Store bottles upright.

Do not freeze.

Do not expose substrate to direct sunlight.

Diluted conjugate is stable for 4 weeks at 4 °C

Diluted wash buffer is stable for 4 weeks at 4 °C

Unused coated strips are stable for 4weeks at 4 °C if stored in the re-sealable bag provided.

7 EQUIPMENT REQUIRED

Properly calibrated and maintained pipetting devices capable of delivering volumes of 50 microlitres (specimens and reagents) and approx 300 microlitres (wash fluids).

Plate or strip reader to read at 450 nm and (optionally) at a wavelength between 620 and 690 nm.

37 °C incubator

The Malaria ELISA may be automated for both liquid handling and result interpretation. A variety of systems have been used for this – please consult the manufacturers of both the kit and the automation system for advice on automation.

Equipment should be able to support the following tolerances:

Volume dispensed	+/- 10%
Incubation temperature	+/- 2 °C
Incubation time	+/- 2 minutes.

8 SPECIMENS

Serum or plasma (collected into EDTA, sodium citrate, or heparin) specimens should be free of blood cells and of obvious microbial contamination.

They may be stored at 2-8 °C for up to 7 days before testing. Specimens needing longer storage should be frozen at –20 °C or lower.

Frozen specimens should be thawed and well mixed before testing.

9 ASSAY PROTOCOL (MANUAL)

Bring all reagents and specimens to room temperature prior to use.

Dilute Wash Buffer 1 in 20 with distilled or deionised water prior to use.

Assay controls

The Negative control must be tested three times with each lot of tests, and the Positive control twice.

Verification of Specimen addition

Verification is by detecting photometrically the difference between an empty well and a well containing serum or plasma at a wavelength of 450 nm. Wells containing specimen will have an A_{450} reading of between 0.050 and 1.000.

Procedural notes

Washing must be thorough, with complete filling and emptying of the wells at each cycle

Procedure

1. Add **50 µL of the undiluted sample** (or control – see "Assay Controls" above) to a coated well.
   Mix on a plate shaker for 30 seconds.
   Incubate (covered) at 37° C for 30 minutes.

2. **Wash** 5 x with working strength wash buffer. A short soak time of about 30 seconds is recommended between each wash cycle. Tap out excess liquid.

3. **Conjugate Incubation**
   Dilute conjugate **1 + 10** in Conjugate Buffer. (50 µL + 500 µL per 10 wells)
   Add **50 µL diluted conjugate** to each well.
   Incubate (covered) at 37° C for 30 minutes.
   Addition of conjugate is verified by reading at 450/ nm. A well with conjugate added must have an OD greater than 0.2

4. **Wash** 5 x with working strength wash buffer. A short soak time of about 30 seconds is recommended between each wash cycle. Tap out excess liquid.

5. **Substrate Incubation**
   Add **50 µL** substrate/chromogen mixture to each well.
   Incubate at room temperature for 30 minutes.
   There is a clear difference of colour between an empty well and a well containing substrate.
   Addition of substrate is verified by reading at 550 nm. A well with substrate added must have an A_{550} of greater than 0.080
   As the substrate is photosensitive, it is recommended that the plate be protected from light during this incubation.

6. **Stop Colour Development**
   Add **50 µL** 0.5M sulphuric acid to each well. (Blue colour changes to yellow).

7. **Read Results**
   Read at 450 nm (A_{450}) Use of a reference filter at 620 – 690 nm will eliminate effects of scratches, bubbles, etc

10 CUT-OFF VALUE

Calculated as the mean of the negative control values plus 0.100

i.e. $\frac{\text{Negative Control 1 + NC2 + NC3}}{3} + 0.100$

Example: $\frac{0.030 + 0.025 + 0.035}{3} = 0.030$

Cut-Off Value = 0.030 + 0.100 = 0.130

11 ASSAY VALIDATION

A_{450} of each Negative Control should be lower or equal to 0.080 If one control is above this value the reading should be ignored and the cut–off calculated using the remaining two.

A_{450} of each Positive Control should be greater than or equal to 1.000

12 INTERPRETATION

Samples with an A_{450} value less than the Cut-off value are considered ***negative*** by Malaria EIA.

Samples just below the Cut-off (C.O. –10% A_{450}) should however, be interpreted with caution.

It is advisable to ***retest*** the corresponding samples in duplicate when the systems and laboratory procedures permit.

Re-tested samples that are above the cut-off in at least one duplicate are considered ***positive*** and should be investigated further.

Samples that are below the cut-off in both duplicates are considered to be negative.

13 PERFORMANCE CHARACTERISTICS

Specificity

External data from 13,608 donor samples deemed at risk to malaria infection gave 96.21% specificity.
(95% confidence limits 93.63% – 98.79%)

Sensitivity

External data for 76 acute *P.falciparum* cases showed 92.5%
(95% confidence limits 90.5% - 94.5%)

External data for 258 IFAT ≥ 80 for *P.falciparum* showed 94.4%
(95% confidence limits 92.44% - 96.38%)

Internal data for *P.vivax* *showed* 100%
(95% confidence limits 97.63% - 100%)

Only small numbers of samples from *P.ovale* and *P.malariae* infections have been studied. Sensitivity for these was 80% and 67% respectively. Numbers were too small to allow meaningful statistical analysis. These figures will be updated as more samples from these infections are tested

Precision

Specimen No.	No. of replicates	Mean A_{450}-A_{620}	Intra-assay CV (%)	Inter-assay CV (%)
1	16	2.402	2.28	3.78
2	16	1.316	3.83	5.17
3	16	0.672	3.83	5.52
4	16	0.353	4.06	6.15
5	16	0.195	3.19	6.16
6 (Negative)	16	0.046	6.95	6.84

14 BIBLIOGRAPHY

1. Kitchen A.D. et al. Evaluation of a malarial antibody assay for use in the screening of blood and tissue products for clinical use. Vox Sanguinis (2004) **87**, 150 - 155
2. Seed C.R. et al. The efficacy of a malarial antibody enzyme immunoassay for establishing the reinstatement status of blood donors potentially exposed to malaria. Vox Sanguinis (2005) **88**, 98 – 106
3. Kitchen A.D. et al. Tranfusion transmitted malaria: current donor selection guidelines are not sufficient. Vox Sanguinis (2005) **88**, 200 – 201

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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..