PAN MALARIA ANTIBODY CELISA

INTENDED USE AND PRINCIPLE OF THE TEST

The Pan Malaria Antibody CELISA is for the detection of specific IgG antibody against *P. falciparum, P. vivax, P. malariae* and *P. ovale in human serum or plasma samples.* The indirect or sandwich ELISA principle is used. Microwells are coated with a panel of recombinant *malaria* antigens. A conjugate of enzyme labelled anti-human globulin is incorporated into the kit. Diluted serum or plasma samples are added to the coated wells, which are then incubated to allow antibodies present to bind to the antigen mix. Other serum components are then removed by a wash step. The conjugate is then added, binding to any antibody fixed to the well. The well is washed and enzyme substrate solution is added. The amount of colour generated is proportional to the amount of malarial antibodies present in the serum under test.

This assay is for the purpose of defining an infection status. Not for use in Australia as a screening assay for the detection of malaria antibodies in blood or organ donations.

CONTENTS OF THE KIT

MBCMW	Celisa Plate - 1x 96 wells - (single use only)	2 plates
CONTROL +	Positive Control	0.10 mL
CONTROL -	Negative Control	0.10 mL
MBCPO	Enzyme Conjugate (200x)	0.15 mL
MBCPT	PBS/Tween (20x)	125 mL
MBCSC	Substrate Chromogen (20x)	1.2 mL
MBCSB	Substrate Buffer	24 mL
MBCSS	Stopping Solution	12 mL

Store all components at 2-8^{O}C.Expiry dates are clearly marked on each kit component and on the box. Expiry dates do not change once opened.

MATERIALS REQUIRED BUT NOT PROVIDED

Micropipettes and tips, clean glassware or plastic containers for solutions, distilled water, humid chamber, ELISA washer, Spectrophotometer to read absorbances at a single wavelength of 450nm, or at dual wavelengths of 450nm and 620nm.

PRECAUTIONS

For in vitro diagnostic use only. Reagents should not be used after the expiry date shown on the label. If protective packaging is damaged, contact your local distributor and ask for a replacement. Do not mix reagents from different kits. Thimerosal preservative added to some components is a poison. Exercise caution when handling these components. The stopping solution is corrosive. Avoid contact with skin, eyes and mucous membranes. Dispense all reagents with care to avoid cross contamination of wells. Avoid exposure of the substrate to light. Treat all clinical and control material as though potentially infectious and dispose of in accordance with local operating regulations. For further information, please refer to the Material Safety Data Sheet.

INSTRUCTIONS FOR USE

Preparation of Wash Buffer

If crystals are present in the concentrate, warm to dissolve. For each microplate, add 50mL PBS-Tween concentrate MBCPT to 950mL of distilled water. Label the bottle WASH BUFFER. Store at 2-8^{o}C. Use WASH BUFFER to dilute samples, conjugate concentrate MBCPO and washing the plates.

Preparation of samples

Fresh, refrigerated or frozen samples of serum or plasma may be used. Avoid contamination by collecting aseptically. Prepare the samples by making a 1/100 dilution of the CONTROL +, the CONTROL - and the test (patient specimen) samples in WASH BUFFER, ensuring proper mixing.

Assay Procedure

1. Bring all reagents to room temperature (18-25 ^{o}C) before use.
2. Prepare WASH BUFFER (see Preparation of Wash Buffer), diluted CONTROL +, diluted CONTROL - and diluted sample (see Preparation of samples).
3. Remove required number of MBCMW strips. Reseal the foil bag containing unused microwell strips immediately with tape.
4. Pipette 100µL of diluted CONTROL +, diluted CONTROL - and DILUTED SAMPLE into individual microwells. Include two positive and two negative controls in each assay run. Cover and incubate for one (1) hour at room temperature (RT) (18^{o}C – 25^{o}C) in a humid chamber.
5. In the last 10 minutes of the incubation period, prepare the working strength CONJUGATE. Add 5µL of Enzyme Conjugate MBCPO to 995µL of WASH BUFFER and mix thoroughly (allow 1mL per strip of 8 wells).
6. Wash the wells preferably using an automatic plate/strip washer or manually as follows:
   -Empty contents from the wells. Refill with the WASH BUFFER.
   -Repeat this process a further three (3) times. Shake out well contents at the end of the fourth wash.
   -NB: take care when flicking out plates, hold side of frame firmly to hold strips in place.
7. Add 100µL of CONJUGATE to each well. Incubate for one (1) hour at room temperature (RT) in a humid chamber.
8. In the last 10 minutes of the incubation period, prepare the working strength SUBSTRATE. Add 50µL of Substrate Chromogen MBCSC to 950µL of Substrate Buffer MBCSB and mix thoroughly (allow 1 mL per strip of 8 wells).The stability of the solution is 30 minutes.
9. Repeat washing as in step 6.
10. Add 100µL of fresh SUBSTRATE and incubate in the dark (covered) at room temperature for 15 minutes.
11. Add 50µL of Stopping Solution MBCSS. Tap the plate to mix.
12. Read the results visually or in a spectrophotometer at 450nm, or 450nm/620nm, blanking the machine on air.

READING AND INTERPRETATION OF RESULTS AND DIAGNOSIS

Visually

Observe the colour intensity of the control and specimen wells. The Positive Control should be blue before, and yellow after stopping.

Photometrically

Read the microwell plate at 450nm or 450nm / 620nm in a compatible ELISA plate reader, blanked against air. For the test results to be accepted the controls must read as follows:

	O.D Value (450nm)	O.D Value (450/620nm)
Positive Control	>1.500 OD	>1.500 OD
Negative Control	<0.250 OD	<0.200
Cut-Off level (COV)	= Negative Control OD + 0.1	

If controls do not satisfy above criteria, repeat the test.

Negative samples should give optical density readings below 0.250 OD units at 450nm or below 0.200 OD units at 450/620nm. The COV calculation shown above is based on internal studies of pools of normal serum from non-endemic malaria countries with a mean OD +3SD of less than OD 0.2. The addition of +0.1 OD takes into consideration those equivocal samples. To allow for inter-laboratory variation we strongly recommend that each laboratory run a number of known local negative blood samples to increase

assay accuracy. COV can be calculated by using the mean OD of negative samples + 3 standard deviations (+3SD). Those specimens giving absorbance values below the COV are regarded as negative i.e. do not contain amounts of antibody measurable by this test. Those specimens giving absorbance values above the COV may contain antibody and are generally considered to be at or above the significant level. Serum samples that give values above the COV should be considered as positive for malaria antibody. This suggests that the donor has or has had malaria. It does not imply in any sense that the donor is carrying malaria parasites at this particular time.

WASTE DISPOSAL

Dispose of any unused components as biohazardous waste. For more information, please refer to the MSDS.

DATA ON THE PAN MALARIA ANTIBODY CELISA

Refer to summary table at end of insert. All data on the Pan Malaria Antibody CELISA can be obtained in the product information sheet. Please ask your local distributor or contact Cellabs.

INDEMNITY NOTICE

Modifications or changes made in the recommended procedure may affect the stated or implied claims. A positive or negative result does not preclude the presence of other underlying causative agents. Cellabs and its agents and distributors shall not be liable for damages under these circumstances

FIGURE 1 PAN MALARIA DIAGRAM FOR USE

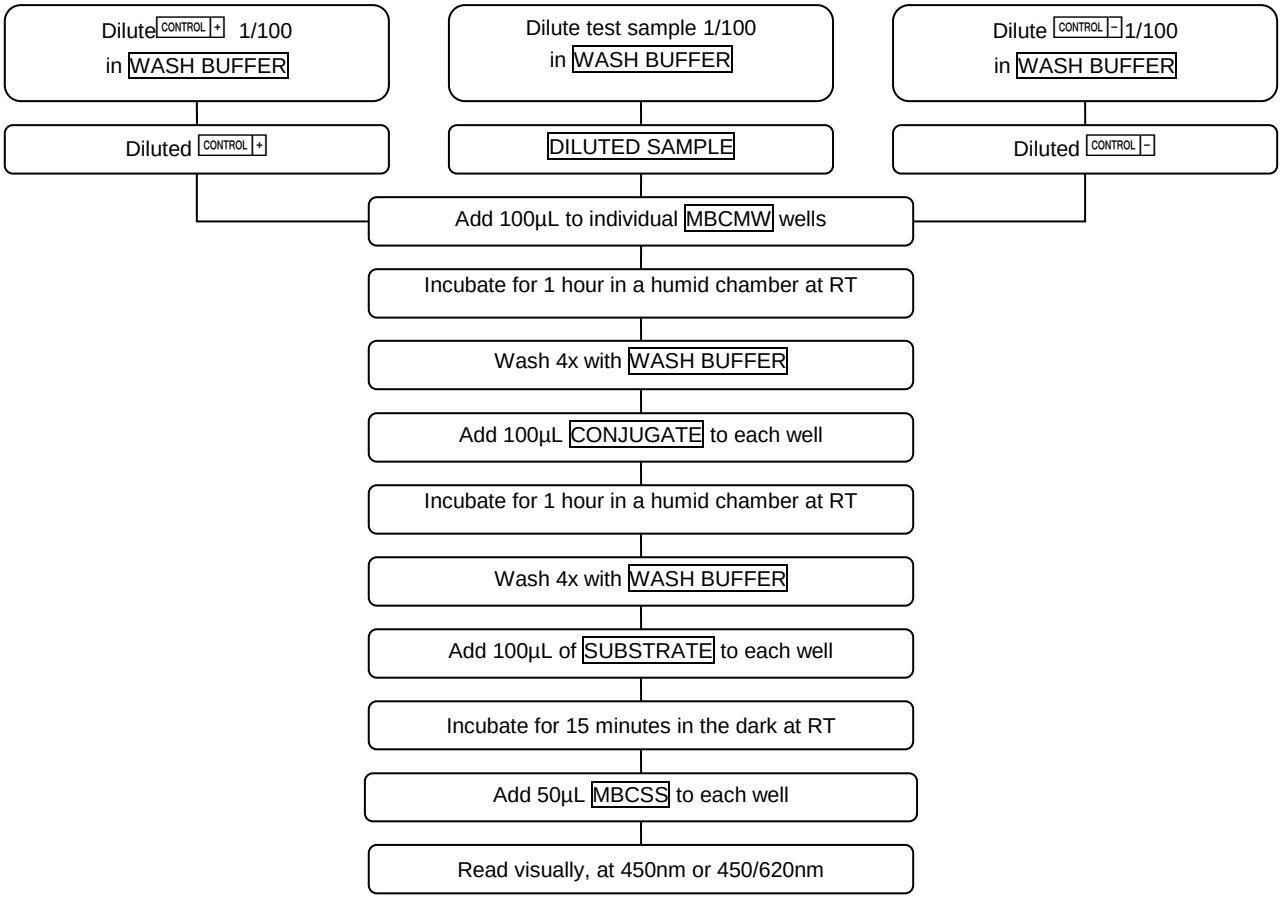

TABLE 1: SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE PAN MALARIA CELISA

Trial	Sensitivity	Specificity	Repeatability	Reproducibility
A	94%	100%	-	-
B	-	-	Positive CV = 2.75%	Positive CV = 5.81%
	Not cross reactive with Toxocara sp., *T. cruzi*, Leishmania sp., *W. bancrofti*, Dengue virus.			

EXPLANATION OF SYMBOLS

Consult Instructions for Use

In Vitro Diagnostic Medical Device (IVD)

Temperature Limitation (2℃ – 8℃)

Batch (LOT)

Control Positive (CONTROL +)

Control Negative (CONTROL -)

Use By/Expiration Date

Do Not Re-use

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