Lymphatic Filariasis Bm14 Antibody CELISA

BACKGROUND

Lymphatic filariasis (LF), commonly known as elephantiasis is one of the oldest diseases known to man. LF is caused by a microscopic, thread-like parasitic nematode worm that is transmitted through the bite of a mosquito vector. LF has a very wide spectrum of manifestations from lymphatic damage without any apparent symptoms, through to chronic and acute forms. The condition is the fourth leading cause of disability worldwide, affecting the health and wellbeing of those who are affected and impacting on the socioeconomic status of endemic communities. The three filarial species infecting man are *Wuchereria bancrofti,* accounting for 90% of disease worldwide*, Brugia malayi*, accounting for 9%, found in Asia and the Pacific and, *Brugia timori* accounting for 1%, found in the islands of *Indonesia*. There are 1.39 billion people at risk in 73 endemic countries worldwide with 120 million infected. The disease has been targeted for elimination by the year 2020 through preventive chemotherapy (PCT), a WHO elimination programme supported by the Global Programme to Eliminate Lymphatic Filariasis (GPELF).

The Bm14 Antibody CELISA is based on the recombinant antigen Bm14, a highly specific antigen that targets both bancroftian and brugian filariasis. It cannot be used in areas where co-infection with Onchocercariasis exists due to cross-reactivity. The Bm14 Antibody CELISA is a reliable diagnostic test for the exposure to LF, it is useful in assessing the progress of preventive chemotherapy (PCT) programmes that assist programme managers in determining the end-point for stopping PCT. When used in transmission assessment surveys (TAS), the Bm14 Antibody CELISA is a suitable diagnostic tool for the surveillance of transmission. The sensitivity of antibody detection tests can provide an early indication of ongoing transmission; this sensitivity cannot be achieved by using antigen detection alone due to the changes of transmission rates in a community where antigen is at a significantly lower rate after the implementation of PCT programmes. The Bm14 Antibody CELISA can be used as part of a comprehensive diagnostic approach for transmission assessment programmes.

INTENDED USE AND PRINCIPLE OF THE TEST

The Bm14 Antibody CELISA is an indirect ELISA for the detection of exposure to LF infection. The test is based on the recombinant antigen Bm14, specific for detecting antibodies to both, bancroftian and brugian filariasis. The test can be used with serum, plasma and blood spot eluates, collected on filter paper in the field.

The test is based on the principles of ELISA. The recombinant antigen is coated on the inner surface of each microwell that binds the antibody present in a sample. An enzyme peroxidase-conjugated antibody to human IgG4 is added to form a binding complex. A chromogenic substrate is added to develop the reaction, turning a well with a positive sample to a blue colour. The colour intensity is directly proportional to the amount of antibody contained in the sample. The addition of a stopping solution changes the blue colour to yellow spectrophotometer reading at 450nm single wavelength or 450/620nm dual wavelength.

CONTENTS OF THE KIT

FAMW	CELISA Plate – 1x96 wells - (single use only)	5 plates
CONTROL +	Positive Control	0.05mL
CONTROL -	Negative Control	0.05mL
FASD	Sample Diluent (10x)	60mL
FAPO	Enzyme Conjugate (100x)	0.6mL
FACD	Conjugate Diluent	60 mL
FAPT	PBS/Tween (20x)	250mL
FASC	Substrate Chromogen (TMB) (20x)	3.0mL
FASB	Substrate Buffer	60mL
FASS	Stopping Solution	30mL

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, humid chamber, ELISA washer, and spectrophotometer to read absorbances at a wavelength of 450nm or dual wavelength of 450/620nm.

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. Exercise caution when handling these components and appropriate protective clothing. 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 Safety Data Sheet.

INSTRUCTIONS FOR USE

Preparation of Wash Buffer

If crystals are present, warm the concentrate to dissolve. For each microplate, add 50mL PBS-Tween concentrate FAPT to 950mL of distilled water. Label the bottle WASH BUFFER. Store at 2-8^{o}C.

Preparation of Sample Diluent

Prepare the working Sample Diluent FASD (1x) by diluting the 10x Concentrate in distilled water. Mix the buffer thoroughly before using to dilute test samples.

Collection and Preparation of Samples

Serum or Plasma

Collect patient blood samples by standard venepuncture procedure. The test may be used with serum or plasma. The serum or plasma should be stored below -10^{o}C if the analysis is delayed. Prepare a 1:100 dilution of the positive control CONTROL +, the negative control CONTROL - and the test samples using the prepared Sample Diluent FASD (1x) above, ensuring proper mixing. Record the position of each diluted SAMPLE on a work sheet.

Blood Spot Eluates

The WHO format Tropbio filter paper disks (Tropbio Cat.No. FP 05-002-12) available from Cellabs Pty Ltd is the standard method for finger prick blood collection. The Bm14 Antibody CELISA has been optimised for use with the Tropbio filter paper disks method. Using different filter paper for blood collection is not recommended and may give incorrect results due to different thickness and different sample elution capacities. Collect blood following the method as described in the filter paper product insert. Each filter paper protrusion absorbs ~10uL of blood. Ensure that filter paper blood spots are thoroughly dried, a minimum of two hours but preferably overnight. Storage is very important if not testing the next day. Store dry filter paper blood spots in individual plastic snap-lock bags. Place individual plastic bags in a large foil pouch with silica gel sachets and seal for short-term storage and transportation up to two weeks. For longer storage more than 4 weeks, store at -20^{o}C up to 3 months.

Prepare to elute the filter paper blood spot samples by carefully removing one circle and completely immersing in a tube of 500uL of prepared working Sample Diluent FASD (1x). Leave the samples refrigerated at 2-8^{o}C overnight. The following day, vortex the contents of the tube to ensure complete elution of sample. The eluate is a 1:50 dilution and approximately equivalent to a 1:100 serum dilution for direct testing in the ELISA.

Assay Procedure

1. Bring all reagents to room temperature (18-25°C) before use.
2. Prepare WASH BUFFER (see Preparation of Wash Buffer).
3. Prepare the working Sample Diluent FASD (1x) (See Preparation of Sample Diluent).
4. Remove required number of FAMW strips. Reseal the foil bag containing unused microwell strips immediately with tape.
5. Pipette 100µL of the test SAMPLE, CONTROL + and CONTROL -, into individual microwells. Include two positive and two negatives in each assay run. Cover and incubate for one (1) hour at 37°C in a humid chamber. (see Preparation of Samples using serum, plasma or blood spot from filter paper).
6. In the last 10 minutes of the incubation period, prepare the working strength CONJUGATE. Add 10µL of Enzyme Conjugate FAPO to 990µL of FACD and mix thoroughly (allow 1mL per strip of 8 wells).
7. 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. After the fourth wash, bang inverted wells dry on absorbent tissue.
   - NB: take care when flicking out plates, hold side of frame firmly to hold strips in place.
8. Add 100µL of CONJUGATE to each well. Incubate for 30 minutes at 37°C in a humid chamber.
9. In the last 10 minutes of the incubation period, prepare the working strength SUBSTRATE. Add 50µL of Substrate Chromogen FASC to 950µL of Substrate Buffer FASB and mix thoroughly (allow 1mL per strip of 8 wells). The stability of the solution is 30 minutes.
10. Repeat washing as in step 7.
11. Add 100µL of fresh SUBSTRATE and incubate in the dark (covered) at room temperature for 15 minutes.
12. Add 50µL of Stopping Solution FASS. Tap the plate to mix.
13. Read the results visually or in a spectrophotometer at dual wavelength 450/620 nm. *Single wavelength 450nm can also be used but note that 450nm will give a slightly higher reading. Use the appropriate OD values for interpretation of results.*

READING AND INTERPRETATION OF RESULTS AND DIAGNOSIS

Photometrically

For the test results to be accepted the controls must read as follows:

Control	OD Value (450/620nm)	OD Value (450nm)
Serum/Plasma samples		
Negative Control	< 0.20	<0.25
Positive Control	> 2.5	>2.5
Cut-off value	0.25	0.35
Filter paper blood eluates		
Negative Control	<0.25	<0.30
Positive Control	>2.5	>2.5
Cut-off value	0.3	0.35

Note: The cut-off value is based on 140 non-endemic serum samples. A cut-off value can be determined for each assessment unit by testing a panel of known negative samples. Calculate the cut-off value (COV) by: COV = mean + 3 Standard Deviations (+3SD). A pilot study conducted in- house with 50 blood spot eluates from Australian residents without prior exposure to LF gave a cut-off of 0.25 OD units (mean +3SD).

Visual Reading

Results can also be read visually. Note colour of the specimen wells and compare with the colour of the negative control. If the colour, read visually, of a specimen well is above that of the negative control, then the specimen contains antibody.

WASTE DISPOSAL

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

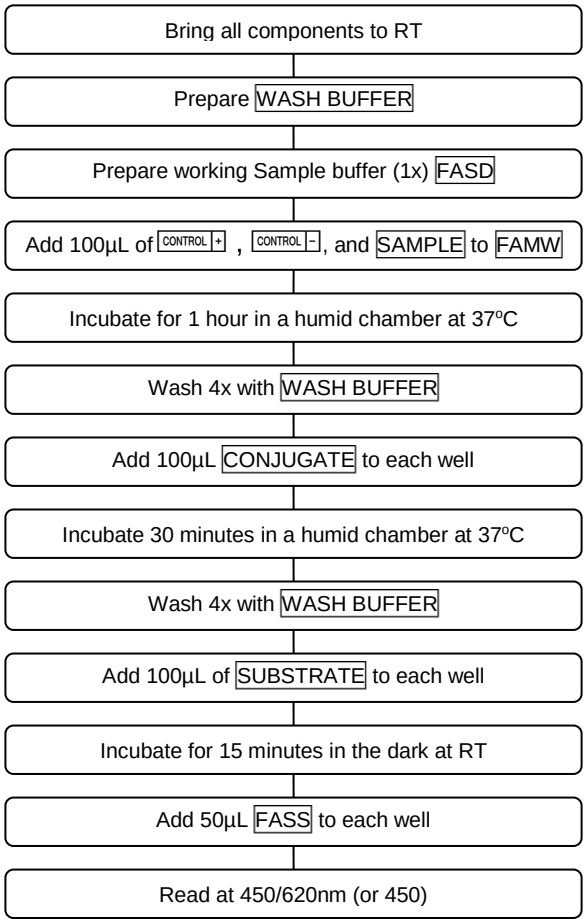
SENSITIVITY, SPECIFICITY, & OTHER DATA ON THE BM14 ANTIBODY CELISA KIT

Data on the Bm14 Antibody CELISA test performance can be obtained from your local distributor or by contacting 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 Lymphatic Filariasis Bm14 Antibody CELISA DIAGRAM FOR USE



EXPLANATION OF SYMBOLS
EXPLICATION DES SYMBOLES

	Consult Instructions for Use Consultez le mode d'emploi
IVD	*In Vitro* Diagnostic Medical Device Diagnostic medical *in-vitro*
2°C 8°C	Temperature Limitation Limites de temperature
LOT	Batch Lot
CONTROL +	Control Positive Contrôle positif
CONTROL -	Control Negative Contrôle négatif
	Use By/Expiration Date Date de péremption
	Do Not Re-use Ne pas ré-utiliser

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