



Development of prototype ELISA and RDT sentinel assays for monitoring exposure to lymphatic filaria infection.

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16 Pacific Countries

Background

The Global Alliance to Eliminate Lymphatic Filariasis (GAELF) was formed in 2000 to facilitate the control and eradication of lymphatic filariasis. A programme of mass drug administration (MDA) was instigated as an effective way of eliminating the disease and protecting more than 1 billion people who are at risk in more than 80 countries. Post-MDA surveillance of LF infection is essential for confirming the interruption of transmission. Detection of filaria specific IgG4 antibody is a reliable diagnostic marker for LF infection^{1,2,4}. We present data on two prototype diagnostic devices based on Bm14³ a recombinant LF antigen. It is hoped that these devices will prove useful as sentinel assays for surveillance of LF following the MDA.

Materials and Methods

Development of Bm14 recombinant antigen based IgG4 filaria antibody detection devices:

LF recombinant antigens were kindly provided by Dr Gary Weil of Washington University School of Medicine St Louis USA. Antigens expressed in two different vectors (-GST and -HIS) were evaluated for sensitivity and specificity. The Bm14-HIS antigen was found to be highly specific and by using this antigen two prototype antibody detection devices were developed at Cellabs.

- (1) IgG4 ELISA for quantifying exposure
- (2) RDT assay for initial field screening

Antibody detection ELISA: How was it developed?

- (1) Two forms of Bm14 (-GST and -HIS) recombinant antigens were initially evaluated.
- (2) Frozen serum samples from Egypt, Sri Lanka, PNG (provided by **Dr Gary Weil**) were used in the evaluation.
- (3) Blood samples collected by using Whatman filter paper discs (TropBio; **Fig 1**) were also used.
- (4) In addition a panel of defined sera samples was provided by **Dr Wayne Melrose** for validating the assay.
- (5) Based on the above, a prototype Filaria Bm14-HIS ELISA was optimised (**Fig 2**) for detection of LF specific IgG4 antibodies in blood and serum samples.

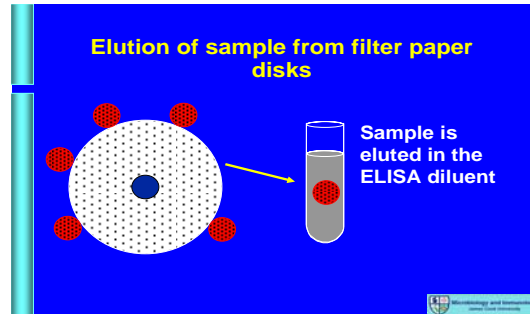


Fig 1: Blood collection discs. Each filter disc absorbs 10 uL blood (~5uL sera). To obtain 1:100 sample dilution, each disc is eluted with 500uL sample diluent.

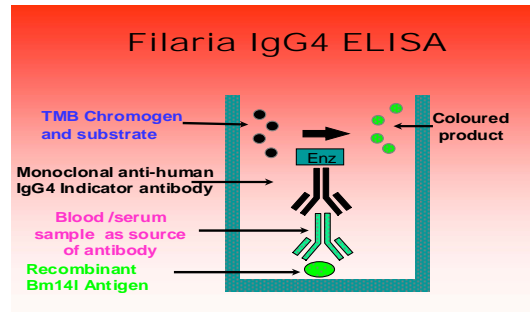
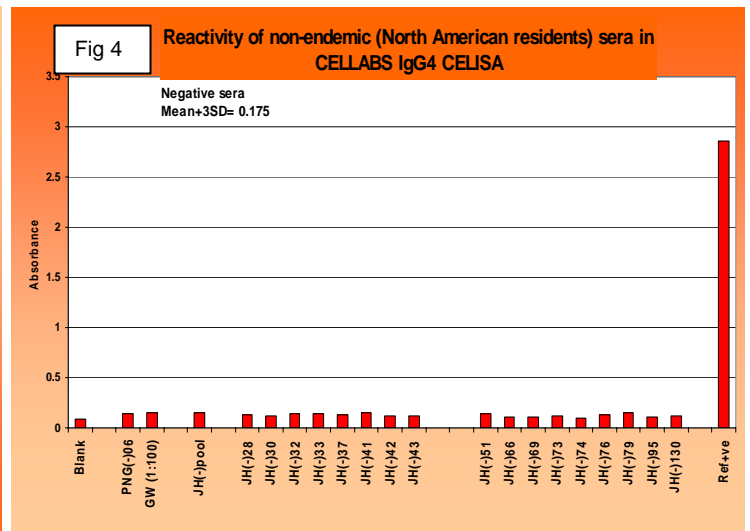
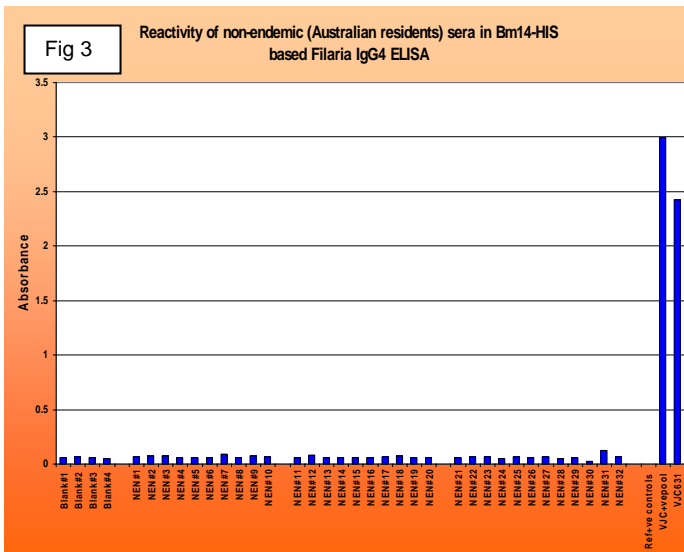


Fig 2. Indirect antibody detection ELISA



Observation:

(3) Serum samples from Egypt, Sri Lanka and PNG were tested in the Prototype (Bm14-HIS) ELISA and compared with the results of the original GW Bm14-GST assay. Absorbance values (Fig 5) and S/N ratio (Fig 6) were compared. A point to note that most of PNG samples tested were from Mf negative subjects and these sera gave weak or borderline results in GW original assay.

(4) The Bm14-HIS prototype assay generated lower BG and higher S/N ratios than the original assay and was found to be reliable for detecting filaria specific circulating IgG4 antibodies in the samples.

Fig 6 Comparative absorbance values (GW original Bm14-GST versus Celllabs Bm14-HIS)

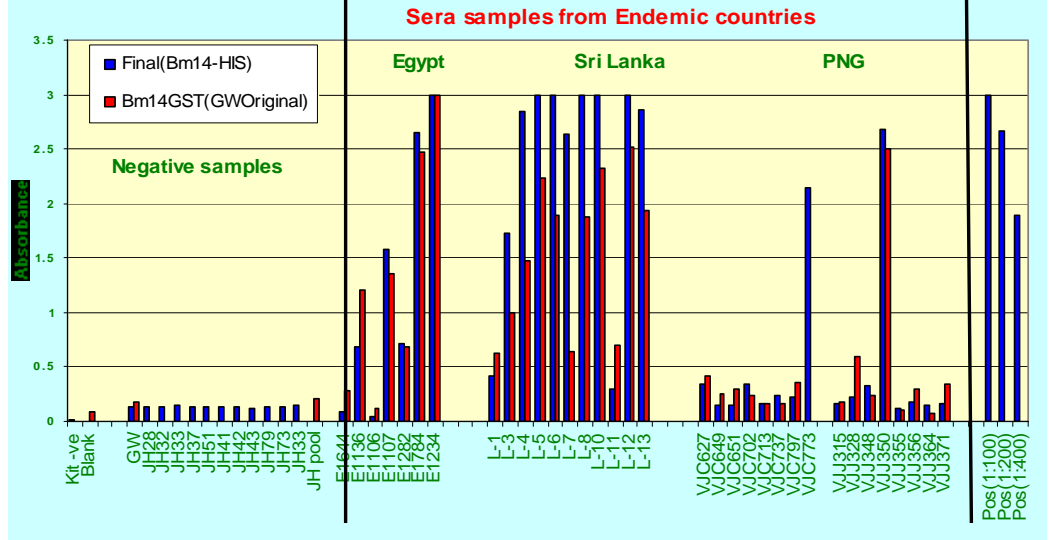


Fig 6 S/N Ratio Comparison (GW original Vs Celllabs)

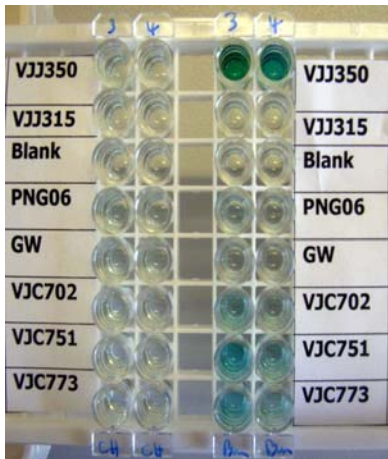
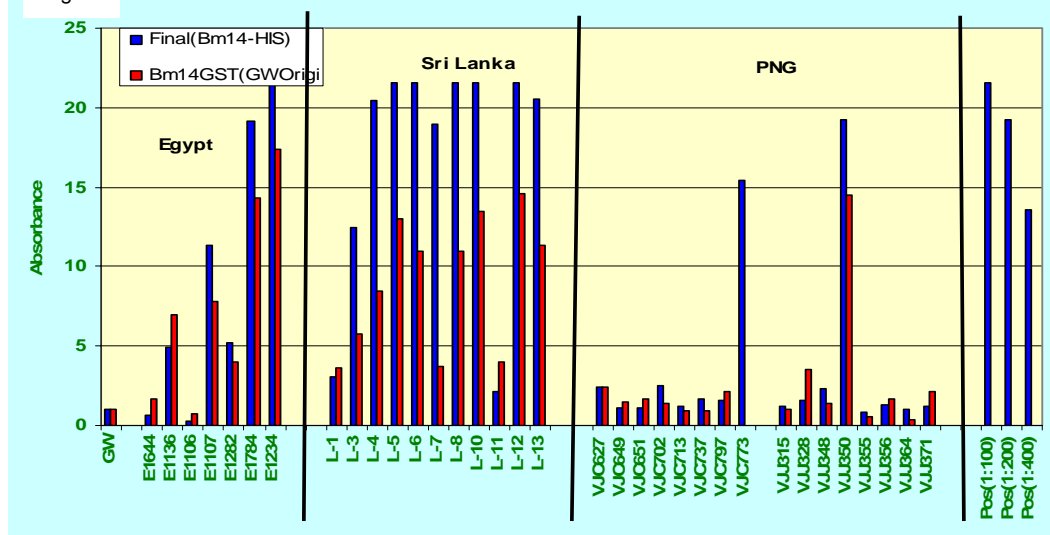


Fig 7. Visual IgG4 CELISA

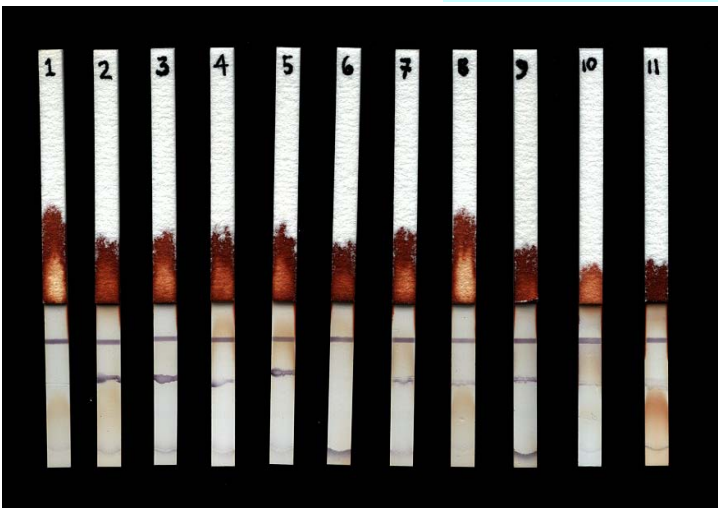


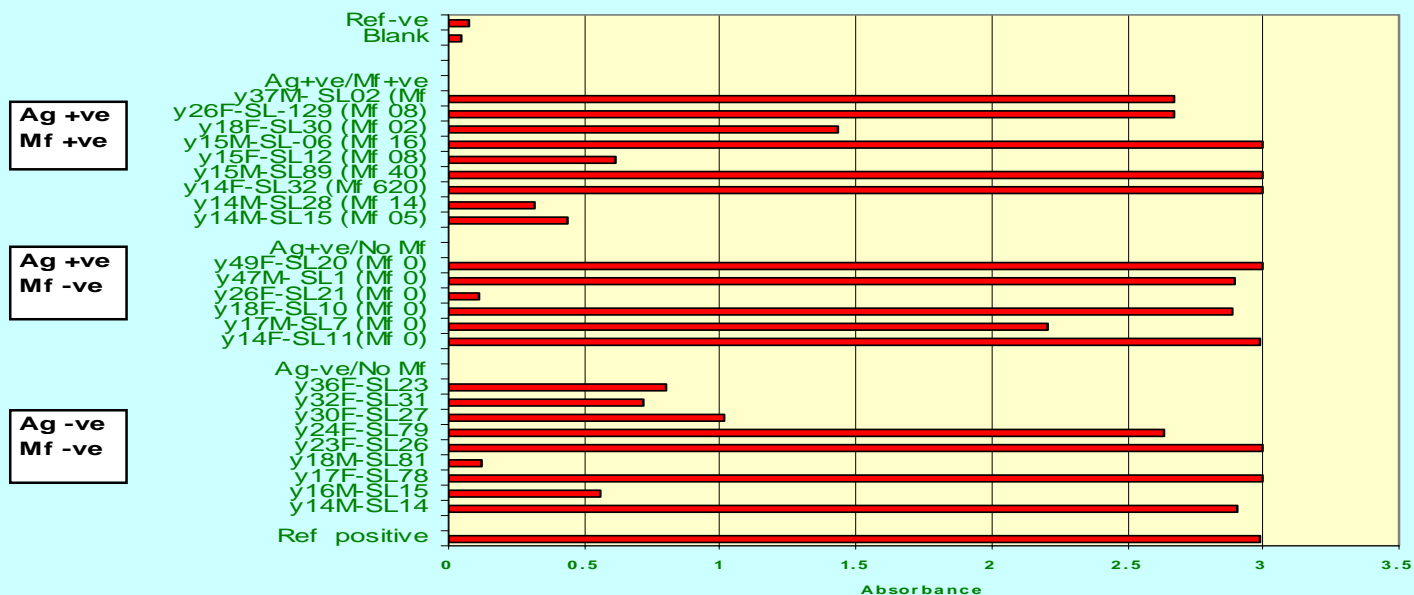
Fig 8. Bm14 – RDT Assay Celllabs has also developed an immunochromatography – based lateral flow dipstick assay. Fig 8 shows the results of n = 11 blood samples tested by the prototype assay.

Sample	Bm14-GST based ELISA (GW)	Bm14-HIS Based CELISA	Bm14-HIS based RDT (Attached figure)
PNG-01	0.011	0.068	Negative
PNG-02	0.8	2.206	Positive(4+)
PNG-03	1.99	2.353	Positive(3+)
PNG-04	1.79	0.479	Positive (2-3+)
PNG-05	2.42	2.999	Positive (2-3+)
PNG-06	0.012	0.068	Negative
PNG-07	0.582	0.54	Positive (1+)
PNG-08	0.33	0.401	Faint
PNG-09	0.76	0.441	Positive (2+)
PNG-10	0.68	0.696	vFaint
PNG-11 (Blood only)	ND	ND	Positive (1+)

Table 1. Comparative CELISA values and RDT results. A good correlation exists between RDT and CELISA

Fig 9

IgG4 antibody level seen in defined LF-endemic sera samples

**Features of IgG4 CELISA:**

- Recombinant antigen based assay.
- Assay can be performed with blood/ sera samples
- Uses monoclonal anti-human IgG4¹ as the marker antibody. Highly specific to filaria IgG4 antibody.
- Assay format: 96 well plate, required reagents supplied for performing the assay.
- Assay time 2 hours. Assay is calorimetric/ qualitative/and quantifiable). Results can be checked visual (Fig 7).
- Manufactured under GMP accreditation.

The Filaria IgG4 ELISA can be used in field support laboratories in two formats:

1. A visual ELISA requiring minimal instrumentation (Fig 7).
2. A quantitative ELISA requiring a photometer

Features of Bm14-RDT

- Suitable for screening LF exposed individuals under field conditions.
- It requires 10 µl whole blood, serum or plasma.
- Results obtained within 15 minutes.
- Individually foil wrapped.
- Cellabs Filaria IgG4 RDT assay can be used directly under field conditions (Fig 8).

Summary:

1. Cellabs Pty Ltd has developed Filaria IgG4 CELISA and Filaria RDT assays based on the BM14-HIS recombinant antigen system.
2. The prototype assays showed promising results and both ELISA and RDTs may be suitable for filaria surveillance following MDA.
3. The BM14-HIS ELISA and RDTs are currently undergoing final evaluation.

- **Bm14 assay validation for identifying LF exposed individuals**

A panel of defined samples supplied by Dr Melrose was evaluated in this assay (Fig 9). The assay was further validated for its sensitivity in detecting Filaria specific IgG4 in exposed individuals who were not positive either for antigen or for microfilariae. In other words, the prototype filaria IgG4 CELISA can reliably identify individuals exposed to infection in absence of any other confirmatory evidence.

- **How could such an assay be applied for post MDA surveillance?**

It has been proposed that primary school children would be the ideal sentinels for exposure as they should have no detectable antibody if transmission has been successfully interrupted.

- **Would Bm14 assays be suitable for post MDA surveillance?**

We have demonstrated that the Bm14 assay can detect LF exposure in endemic residents who were negative for parasites. Based on this, the Bm14 assay could be useful as a tool for post-MDA surveillance.

- **What further evaluation is required for these diagnostic assays prior to use as sentinel assays during post MDA surveillance?**

The Bm14 prototype assays need to be tested for their ability to detect antibody levels in primary school children in different countries where the MDA programme is underway.

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