EVALUATION OF SD BIOLINE ONCHO/LF IGG4 BIPLEX AND SD BIOLINE LYMPHATIC FILARIASIS IGG4 SCREENING TOOLS IN FILARIAL-ENDEMIC REGIONS OF CAMEROON

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INTRODUCTION

Lymphatic filariasis (LF) is a disfiguring disease caused primarily by *Wuchereria bancrofti* and can be co-endemic with the filarial parasites *Loa loa*, *Mansonella perstans*, and *Onchocerca volvulus* in central Africa. Commercial antigen detection tests for *W. bancrofti* (Wb) may cross-react with sera from these individuals, particularly those heavily infected with *L. loa*.

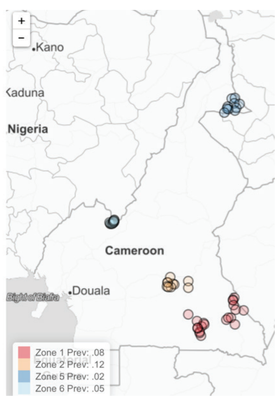
Two new IgG4 rapid diagnostic tests (RDTs) incorporating Wb123 are available from Standard Diagnostics, Inc.: the SD BIOLINE LF IgG4 rapid test (Wb123) and the SD BIOLINE Oncho/LF IgG4 biplex rapid test (Ov16/Wb123). The aim of this research was to determine the specificity of the Wb123 RDTs in co-endemic zones.

METHODS

- Fingerstick blood samples from 5,001 individuals were evaluated by the Wb123 RDT, Ov16/Wb123 RDT (30 minutes and 24 hours result), Alere™ Filariasis Test Strip, microscopy to detect *Mansonella* and *Loa* microfilariae, and Wb123 enzyme-linked immunosorbent assay (ELISA) using fingerstick-derived dried blood spots (DBS), collected in CeLLabs' TropBio filter paper.
- Wb-positive rapid test results were confirmed by night-blood microscopy and quantitative polymerase chain reaction (qPCR) to detect *W. bancrofti*.
- The Inbios International, Inc. Filaria *Detect*™ anti-Wb123 human IgG4 ELISA was used according to the manufacturer's protocol and the following:
  - Each 6 mm DBS was eluted in 250 µL of sample diluent overnight at 4°C and run in duplicate wells.
  - Kit controls were diluted 50-fold and run in duplicate wells on each plate.
  - ELISA result classified positive if normalized absorbance greater than threshold found by expectation maximization, an iterative technique to find the best fit of two variable Gaussian distributions to normalized ELISA data.
- The ELISA dataset of normalized ELISA values (sample avg./plate low positive control avg) comprised all samples that passed the quality criteria:
  - Plate passed if controls met product insert criteria AND the low-positive plate control duplicate well difference was <0.125 absorbance units.
  - Sample passed if both duplicate absorbance values were <0.2, or duplicate well absorbance percent difference <37% (difference value/duplicate well average).

If DBS available, ELISA was repeated if initial results failed criteria. Repeated ELISA results were used to replace the initial results if passed criteria; otherwise samples were excluded from analysis.

Figure 1. Map of the 50 villages in 4 regions of Cameroon that were included in this cross-sectional study. Seropositivity for each zone (Prev) determined by Wb123 ELISA.



RESULTS

Figure 2. Wb123 seropositivity by age groups, measured by ELISA.

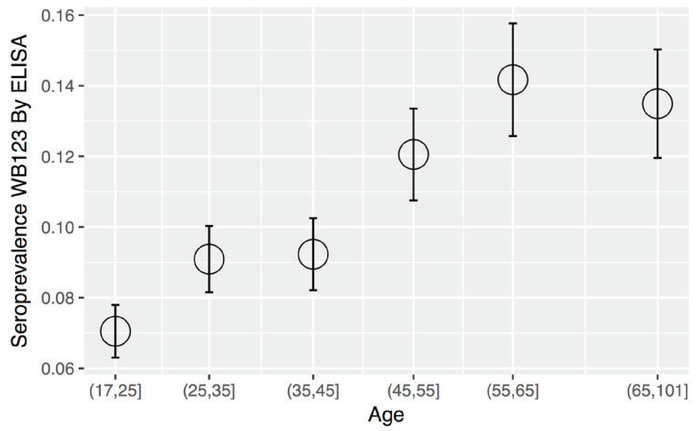
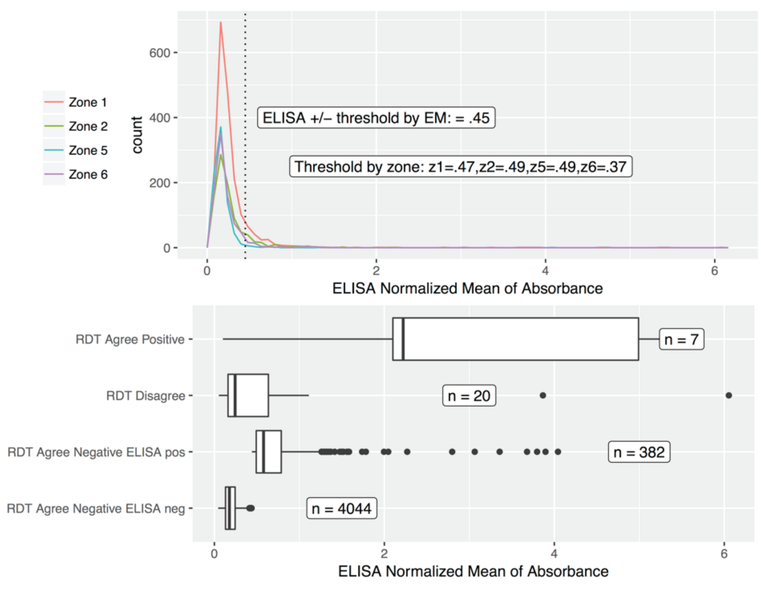


Figure 3. (Top) Distribution of normalized Wb123 ELISA values with thresholds identified in total set and by zone. (Bottom) Distribution of ELISA values for different categories of RDT-positivity. RDT Agree Positive: both LF and biplex RDT results were Wb123-positive at all time points tested. RDT Disagree: discordance between LF and biplex and/or different time point Wb123 results. RDT Agree Negative: both LF and biplex results were Wb123-negative at all time points.



Abbreviation: EM, expectation maximization.

Table 1. Description of Wb123 rapid tests and ELISA results.

Wb123 results	LF rapid test	Oncho/LF rapid test	ELISA
Negative at 30 minutes and 24 hours	4,970	4,968	4,077
Positive at 30 minutes and 24 hours	9	18	396
Positive at either time point	8	6	n/a
Proportion positive among valid results[1]	0.34% (17/4,996)	0.48% (24/4,998)	8.85% (396/4,473)
Invalid or missing result	5	3	528

[1]Rapid test positive if positive at any time point.

Table 2. Specificity of LF rapid tests compared to Wb123 ELISA, by filarial co-infection.

Percentage specificity compared to ELISA[1]	Wb123 line on LF rapid test[1] (n=4,996)	Wb123 line on Oncho/LF rapid test[1] (n=4,998)	Filariasis Test Strip[2] (n=4,945)
Overall	99.8 (99.6-99.9)	99.8 (99.6-99.9)	99.0 (98.6-99.3)
***L. Loa*[2]**			
No *L. loa* (3,930)	99.9 (99.7-100)	99.8 (99.6-99.9)	99.7 (99.5-99.9)
Low *L. loa* (929)	99.9 (99.7-100)	99.7 (98.8-100)	99.0 (97.9-99.6)
High *L. loa* (141)	98.6 (92.6-100)	100	71.1 (60.1-80.5)
***Mansonella*[2]**			
No *Mansonella* (4,787)	99.9 (99.7-100)	99.8 (99.6-99.9)	99.2 (98.8-99.4)
Some *Mansonella* (213)	99.9 (99.7-100)	99.3 (96.2-100)	95.2 (90.7-97.9)

[1]Rapid test positive if any time point is positive.

[2]A total of 881 *Loa* infections (high *L. loa* ≥8,000 mf/mL), 24 *Mansonella* infections, and 189 *Loa* and *Mansonella* co-infections.

CONCLUSIONS

- Specificity of the Wb123 RDTs was >99% against ELISA, and no cross-reactivity with *L. Loa* or *Mansonella* was detected.
- The proportion positive by either Wb123 RDT was less than 1% and corresponding microscopy and qPCR results were Wb-negative.
- The proportion categorized as positive by Wb123 ELISA was 8.85%.
- Wb123 rapid test result conversion and reversion and/or result discordance between biplex and LF RDTs may be associated with lower anti-Wb123 antibody quantities as determined by ELISA.
- Distribution of normalized Wb123 ELISA values varied by region, and did not display clear bimodal positive and negative populations.
- Determining an ELISA-positive cutoff in the absence of a significant detectable positive population raises a challenge in determining a clinically meaningful level of anti-Wb123 antibodies.