ANALYTICAL AND DIAGNOSTIC PERFORMANCE OF A HIGH-SENSITIVITY HISTIDINE-RICH PROTEIN 2 ELISA FOR DETECTION OF *PLASMODIUM FALCIPARUM* MALARIA

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INTRODUCTION

- In low transmission settings, individuals with submicroscopic *Plasmodium falciparum (Pf)* infections remain undetectable by current histidine-rich protein 2 (HRP2)-based rapid diagnostic tests (RDTs) and enzyme-linked immunosorbent assays (ELISAs).
- Detection and treatment of this reservoir population is essential. It would be beneficial to have a highly sensitive laboratory reference assay to confirm *Pf* infections.
- Recently, Alere™ developed a high-sensitivity Malaria Ag P.f ELISA (HS ELISA).

The aim of this study was to characterize the analytical and diagnostic performance of the HS ELISA.

METHODS AND MATERIALS

- Alere ™ high-sensitivity Malaria Ag P.f ELISA (HS ELISA)
- Analytical sensitivity and specificity
  - *Pf* recombinant GST-W2 HRP2: 5-800 pg/mL.
  - *Pf* native culture strains: 0.01-2000 p/µL.
    - ITG, W2, 3D7: hrp2+, hrp3+
    - Dd2, D10: hrp2-, hrp3+
    - HB3: hrp2+, hrp3-
    - 3BD5: hrp2-, hrp3-
    - Normalized absorbance ratio: absorbance value of each sample/average absorbance of calibrator in same plate.
  - All specimen types and concentrations were tested in duplicate.
- Diagnostic sensitivity and specificity
  - Frozen whole blood specimens from asymptomatic study participants in Karen Village (TOT), Myanmar (April-May 2015) and Nagongera District, Uganda (April-August 2015).
  - 100 *Pf*-positive from Uganda and 200 *Pf*-negative from Myanmar.
    - Positive or Negative for *Pf* by both microscopy and quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

RESULTS

- HS ELISA reliably detected 100% of *Pf* recombinant GST-W2 HRP2 concentrations >25 pg/mL (Figure 1), and all negative specimens were non-reactive (data not shown).
- Normalized absorbance ratios (Table 1)
  - Highest for wild-type (ITG, W2, 3D7) and *hrp3* deletion (HB3) strains: 31.8-40.1 (0.01-0.90 p/µL).
  - Lowest for *hrp2* deletion strains (Dd2, D10): 1.5-2.9 (74.1 p/µL).
  - Non-reactive against *hrp2* and *hrp3* double deletion (3BD5) strain at all parasitemias.
- Clinical specimens from Myanmar and Uganda by HS ELISA: 104 specimens were positive, 173 specimens were negative, and 23 specimens were discordant (Table 2).
  - Of the discordant specimens, 5 lacked the volume for retesting and were removed from sensitivity and specificity measurements. The remaining 18 specimens were negative after repeat testing.
  - Sensitivity (95% CI): 100% (96.4-100)
    Specificity (95% CI): 97.9% (94.8-99.4)

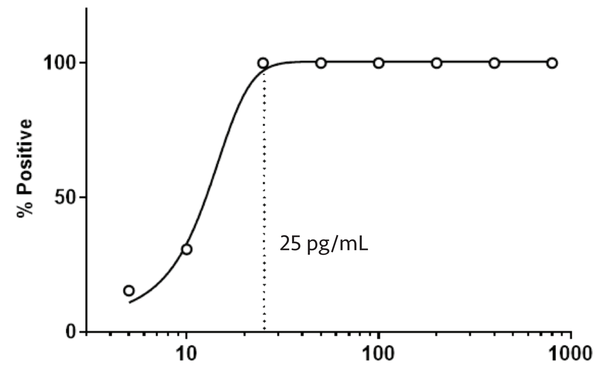
Table 1. Normalized HS ELISA absorbance ratios of *Pf* native culture strains across parasitemias.

	Normalized absorbance ratio						
Parasites/ µL	ITG	W2	3D7	HB3	Dd2	D10	3DB5
2000	43.9	44.1	40.1	31.8	40.1	31.8	0.8
666.7	44.1	44.1	40.1	31.8	32.3	23.5	0.8
222.2	44.0	44.0	40.1	31.8	10.0	7.3	0.8
74.1	32.1	37.3	40.1	31.8	2.9	1.5	0.8
24.7	16.0	19.2	40.2	28.9	1.0	0.7	0.8
8.2	6.2	8.0	31.3	13.7	0.8	0.6	0.8
2.7	2.5	3.4	17.0	6.0	0.9		0.9
0.9	1.5	1.9	7.5	3.0	0.8		0.8
0.3	0.9	1.2	3.5	1.6			
0.1	0.8	0.9	2.3	1.0			
0.03	0.8	0.9	1.5	0.8			
0.01	0.7	0.9	1.3	0.5			

Table 2. HS ELISA diagnostic performance against clinical whole blood specimens from Myanmar and Uganda.

		Microscopy and qRT-PCR	
		Positive	**Negative**
Alere™ high-sensitivity Malaria Ag *P.f* ELISA	**Positive**	100	4
	Negative	0	191
	Total	100	195

Figure 1. Analytical sensitivity of HS ELISA using Pf recombinant GST-W2 HRP2.



CONCLUSION

- The HS ELISA was able to detect low levels of HRP2 and parasitemia against *Pf* recombinant and native culture strains respectively.
- Reactivity to *Pf* native culture strains varied and showed high specificity for HRP2.
- Excellent sensitivity and specificity against clinical specimens that were *Pf*-positive or -negative by both qRT-PCR and microscopy.
- Similar workflow to current commercial ELISAs and therefore can be easily implementable.
- HS ELISA may be useful in research-use only settings for assessing new diagnostics, epidemiological and drug sensitivity studies due to its lower limit of detection for *Pf*.
- Future work
  - Parallel testing of HS ELISA and current commercial ELISAs to further define performance.
  - Field studies to demonstrate the utility of the HS ELISA in malaria control and elimination.
  - Development of next-generation ELISA to address *pfhrp2/pfhrp3* deletion strains.

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