1585

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NEW MULTIPLEX ASSAY FOR ASSESSING *PLASMODIUM VIVAX* AND *PLASMODIUM FALCIPARUM* INFECTION

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INTRODUCTION

Asymptomatic infection in many endemic areas presents new challenges for malaria prevention and control strategies as the individuals with asymptomatic infection constituting the human parasite reservoirs contribute to active transmission.[1] For a successful elimination strategy, it is crucial that asymptomatic and submicroscopic reservoirs of *Plasmodium* species be identified and treated for targeted intervention. Here we sought to develop a highly sensitive tool to detect *P. falciparum* and *P. vivax* infection.

MATERIALS AND METHODS

Clinical studies[2]

Induced blood-stage malaria challenge study, Queensland, Australia.

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Enrollment of human volunteer.

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Inoculation of 3D7 *P. falciparum*.

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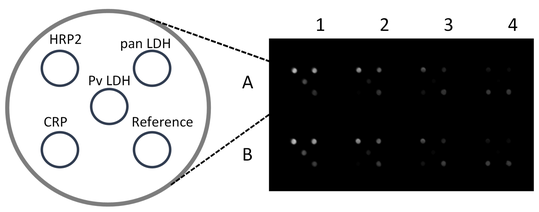
Collection of blood during D0-D11. Drug treatment on D7.

Q-Plex multiplex assay

(Quansys Bioscience, Utah, USA) was utilized to quantify:

- Histidine-rich protein 2 (HRP2).
- Pan-specific lactate dehydrogenase (Pan LDH).
- *P. vivax*-specific LDH (Pv LDH).
- C-reactive protein (CRP).

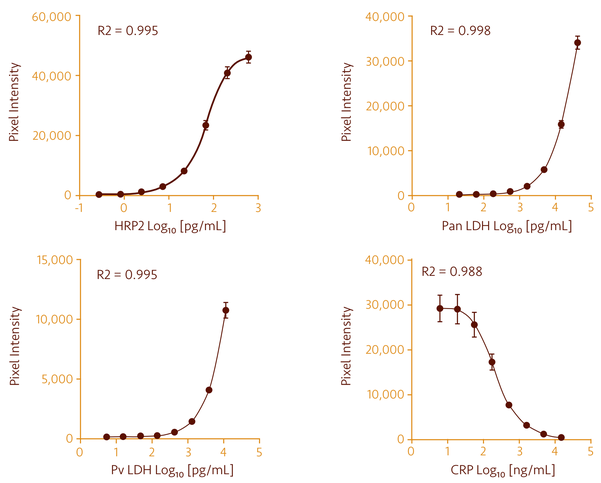


Data analysis was performed using a five-parameter logistic curve fit.

RESULTS: VALIDATION

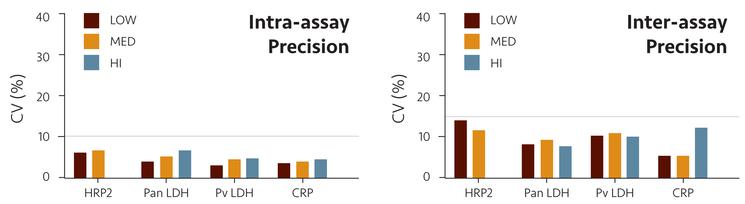
CALIBRATOR CURVES

- The calibration curves (n = 5) showed good linearity and acceptable results of the back-calculated concentrations (80-120%; data not shown).



PRECISION

- In intra-assay precision and inter-assay precision analyses, the assay coefficient of variation (CV) was in acceptable range, <10% and <15% , respectively.



RECOVERY

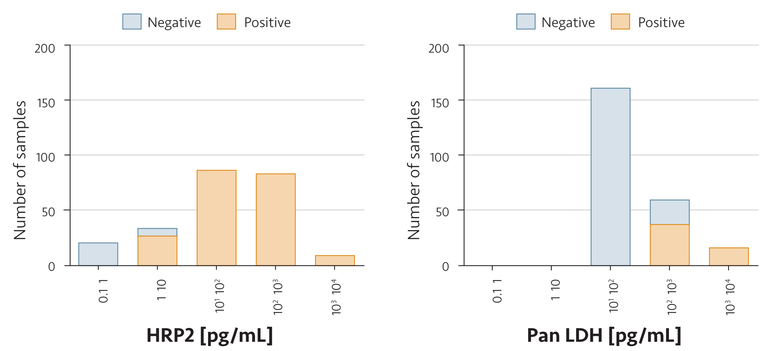
- As % recovery for analytes in some samples were not within acceptable range (80-120%), there may be interference in quantification

Spike-in (n=5)	Analyte	Mean Concentration	Avg. % Recovery	Range (%)
Recombinant proteins	HRP2	135.1 pg/mL	110.2	89.9-126
	Pan LDH	19,215 pg/mL	94.7	84.4-112.9
	Pv LDH	19,509.9 pg/mL	109.9	65.3-131.9
	CRP	NA	NA	NA
***P. falciparum* ITG strain parasitized red blood cells**	HRP2	102.0 pg/mL	89.7	77.3-101.3
	Pan LDH	355.5 pg/mL	109.2	98.5-122.8
	Pv LDH	<LLOQ	NA	NA
	CRP	NA	NA	NA

RESULTS: CLINICAL STUDIES

SPECIFICITY SENSITIVITY

- Cut-off: 2.3 pg/mL for HRP2, and 204.5 pg/mL for Pan LDH, calculated by Receiver Operating Characteristic analysis with a data set from Myanmar and Uganda clinical tests (data not shown).



- In comparison to HRP2, sensitivity for Pan LDH was poor.

D0-D7		qRT-PCR	
		Positive	**Negative**
Q-Plex	**Positive**	85[$]	2[%]
	Negative	7	17
	Total	92	19

Sensitivity = 92.4% (96.9%, 85.0%)
Specificity = 89.5% (98.7%, 66.9%)

[$] [%] *A positive signal on any one of HRP2 or Pan LDH detection elements from confirmed positive and negative samples is considered as true positive and false positive, respectively.*

[%] *Two false positive samples were on day 4.*

DISCUSSION AND CONCLUSIONS

- This multiplex system is a new tool for assessing *P. falciparum* and *P. vivax* infection.
- This system allows screening of multiple targets at low concentration in the same sample, resulting in significant savings in cost and time.
- Future work will include the development of an assay for non-HRP2, non-LDH-based detection of *Plasmodium* species to increase the screening potential of the assay.

REFERENCES

1. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. *Nature Reviews Microbiology.* 2014;12(12):833-840. doi:10.1038/nrmicro3364.

2. Das S, Jang I, Barney B, Peck R, Rek JC et al. Performance of a high-sensitivity rapid diagnostic test for Plasmodium falciparum malaria in asymptomatic individuals from Uganda and Myanmar and naïve human challenge infections. *The American Journal of Tropical Medicine and Hygiene.* 2017 Epub ahead of print]. doi:10.4269/ajtmh.17-0245.

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