P0216

Detection and quantification of *Plasmodium falciparum* and *Plasmodium vivax* in a human challenge model using the Karius® Test, a cell-free DNA sequencing pathogen assay

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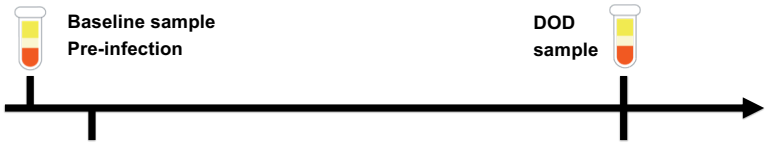
Background

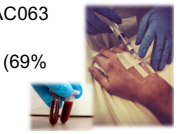
Controlled human malaria infection (CHMI) models[1] provide a means to assess the performance of novel diagnostics. We report for the first time, the detection and quantitation of *Plasmodium falciparum* and *P. vivax* using the Karius Test®, a plasma next generation sequencing (NGS) cell-free DNA pathogen assay[2,3].

Controlled Human Malaria Infection (CHMI)



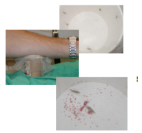
Infection with:

P. falciparum (3D7 clone) – VAC063 (NCT02927145)
- 1000 parasitized erythrocytes (69% viability)
- 690 parasites (effective dose)



OR

P. vivax – VAC068 (NCT03377296)
- Sporozoites delivered by mosquito bite (5 mosquitoes per participant)



Day of Diagnosis (DOD)

VAC063
- Positive thick film + symptoms
- Positive thick film, asymptomatic, whole blood qPCR ≥ 500 parasites/ml
- Symptomatic, qPCR ≥ 500

VAC068
- Whole blood qPCR >5000 genome copies/ml

The Karius® Test

- The Karius Test identifies microbial cfDNA in plasma from bacteria, DNA viruses, yeasts, mold, and protozoa using next-generation sequencing[2]
- Next day results* are reported from a single blood draw processed at a CAP-accredited and CLIA-licensed laboratory in Redwood City, CA, USA
- Database and algorithm can identify *Plasmodium falciparum, P. vivax, P. ovale, P. knowlesi, P. cynomolgi.*

*About 80% of specimens received by 8:30 AM (PT) Monday through Saturday are reported the next day

Results

The Karius Test can accurately detect *Plasmodium falciparum* and *Plasmodium vivax*

Karius Test (restricted to *Plasmodium*)

	CLIA		Higher Sens (RUO)	
	Pos	Neg	Pos	Neg
Plasmodium falciparum (Day of Diagnosis)	11 (73%)	4	15 (100%)	0
Baseline (pre-infection)	0	15 (100%)	0	15 (100%)
Plasmodium vivax (Day of Diagnosis)	2 (100%)	0	2 (100%)	0

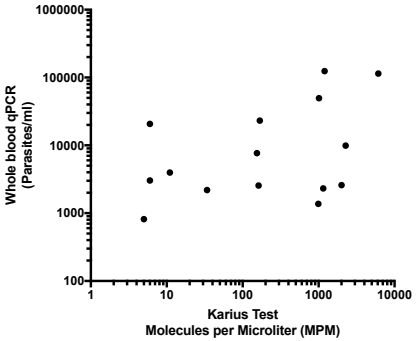
No *P. falciparum* reads identified in baseline samples

While there is good agreement between the Karius Test and qPCR for the detection of *P. falciparum,* there is no correlation in quantity

Quantity of *P. falciparum* was measured by the Karius Test (expressed in Molecules per Microliter – MPM) and whole blood qPCR (expressed in parasites/ml).

There was no clear relationship between MPM and parasites/ml

P = 0.118 Spearman Correlation; r = 0.422



Conclusions

- The Karius Test is a novel method to detect and quantitate *Plasmodium* species.
- The Karius Test had high agreement with a *Plasmodium* whole blood quantitative PCR, however there was no clear correlation between quantities measured by qPCR vs. the Karius Test.
- This is a novel method to detect infection in settings of low-level parasitemia and can also identify potential co-infections or pathogens that may mimic symptoms of acute malaria.

References

1. Payne RO et al, Efficacy of *P. falciparum* Apical Membrane Antigen 1 Vaccine, FMP2.1/AS01. *J Infect Dis* 2016;213:1743-51.
2. Blauwkamp TA et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat Microbiol* 2019; 4:663-674.
3. Hong DK et al. Liquid biopsy for infectious diseases: Sequencing of cell-free plasma to detect pathogen DNA in patients with invasive fungal disease, *Diagn Microbiol Infect Dis* 2018; 92(3):210-213.