Clinical trial of a novel molecular RDT for the detection of *Plasmodium falciparum* in whole blood in low resource setting.

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Background: Rapid diagnostic tests (RDTs) and microscopy are the main diagnostic tools used for detecting malaria. Unfortunately, neither can detect parasites at low infection densities observed in asymptomatic patients required for malaria elimination (<150 parasites/µL). This infection reservoir can only be detected through time-consuming molecular testing at centralized facilities. We developed and evaluated a low-resource molecular test to circumvent limitations for point of care testing.

Methods: Our rapid molecular *P. falciparum* test (rPf test) detects parasite DNA in whole blood by combining rapid sample preparation using TNA-Cifer Reagent (BioCifer, QLD), isothermal recombinase polymerase amplification (RPA; TwistDx, UK), and HybriDetect lateral flow strips (Milenia Biotec, Germany). Only a heating block and pipettes for liquid handling are required. We compared the performance of our test to quantitative results obtained by ultra-sensitive polymerase chain reaction (uPCR) which purifies DNA from concentrated red cells. The human blood samples tested were from volunteers within an induced blood-stage malaria clinical trial that were administered *Plasmodium falciparum*-infected erythrocytes intravenously and collected at various time intervals (n = 65 blood samples collected from 7 volunteers; ethics #P3483).

Results: The rPf test took 20 minutes from the start of sample processing to final lateral flow readout. From the 65 human clinical samples analyzed, the limit of detection with 90% positive agreement with uPCR was 50 parasites/µL (95% CI, 70% to 99%) and 25 parasites/µL with 75% positivity agreement (95% CI, 57% to 89%).

Conclusion: Our rPf test was reliable for detecting low-density parasites in blood, with 8x improved sensitivity than the WHO criteria for RDT acceptance (75% positive at 200 parasites/µL). The test can be performed in non-centralized facilities as it does not require any sophisticated equipment and would be of particular benefit in settings where the extra sensitivity of whole-blood PCR testing is required, but difficult to implement.

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