Trio-fluorophore as a tool to analyse artemisinin inducted dormancy of *Plasmodium falciparum*

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INTRODUCTION

Intraerythrocytic dormant stage of *Plasmodium falciparum* is one of the mechanisms to escape the effects of antimalarial drugs by temporary arresting their growth. However, the existence of dormant form of *P. falciparum* in erythrocytes remain controversial in part due to morphological similarity between dormant and pyknotic parasites. The advance in flow cytometric technique, which uses fluorescent DNA stains in combination with anti-CD45 antibodies, has increased speed, accuracy, and sensitivity, which addresses the challenges of conventional microscopic techniques.

OBJECTIVES

The Ultimate objective:

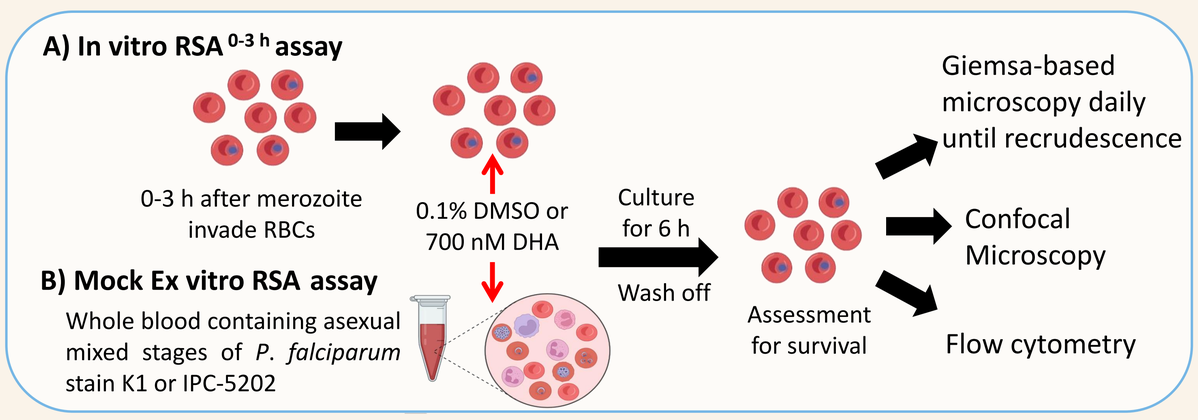
- This study aims to develop a fluorescence-based phenotypic assay for separating live/dead/dormant of *Plasmodium*-infected erythrocytes

The specific objectives:

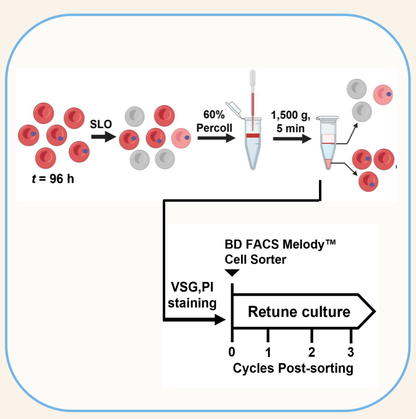
- To determine the fluorescent markers for identifying dormancy of the parasites
- To confirm the effect of the fluorescent probes on recrudescence of after drug exposure
- To develop a single tube method for detection live, dormant and dead *P. falciparum* in sample analysis

METHODS

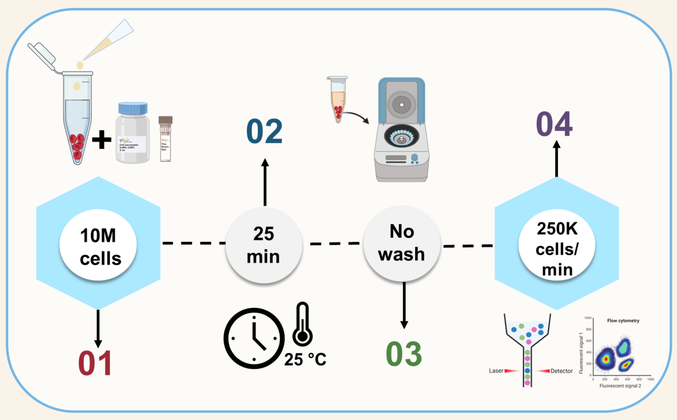
1. Determination of the fluorescent markers for identifying dormancy parasites



2. Confirmation of the probes



3. All-in-one tube assay



RESULTS

1 DHA induces dormant stage in ring-stage *P. falciparum*

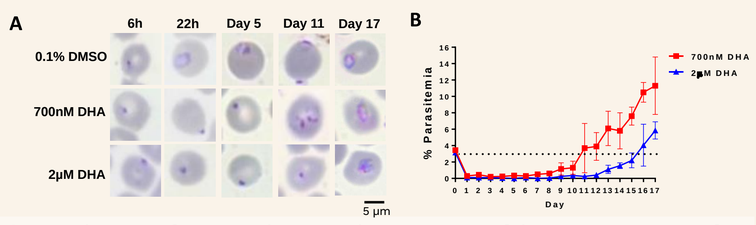


Fig 1. Induction of intraerythrocytic dormancy in *P. falciparum* strain K1 after DHA treatment. (A) Representative microscopic images of Giemsa-stained erythrocytes on thin blood smears. Scale bar = 5 μm. (B) Recrudescence curve of P. falciparum strain K1 exposed to 700 nM and 2 μM.

2 Combination of cell permeability reagent and the use of fluorescent DNA probes to fractionate DHA-exposed ring-stage parasites

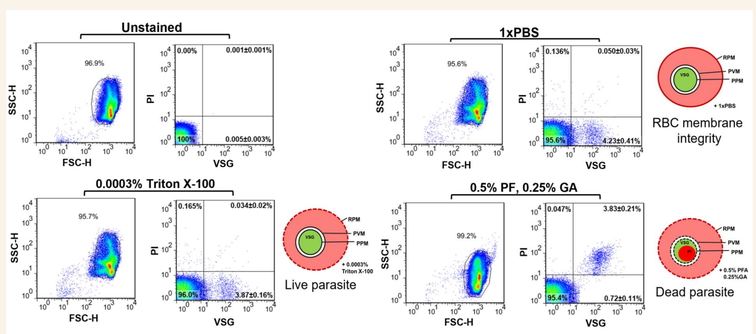


Fig 2. Combination of fluorescent DNA stains and cell permeable buffer can identify live, dormant and dead *P. falciparum.* (A) Dot plot of the representative flow cytometry analysis of *P. falciparum* after exposure to cell membrane-permeating reagents. (B) Representative confocal images of DHA-induced dormant and dead parasites. Cells are displayed according to the bright field, Visafe green (VSG) in green and propidium iodide (PI) in red.

3 The assay facilitates the detection of dormant *P. falciparum*

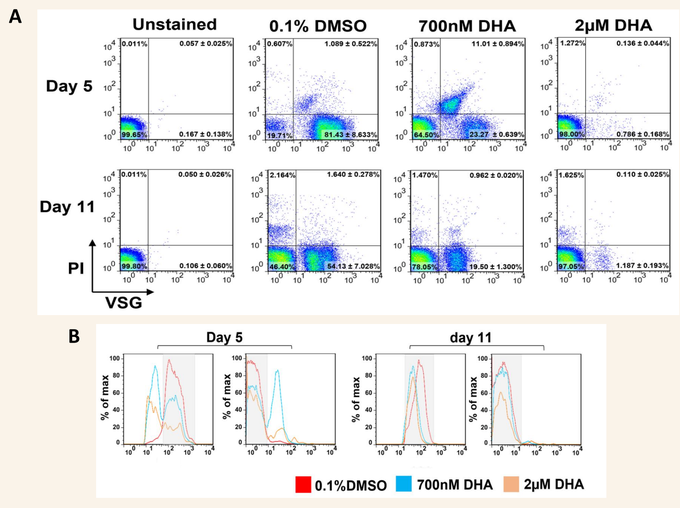


Fig 3. Analysis of DHA-induced dormant and dead parasites using flow cytometry. (A) Representative flow cytometric profiles of parasites exposed to 0.1% DMSO, 700 nM DHA or 2 μM DHA on days 5 and 11 post culture. (B) Histograms showing the fluorescence intensity of VSG and PI among the DMSO-exposed parasites (red line), 700 nM DHA-exposed parasites (blue line) and 2 μM DHA-exposed parasites (orange line) on days 5 and 11. The viable cells are shown in the grey band.

4 The use of new reagent kit does not affect the recrudescence of DHA-induced dormant parasites

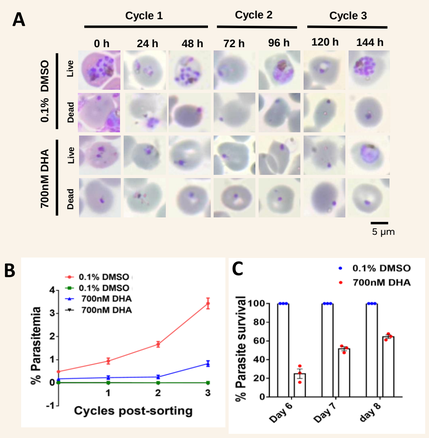


Fig 4. Viability confirmation of dormant *P. falciparum* after DHA exposure. (A) Representative microscopic images of Giemsa-stained erythrocytes show live and dead cells of the sorted parasites after exposure to 0.1% DMSO or 700 nM DHA. (B) Percentages of microscope-based parasitemia after cell sorting and culture for three cycles. (C) Parasite survival rate after DHA exposure.

5 Application of the Trio-fluorophore for the mock *ex vivo* RSA assay

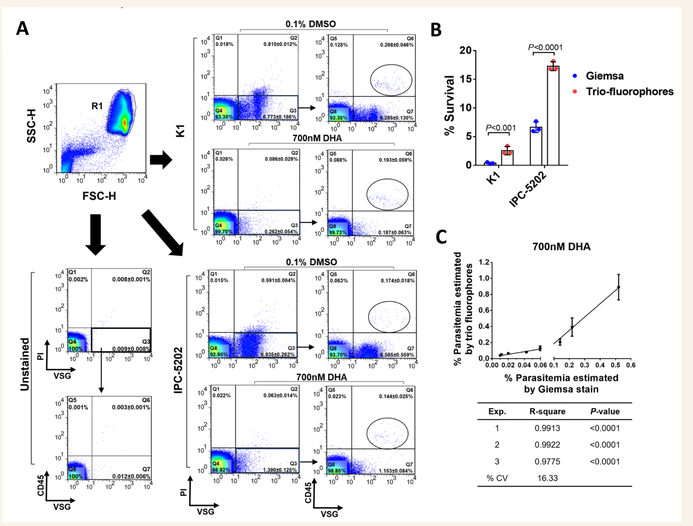


Fig 5. (A) Representative flow cytometric profiles of the cultured parasites 72 hours post culture. Given the presence of WBCs in the mock blood, anti-CD45 antibodies were used in the analysis, generating the trio fluorophore assay. (B) Bar graph comparing survival percentage and (C) Spearman's rank correlation coefficient between the standard microscopy method and the trio fluorophore

CONCLUSIONS

Capability
Identify a fake death
Know drug resistance

Simplicity
No blood smear
No staining
No microscopy

Fast
Semi- automatic

Performance
High accuracy
High sensitivity

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Reference: Kobpornchai, P., Imwong, M. & Kulkeaw, K. Trio fluorophore-based phenotypic assay for the detection of artemisinin-induced growth-arrested *Plasmodium falciparum* in human erythrocytes. Sci Rep 14, 1802 (2024**). Patent No.** 2301000336

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