Malaria gametocytes production in the Wave Bioreactor: optimizing yields and quantification protocols

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ABSTRACT

Malaria is one of the most debilitating mosquito-borne diseases and poses a major health and socio-economic burden in many endemic countries. The sexual stages of this parasite, gametocytes, are solely responsible for malaria transmission when ingested by mosquitoes and the dynamics of transmission are mainly determined by the density and sex ratio of the gametocytes. Molecular methods are critically needed for quantification of gametocytes, particularly when assessing transmission epidemiology and the efficacy of transmission-blocking interventions. The lack of widely available and well-characterized gametocyte quantification reference standards hampers basic research on this critical parasite stage. We aimed to produce mature gametocyte in the Wave Bioreactor with the ultimate goal of making reagents from this crucial parasite stage and optimizing protocols for gametocyte quantification and sex discrimination. A known gametocyte-producing line MRA-1000 (NF-54), available from ATCC through BEI Resources, was used in optimization experiments for generating large quantities of gametocytes. Quantification of gametocyte densities and sex discrimination was performed using reverse transcriptase quantitative PCR assays targeting gametocyte-specific transcripts Pfs25 (female-specific), Pfs230 (male-specific), and Pfs16 (all gametocytes). Gametocyte synthetic standards were generated by amplification of genomic DNA from a *P. falciparum* laboratory strain 3D7 (MRA-102) using sequence-specific primers containing a T7 promoter region that enables RNA transcription from in vitro DNA. RNA was transcribed into gametocyte-specific cDNA. Synthetic cDNA was then used to generate standard curves for quantifying gametocytes harvested from the wave bioreactor. Our results show that gametocytes can be accurately quantified and discriminated based on their sex using these synthetic standards. These findings opened doors for research on this critical parasite stage. Availability of gametocyte-derived reagents and standards through BEI Resources (https://www.beiresources.org), managed by ATCC, will aid research directed towards developing transmission-blocking interventions.

BACKGROUND

- Transmission of *falciparum* malaria requires uptake of sexual stages (gametocytes) from infected individuals by mosquitoes. Gametocytes therefore pose as a logical target for malaria control and intervention studies.
- High-yield production of viable mature stage V gametocytes is a complex and time consuming process. Previously described protocols are labor and resource-intensive and usually generate limited quantities of gametocytes for research. This issue coupled with the absence of widely available and well-characterized gametocyte quantification standards hampers basic research on this critical parasite stage.
- The Wave Bioreactor presents an opportunity to produce vast quantities of gametocytes [1], in a controlled semi-automated system, which could be used for various applications including pre-clinical development of transmission blocking drugs and vaccines.
- The objectives of this study were to (i) optimize and establish a high-yield culture system for gametocyte production in the Wave 25 Bioreactor, and (ii) use synthetic in vitro cDNA standards developed in our lab to quantify and discriminate male from female gametocytes.

MATERIALS & METHODS

In vitro culture of *Plasmodium falciparum* for gametocyte production

- **Parasite strain.** A known gametocyte-producing strain, NF-54 was obtained from the BEI Resources Repository, NIAID, NIH (www.beiresources.org): (BEI RESOURCES MRA-1000).
- **Parasite culture.** *P. falciprum* cultures were incubated at 37°C in leukocyte-depleted human type O+ erythrocytes (in CPDA-1) using RPMI 1640 supplemented with 10% heat-inactivated human type A+ serum, 0.18% Glucose, 0.18 mM Hypoxanthine, 1.77 mM L-Glutamine, 22 mM HEPES buffer 0.21% Sodium Bicarbonate and, 4 µg/mL Gentamicin under standard in vitro P. falciparum culture conditions
- **Gametocyte induction and production.** Gametocytes were produced in 2 L cellbags on the Wave Bioreactor [1] (Figure 1). Induction of gametocytogenesis was performed in the cellbag upon inoculation at a high hematocrit (hct) and no media change for 48 hours.

Detection and quantification of gametocyte-specific transcripts

- Assays are based on detection of gametocyte-specific transcripts pfs16 (expressed in all gametocytes), pfs25 (female-specific) and pfs230 (male- specific) [2,3]
- Total RNA was extracted from gametocytemic cultures propagated in the Wave Bioreactor to make cDNA for RT-qPCR assays
- Synthetic in vitro DNA (ivDNA) standards were generated by amplifying genomic DNA from a standard *P. falciparum* laboratory strain 3D7 (BEI Resources www.beiresources.org) using sequence-specific primers containing a T7 promoter region that enables RNA transcription from in vitro DNA. RNA was transcribed into gametocyte-specific cDNA. (Figure 3). The synthetic cDNA was used to generate standards for gametocyte quantification.
- RT-qPCR assays were performed using gametocyte-specific primers and probes (Life Technologies), and the CFX96™ system (Bio-Rad Laboratories).

TECHNICAL APPROACH SUMMARY

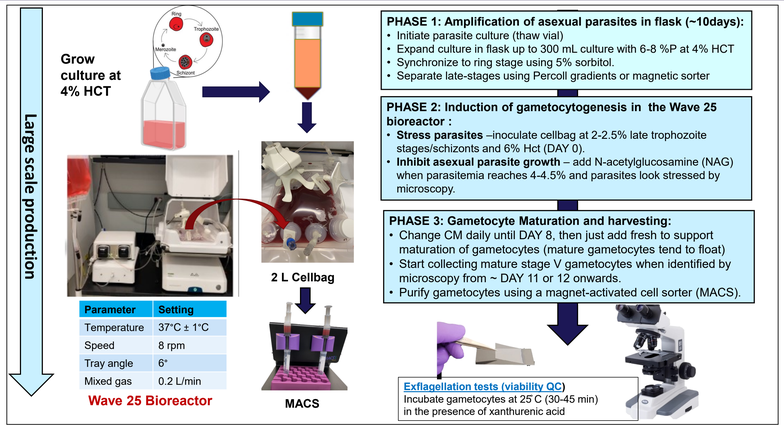

Figure 1. Large-scale in vitro production of mature stage V gametocytes in the wave bioreactor

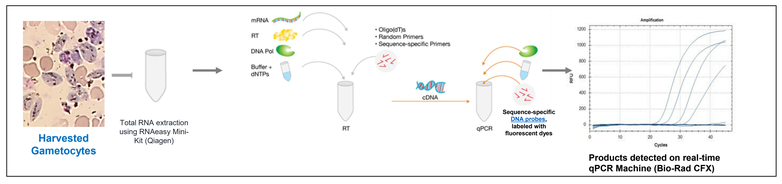

Figure 2. cDNA synthesis from gametocyte samples harvested from the wave bioreactor

RESULTS

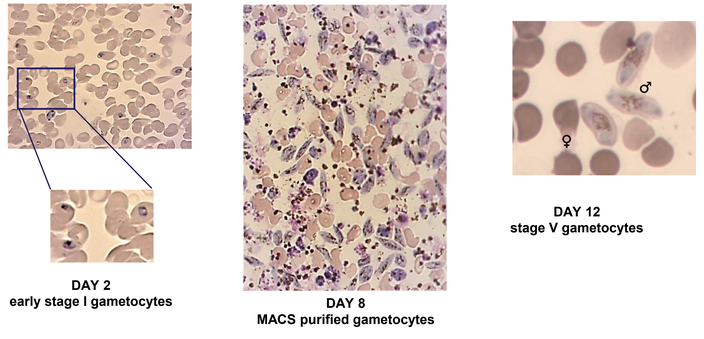

Figure 3. Giemsa-stained blood smears harvested from Cellbag culture in wave bioreactor

RESULTS

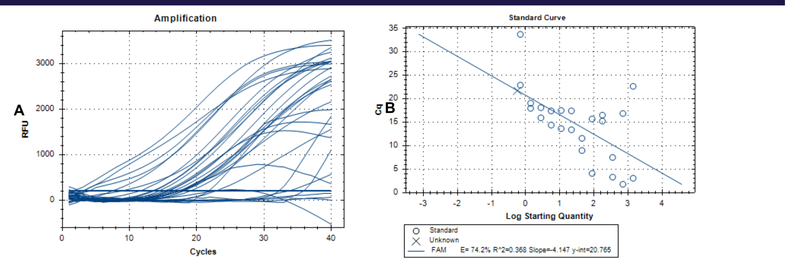

Figure 4. **RT-qPCR Assay for detection and quantification of gametocytes.** cDNA was amplified and quantified using gametocyte-specific primers and probes. Assay based on the detection of gametocyte-specific transcripts produced when parasites undergo sexual development *pfs16* – All gametocytes (non-sex specific) showing the amplification curve (Panel A) and a standard curve generated from a serially diluted synthetic standard for pfs16 (Panel B) .

SUMMARY

- We have optimized a protocol for large-scale production of malaria gametocyte in a semi-automated Wave Bioreactor System.
- We developed synthetic gametocyte quantification standards to detect and quantify all gametocytes and discriminate the two gametocyte sexes.
- These tools will help advance basic research on malaria gametocytes including understanding gametocyte sex ratio allocation and pre-clinical development of transmission-blocking drugs and vaccines.

REFERENCES

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3. Schneider P. et al. Mol Biochem Parasitol. 2015 Jan-Feb;199(1-2):29-33

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