Enrichment of sporozoites from whole mosquitos

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1. Introduction

A child dies from malaria every minute, with more than 240 million cases each year globally. Although the first malaria vaccine was licensed in 2022, it shows only modest efficacy and waning protection over 12 months. The world therefore needs an effective, long-lasting malaria vaccine. To date the best protection gained against malaria challenge has come from immunization with attenuated (radiation treated) whole parasites, the sporozoite form normally injected by a biting mosquito. Isolating sporozoites from mosquitoes is a painstaking process that has only been possible via manual dissection. Scalable production of a whole-parasite vaccine therefore remains challenging and would be greatly facilitated by a dissection-independent isolation method. Here we have developed just such a method using homogenization, filtration and FFE purification of sporozoites from whole mosquitoes.

2. Methods

Parasite preparation: Whole mosquitoes (*Anopheles stephensi*) infected with malaria (either rodent malaria *Plasmodium berghei* or human malaria *P. falciparum*) were homogenized, filter passed and prepped by centrifugation as described (Blight et al, 2021).

FFE-separation separation: The standard FFE-protocol for continuous FF-ZE-pH, based on Histidine/Propionic acid was used. A stepwise pH gradient, as shown below, was preformed by several separation media with different values of conductivity and pH.

Post FFE-analysis: Fractions collected from post-FFE separation were analysed by dot-blot for positivity against the sporozoite surface protein CSP (circumsporozoite protein) and/or visually inspected for sporozoite positivity.

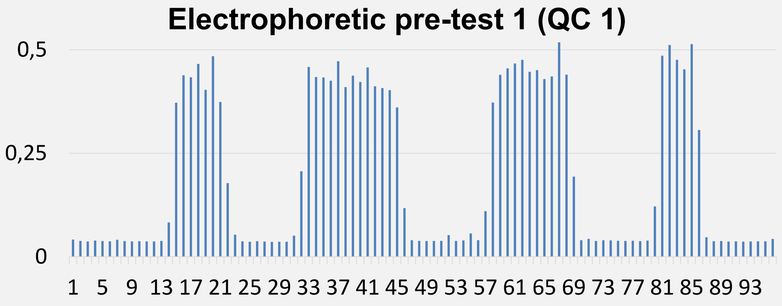
3. Results

1. FFE Quality Control

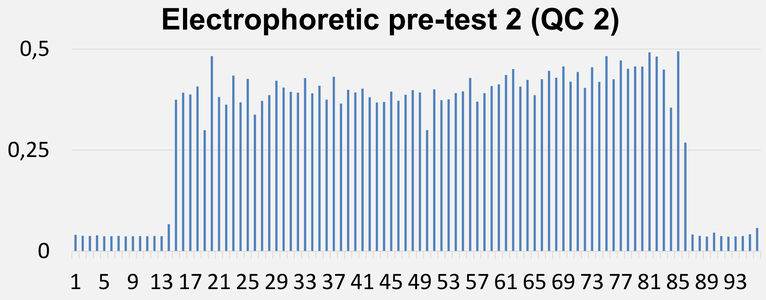
Purpose of test

Electrophoretic pre-tests (QC 1, QC 2) are part of the FFE quality control (QC) procedures to ensure proper FFE instrument set up and media composition. The stripe output of QC 1 and QC 2has always to be uniform and with clear borders.

Electrophoretic pre-test 1 (QC 1)



Electrophoretic pre-test 2 (QC 2)



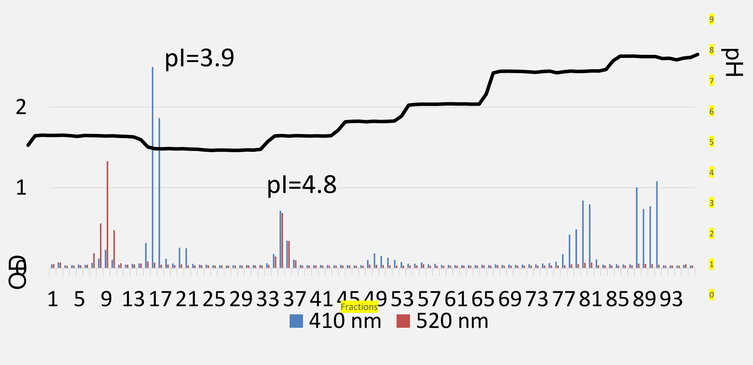
Result

Electrophoretic pre test 1 – QC 1:
Right tubing setup was confirmed by measuring the stripe-profile in the micro titer plate at absorbance 520 nm.
Electrophoretic pre test 2 – QC 2:
No blocked tubes in continuous and uniform stripe area, also verifying right tube and pump adjustments.

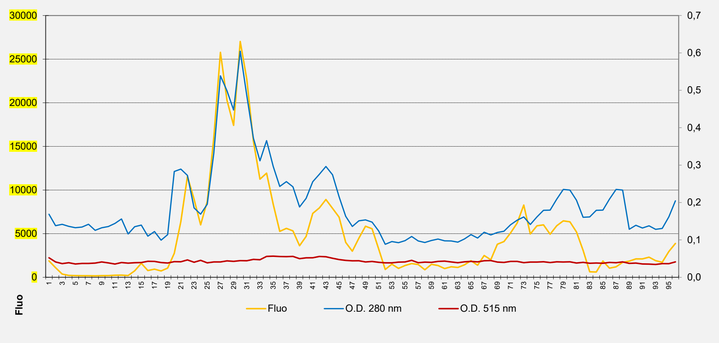
2. FFE performance

Checking the FFE performance (QC 3, QCad) includes the testing with separation media, stabilization media, electrolytes, IEF pI-marker mixture and standard sample, prepurified. This is done to evaluate the quality of the pH gradient created for an experiment and to compare the sample reproducibility between different FFE tests.

Separation of pI marker mixture (QC 3)



Protein pherogram across all FFE-fractions, obtained from human plasma as a reference sample

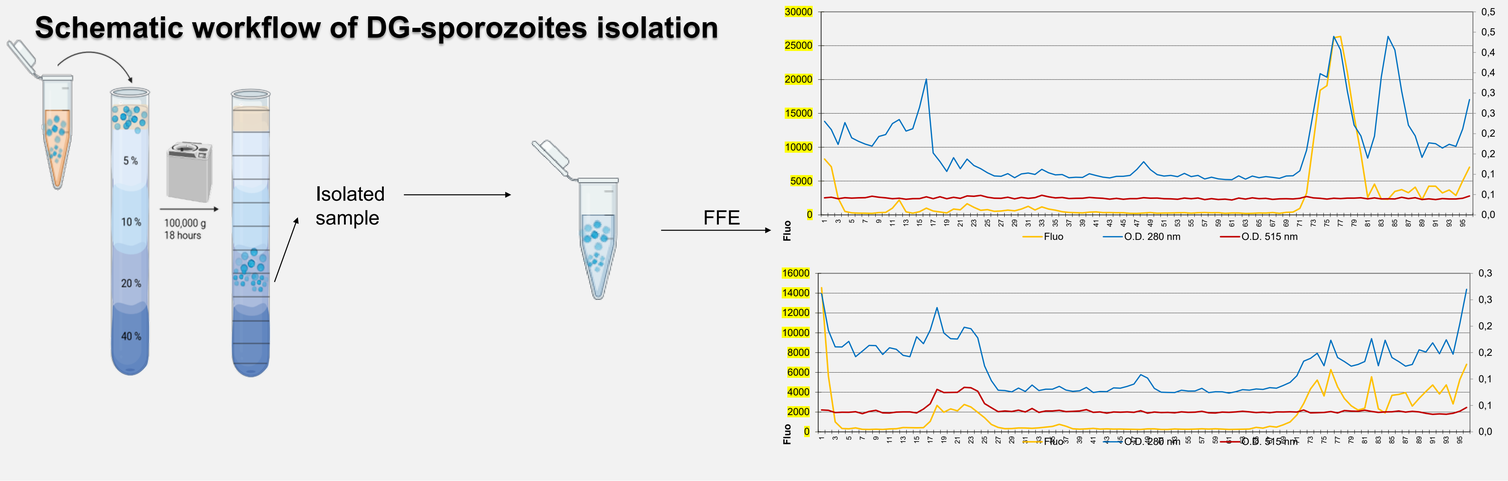


pI marker – QC 3: A known mixture of analytes was separated and deflected towards their isoelectric point (pI) where they stop migration and focus. This is due to the pH gradient created by the IEF buffers, which flow through the electric field.

3. Workflow of enrichment of sporozoites from… by FFE

To get enriched Sporozoites from mosquitoes filtration and Density Gradient Ultracentrifugation purification (Accudenz) was used as described (Blight et al 2021).

Schematic workflow of DG-sporozoites isolation

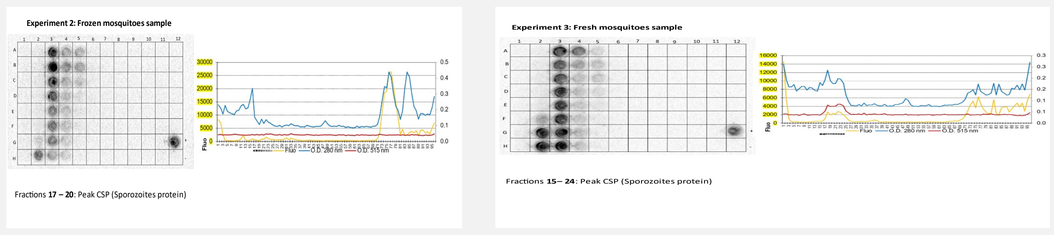


Two samples with differences in concentrations of sporozoites and contaminating proteins were separated and the FFE protein pherograms showed several distinct peaks inside the area between the FFE-fraction 15 and 25 (expected area of separated sporozoites).

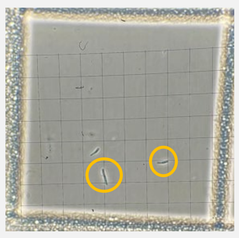
4. Analysis of FFE-fractions

Dot Blot analysis probing with antibodies against the sporozoite protein CSP were undertaken to determine the location of the sporozoite positive fractions from the FFE-separation process.

Dot Blot analysis



Microscopy



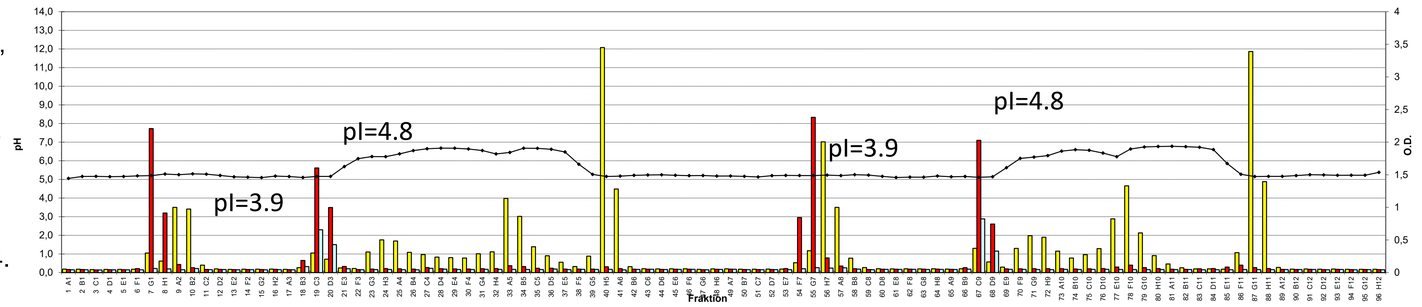
Positive fractions were clearly detectable by dotblot and correlated with intact sporozoites found in each well by microscopy inspection. Little debris was seen suggested pure parasites.

4. Conclusion and Outlook

Here we show that FFE can be used to separate purified sporozoites away from contaminating particles and proteins derived from whole mosquitoes.

Reference: Blight J, Sala KA, Atcheson E, Kramer H, El-Turabi A, Real E, Dahalan FA, Bettencourt P, Dickinson-Craig E, Alves E, Salman AM, Janse CJ, Ashcroft FM, Hill AV, Reyes-Sandoval A, Blagborough AM, Baum J. Dissection-independent production of Plasmodium sporozoites from whole mosquitoes. Life Sci Alliance. 2021 Jun 16;4(7):e202101094. doi: 10.26508/lsa.202101094.

Scale Up of FFE-Process: The sample capacity of the FFE-process can be scaled up, as shown below. The total area inside the separation cell can be divided into 2 separate separation areas. Two samples of pI-markers can be separated simultaneously in a parallel mode of process, as shown above in QC3 as separation process with 1 sample injection. The max. value of sample capacity in case of sporozoites–samples could be about 50 ml/h. Processing a sample with a conc. of sporocytes=7x10^6/ml over a time of 6000 hours up to 2.1x10^{12} sporozoites could be separated per year



5. Acknowledgements

Conflict of interest: G. Weber, is CEO, M. Meckel is employee of FFE Service GmbH, the manufacturer of the FFE instrument

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