Transmission Blocking Drug Screening Assay Development Based on the Ability of *Plasmodium falciparum* Gametocytes to Cross a Spleen

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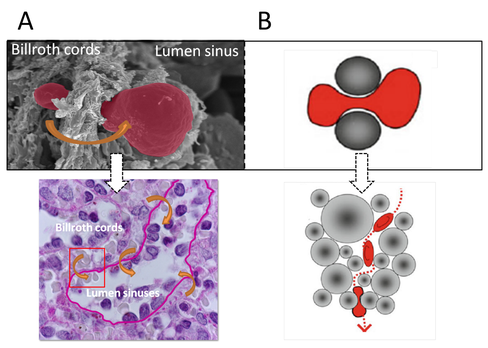
Introduction

In an effort to discover transmission blocking drugs for *Plasmodium falciparum,* we are currently developing a unique drug screening assay based on the ability of gametocytes to cross narrow slits which are present in the human spleen (A). This approach utilizes microsphere filtering to mimic the mechanical challenges imposed on red blood cells (RBCs) in the human spleen (B).[1] During gametocyte maturation from stages II through IV, gametocytes are rigid and non-deformable, thus are retained within the microsphere filter. However, stage V gametocytes undergo a switch to become highly deformable, thereby allowing passage through the filter.[2]

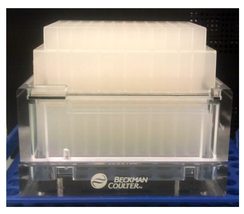
Previously, this microsphere filtration technology was limited to a single sample at a time. To allow for parallel processing of many samples and enable a higher throughput for compound screening we have miniaturized the filtration unit and adapted it to 96 well format. Furthermore, the filtration protocol has been streamlined through the use of large capacity reservoirs and a reduced number of washing step to improve throughput. Importantly, the performance of the multi-well filtration format was validated using stage V gametocytes and retention rates were consistent with previously published results.[2] Automated, high-content image quantification methods were developed using fluorescence detection to enable the rapid and accurate determination of gametocyte numbers up- and down-stream of filtration. Using this screening platform, we plan to screen small molecule libraries for compounds which are capable of modulating the deformability of gametocytes. This novel approach to gametocyte drug screening has the potential to reveal compounds which increase the rigidity of late stage gametocytes thereby inducing splenic clearance and reducing transmission to the Anopheline mosquito vectors.



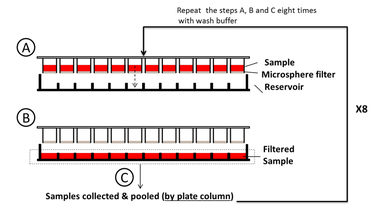
Deplaine et al., Blood, 2011

(A) Normal RBCs squeezing across inter-endothelial slits in the splenic red pulp. B) The geometry of inter-endothelial slits can be mimicked with microspheres.

Screening Compatible Filtration Format



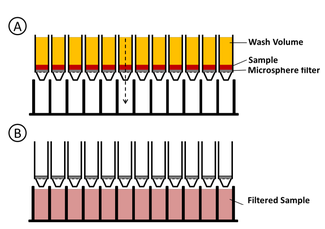
Shallow well format



Sample staining and flow cytometry

~3 hr/plate

Deep well format



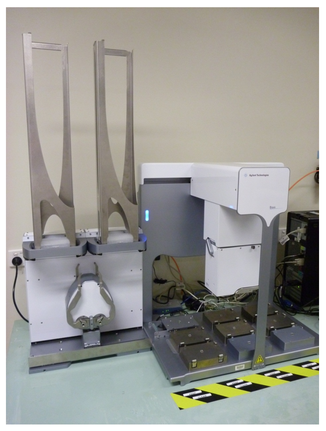
Sample staining and high-content analysis

~20 min/plate

Miniaturization of the microsphere filtration device to 96 well format amenable to compound screening was first demonstrated using a shallow well format. The shallow well format recapitulated the spleen-like filtering of the single column method, however, processing was time and labor intensive, requiring 8 washing steps. A deep-well format filtration device was developed subsequently to address these sample handling limitations. Using the deep well format, sample processing was reduced to a single wash step and plate processing time was reduced 9 fold thereby increasing throughput for compound screening campaigns. mimic

Robotic Handling and Analysis

A

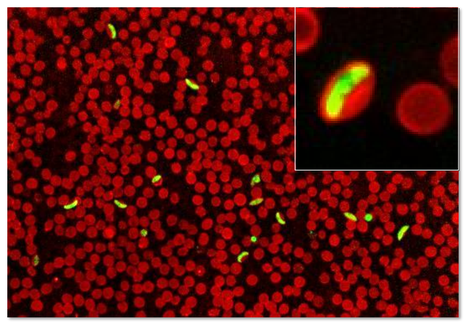


B

(A) The deposition of microspheres into filtering plates, loading of samples to microsphere plates and transfer of the processed samples to imaging formats is fully automated using liquid handling robots (Agilent Bravo® - pictured and Beckman Coulter Biomek® 3000) (B) Analysis and quantification of the samples is performed using the high-content imaging platform, Perkin Elmer Opera® spinning disc confocal.

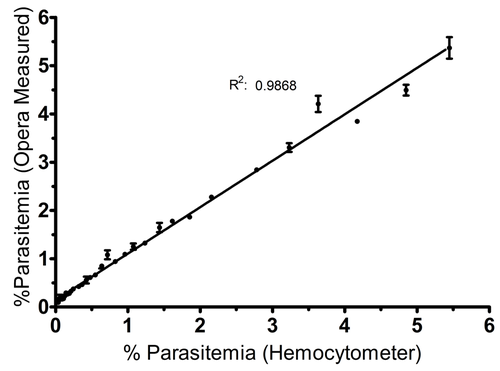
Automated Quantification of Gametocytes

A



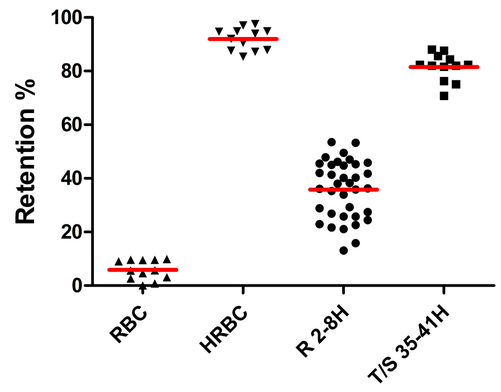
RBCs: Cell Mask PM dye
Stage V Gametocytes: NF54Pfs16 GFP

B



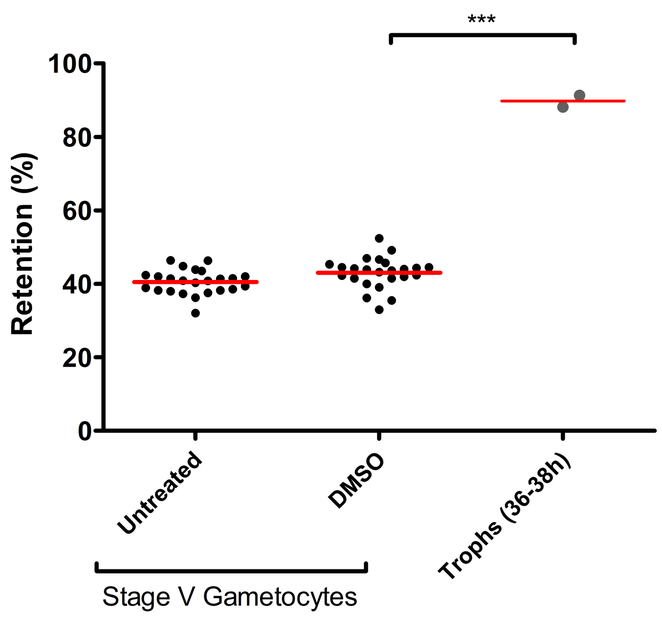
(A) High-content imaging of stage V gametocytes in RBCs was performed using an Opera spinning disc confocal microscope. Stage V gametocytes of the NF54^{Pfs16} GFP transgenic strain[3] were detected by GFP fluorescence. RBCs were labelled using Cell Mask Plasma Membrane Orange® (Invitrogen). The parasitemia was calculated using automated image segmentation algorithms to quantify the number of elongated gametocytes divided by the total number of RBCs. B) Linearity of the automated detection was assessed using manual counting with a hemocytometer across 3 separate cultures and dilution series.

Validation of Filtration using Asexual Stages



To evaluate the spleen-like filtering performance of the device, control conditions were tested using uninfected RBCs and asexual stages. As expected, RBCs were highly deformable and exhibited very low retention. Heated RBCs (HRBC) on the other hand, showed >90% retention, consistent with previously published results. Young rings (R 2-8h) were used as a model for the moderate retention expected for stage V gametocytes (30-50%[2]). Trophozoites and schizonts (T/S 35-41H) served as a surrogate for the highly retained immature gametocytes or predicted non-deformable compound treated stage V gametocytes.

Effects of Compound Treatment on Stage V Gametocytes



	Mean Retention (%)
Untreated	40.51
DMSO	43
Trophs (36-38h)	89.73

Gametocytes undergo a transition at stage V to become much more deformable and capable of passing through the spleen or microsphere splenic mimic. In order to discover compounds which facilitate clearance of stage V gametocytes, we tested two compounds as proof of principle to assess assay performance. Untreated and DMSO (0.4%) treated stage V gametocytes demonstrated modest retention, whereas trophozoites had dramatically higher retention rates.

Conclusions

An assay for small molecule screening to discover compounds which induce stage V gametocyte clearance has been validated and optimized using asexual stages and RBCs. Importantly, stage V gametocytes have significantly lower retention rates than controls demonstrating a favorable assay window and proof of principle demonstration. This screening platform will be used to probe compound libraries, including the Medicines for Malaria Venture (MMV) malaria box, for compounds with the ability to decrease stage V gametocyte deformability and facilitate splenic clearance and block *P. falciparum* transmission cycle.

References

Deplaine, G., et al. (2011). "The sensing of poorly deformable red blood cells by the human spleen can be mimicked in vitro." Blood 117(8): e88-95.

Tiburcio, M., et al. (2012). "A switch in infected erythrocyte deformability at the maturation and blood circulation of Plasmodium falciparum transmission stages." Blood 119(24): e172-180.

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Acknowledgements

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