



Automated counting of bound *Plasmodium falciparum*-infected erythrocytes to screen for chondroitin sulfate A-binding inhibitory antibodies

Pamela A. Magistrado¹, Walther Risom², Davis John³, Deus Makingi⁴, Thor G. Theander¹ and Morten Nielsen¹

¹Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and Department of Infectious Diseases, Copenhagen University Hospital (Rigshospitalet), Denmark; ²DFA Instruments, Copenhagen, Denmark; ³Kilimanjaro Christian Medical Centre, Moshi, Tanzania

INTRODUCTION

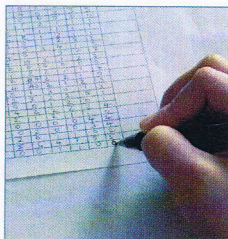
The pathology of pregnancy associated malaria (PAM) involves placental sequestration of *Plasmodium falciparum*-infected erythrocytes through the interaction between the parasite protein, VAR2CSA and the placental receptor, chondroitin sulfate A (CSA). We have recently shown that a single VAR2CSA domain, DBL4-ID4 of the FCR3 sequence, can induce CSA-binding inhibitory antibodies against *P. falciparum* isolates from East- and West-African pregnant women. This identified DBL4-ID4 as the leading vaccine candidate against PAM. Current studies include benchmarking other antibody specificities against DBL4-ID4 antibodies and identification of the most inhibitory vaccine adjuvant/delivery system. The method used for screening CSA-binding inhibitory antibodies is based on a static plate assay where soluble CSA is immobilized on plates, parasites pre-incubated with or without VAR2CSA antibodies are allowed to bind on the CSA spots, unbound cells washed off, bound parasites fixed and stained. Inhibition is assessed by counting the parasites bound on the plate under the microscope manually. This is, however, prone to bias and is tedious. Therefore, we implemented a new automated method of counting CSA-bound parasites under the microscope.

METHOD

- Binding inhibition assay:**
1. *P. falciparum* isolates from pregnant women preincubated with VAR2CSA antibodies
 2. Bound to CSA immobilized on spots on plates
 3. Washed to remove unbound cells
 4. Fixed, Giemsa stained, washed and dried
 5. Counted bound parasites to measure CSA inhibition by VAR2CSA antibodies



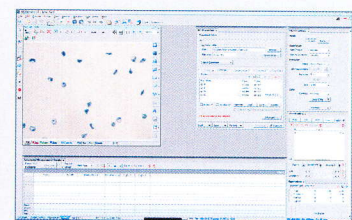
Manual Counting



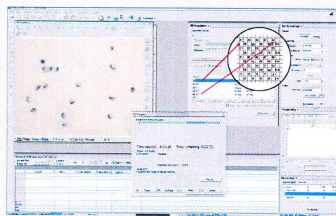
Automated Counting



1. Plate placed under the microscope



2. Parasites focused manually and software opened



3. 50 images taken automatically of 50 fields distributed evenly all throughout the spot (from A1-J10)



4. Infected erythrocytes counted automatically by the software and results exported to excel file

RESULTS AND CONCLUSION

	Manual counting	Automated counting
Bias	High – microscopist chooses the field and might be looking for the specimen involuntarily; the same field might be counted more than once	Low - fields for counting for each spot are pre-programmed and are evenly distributed all throughout the spot
Time to count one spot	5 minutes 40 seconds	13 minutes but while the software is taking 50 images for 8 minutes, the system can be left aside
Lab isolate results		
Clinical isolate results		

Results from manual and automated counting are similar but there is no or less bias with the automated counting since the fields for counting are pre-programmed and evenly distributed.

Since the manual counting is prone to bias, the automated counting method can be used as a better alternative for getting more credible results from binding inhibition assays.

ACKNOWLEDGEMENTS

MCDC
MALARIA CAPACITY DEVELOPMENT CONSORTIUM

DANIDA
Joint Malaria Programme

REFERENCES