

Activity characterization and classification of anti-plasmodial compounds.

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Introduction: It is vital to proactively identify anti-plasmodial compounds with alternative mechanisms of action: not only to Artemisinin but all other previously and currently utilized anti-plasmodial drugs.

Method: A set of novel High throughput confocal fluorescent microscopy image analysis assays.

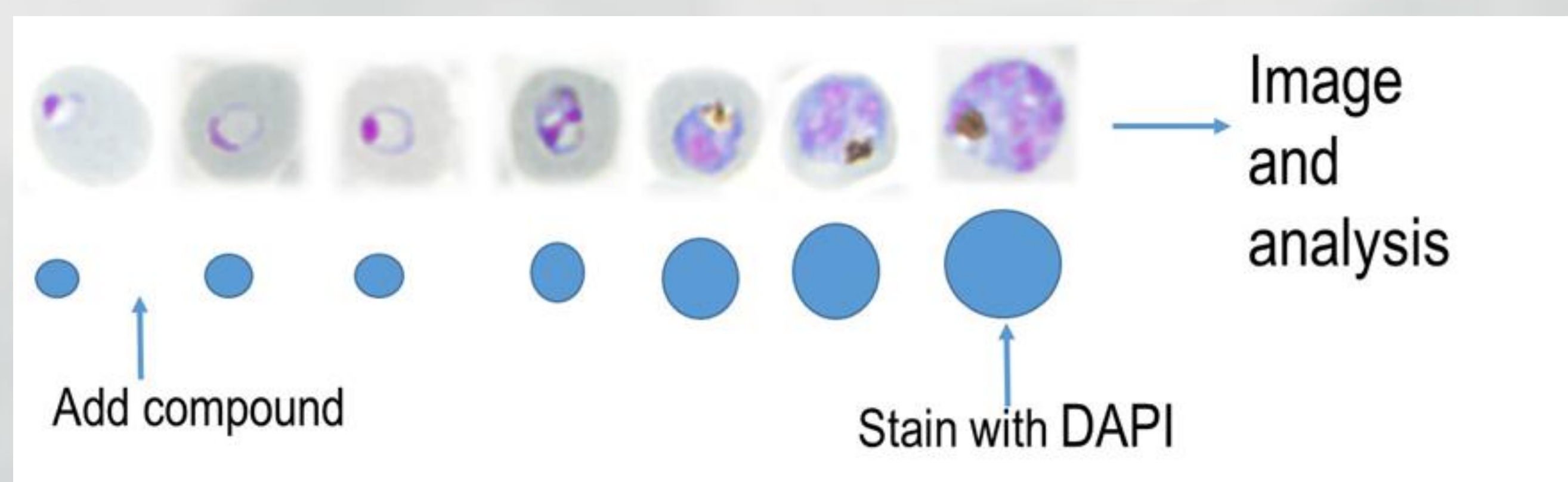
Compounds: Medicines for malaria venture (MMV) open access Pathogen box "malaria set" consisting of 125 compounds.

Results: 125 compounds classified into 8 asexual activity groups. Compounds with ring stage onset of action were more likely to have late stage gametocyte activity.

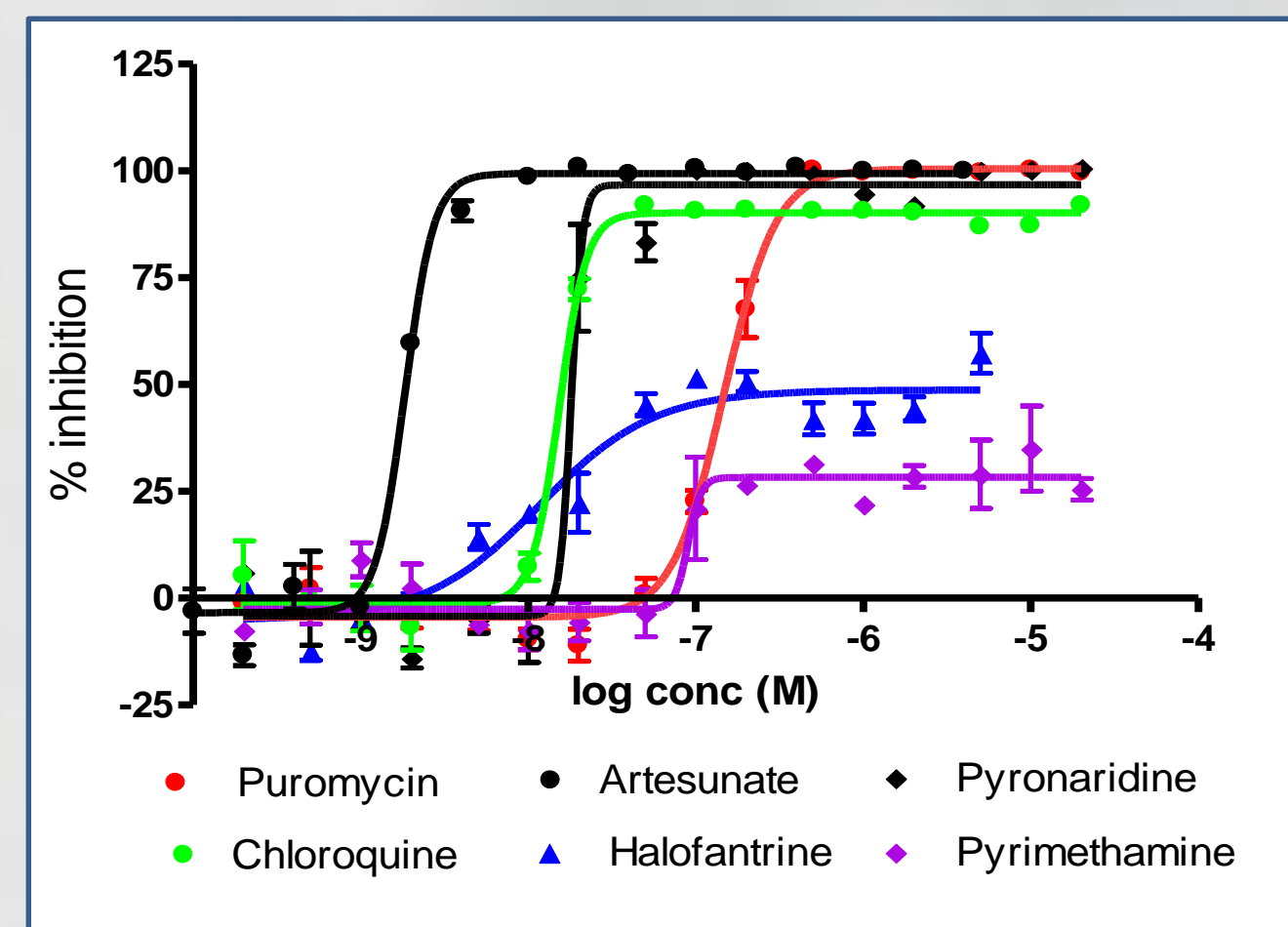
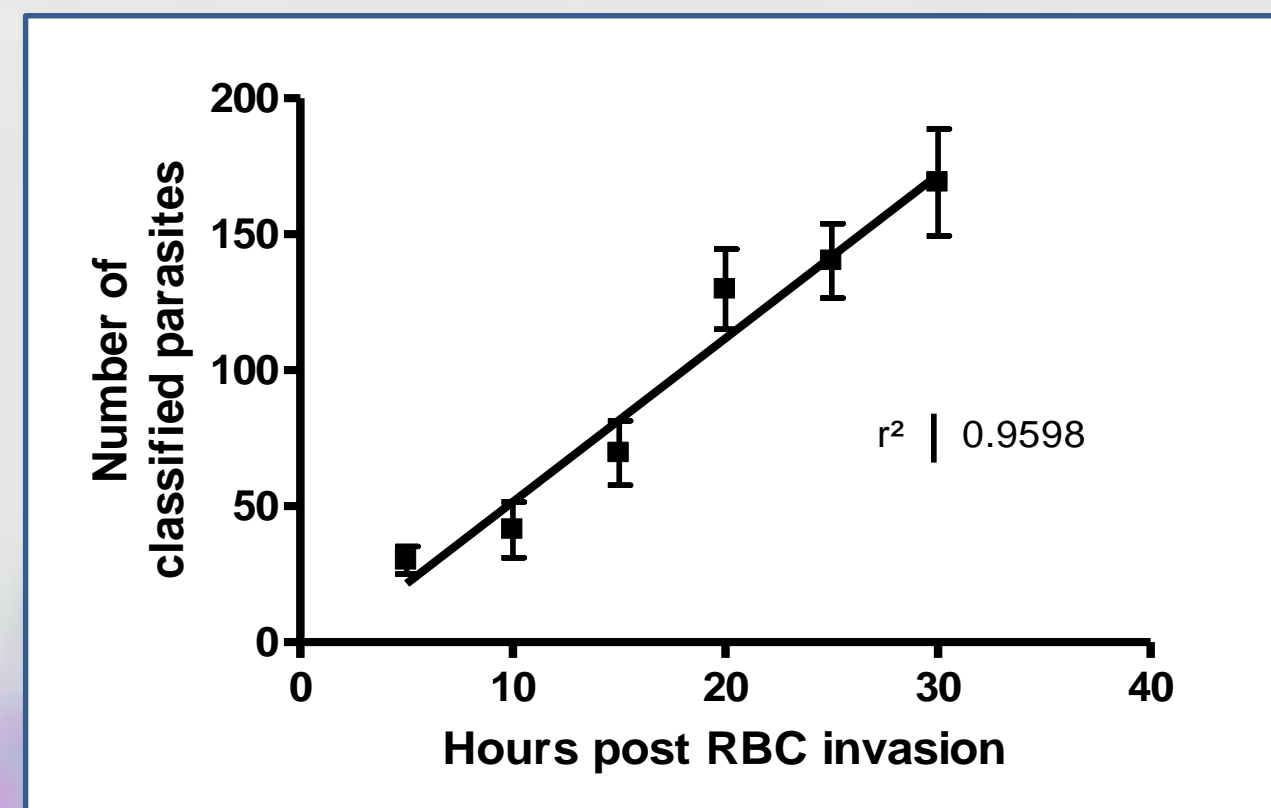
Conclusion: A suite of 7 aligned confocal imaging assays can classify compounds into 8 asexual stage activity groups relating to potential alternative mechanisms of action. Mature stage gametocyte activity was 4 fold more likely for compounds with an onset of action at ring stage than those for more mature forms of the asexual parasite.

Assay Principles

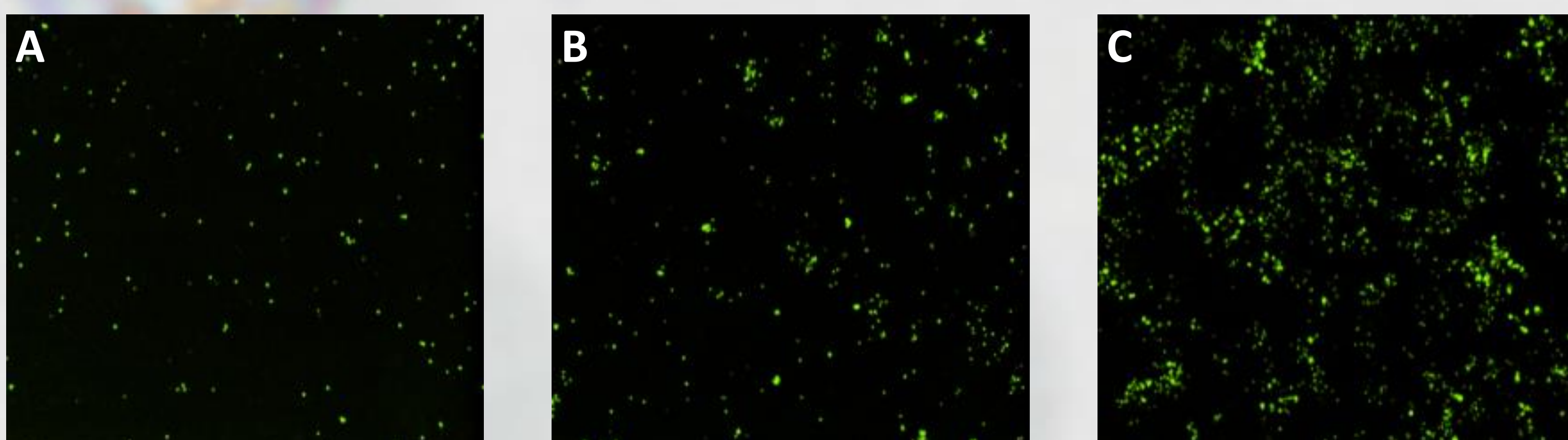
1. Schizont Maturation Imaging Assay (SMIIA)/onset of action



As the asexual parasite grows it becomes larger with greater fluorescent intensity and the number of parasites identified increases. When testing in dose response curves, the Emax of the curve is related to the onset of action of the compound against the parasite.



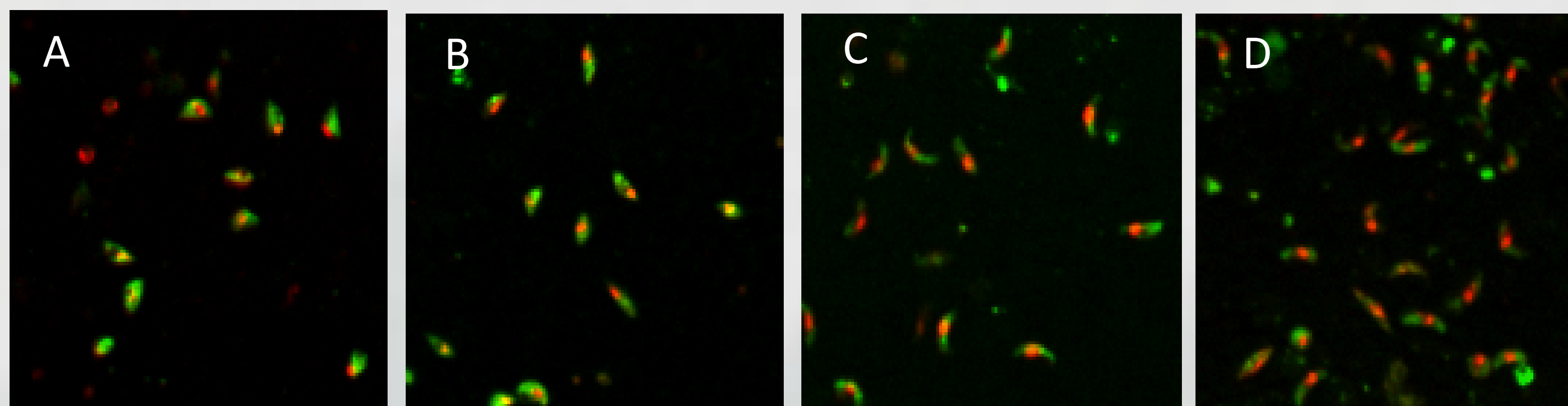
2. First versus second generation asexual activity



| Image | Condition | Spots | % Inhibition |
|-------|-------------------|-------|--------------|
| A | 500nM Artemisinin | 159 | 100 |
| B | 20nM Artemisinin | 332 | 78 |
| C | 0.5nM Artemisinin | 954 | 0 |

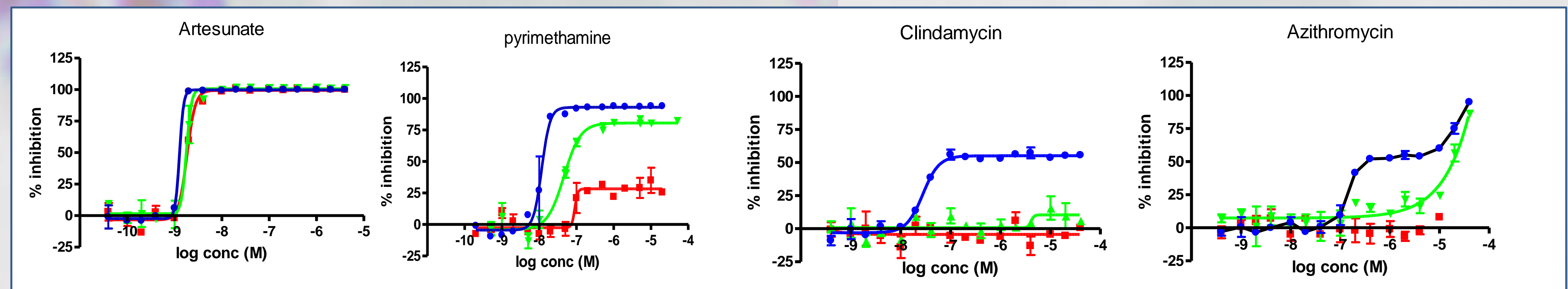
Highly synchronous ring stage parasites are isolated and added to the compounds. After one or two cycles of parasite proliferation DAPI (Fluorescent stain) is added to the imaging plates, the plates are imaged and the number of parasites enumerated using a mathematical algorithm.

3. Gametocyte activity



Compound inhibitory activity is determined for any stage of gametocyte development based on the common staining and script analysis involved in all assay time points. The two parameters determining a viable gametocyte (elongation factor and mitotracker fluorescent intensity) are adjusted for gametocyte development stage **post incubation** ie A. ring stage (II), B. early stage (III), C. late stage (V) and D. mature stage (V).

Compound activity Examples (Red = SMIIA, Green = 1 cycle, Blue = 2 cycle of proliferation)

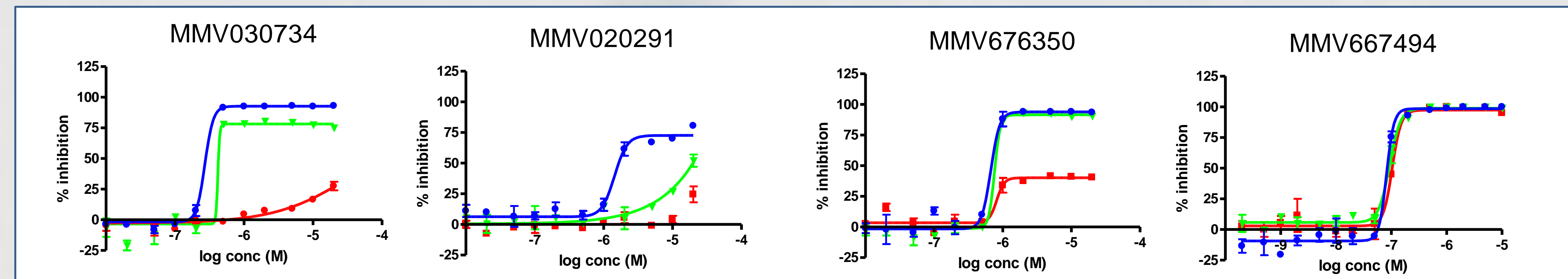


Artesunate: active on ring stage parasites and equally active on the 1st and 2nd proliferation cycle

Pyrimethamine: stage of onset is mature trophozoites, with activity increasing with each parasite cycle of proliferation

Clindamycin: only active against the 2nd cycle of proliferation.

Azithromycin: low activity against the 1st proliferation cycle and biphasic in the 2nd cycle



Pathogen Box exemplars

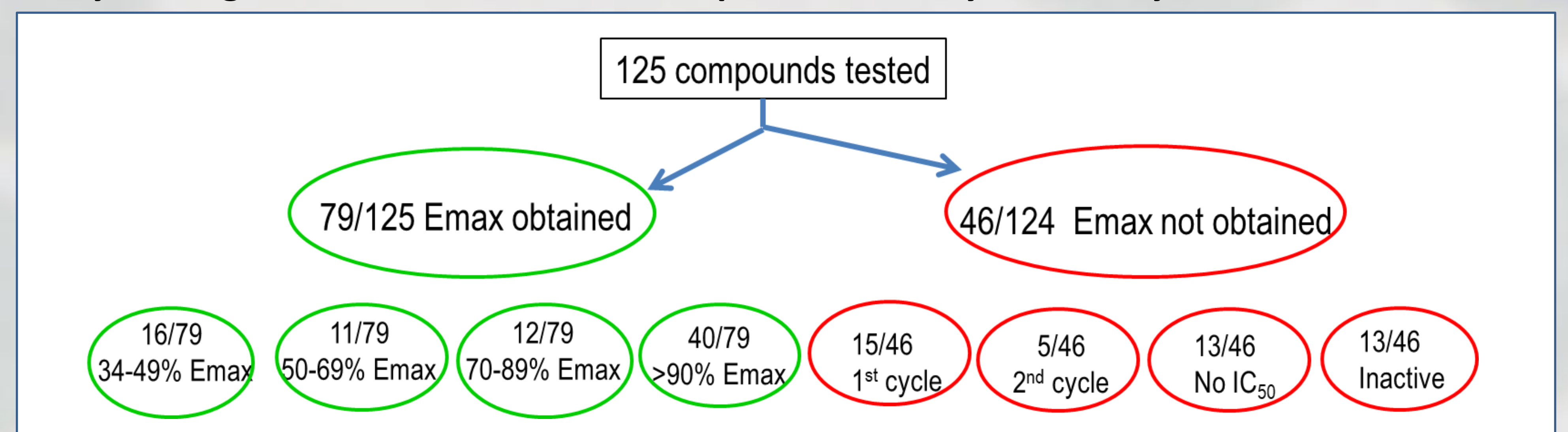
MMV030734 – either active post 35 hours against mature schizonts or inhibits invasion of merozoites into new RBC which is only detected in the 1 and 2 cycle proliferation assays

MMV020291 - 2nd cycle similar to clindamycin or azithromycin.

MMV676350 - shows a typical mature trophozoite onset of action, similar to pyrimethamine, but comparable activity with a single or duplicate replication cycle.

MMV667494 - similar to puromycin or artesunate.

Summary Pathogen Box SMIIA and 1st vs 2nd proliferation cycle activity



Relationship of Asexual and Gametocyte Activity Profiles.

| Asexual classification | % active compounds RSG | % active compounds ESG | % active compounds LSG/MSG* |
|------------------------------|------------------------|------------------------|-----------------------------|
| SMIIA Emax 34-69 | 60 | 26 | 7 |
| SMIIA Emax 70-110 | 60 | 36 | 29 |
| 1 st cycle active | 46 | 33 | 0 |
| 2 nd cycle active | 0 | 0 | 0 |

Compounds with activity against mature gametocyte forms are four fold more likely to have activity against the earlier stages of the asexual parasite. However, activity against "Ring stage gametocytes" is comparable between the two asexual activity groups

Acknowledgements.

Medicines for Malaria Venture for the provision of the pathogen box.
Australian Red Cross Blood Services for the provision of blood.
NHMRC Postgraduate Scholarships - Sandra Duffy - APP1150359, supported in part by a Griffith University Postgraduate Scholarship and a Discovery Biology Post Graduate Scholarship.
Australian Research Council (LP120200557 to V.M.A.)

References.

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