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Lack of resistance of Plasmodium falciparum to dihydroartemisinin in Uganda based on parasitological and molecular assays

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Lack of resistance of *Plasmodium falciparum* to dihydroartemisinin in Uganda based on parasitological and molecular assays

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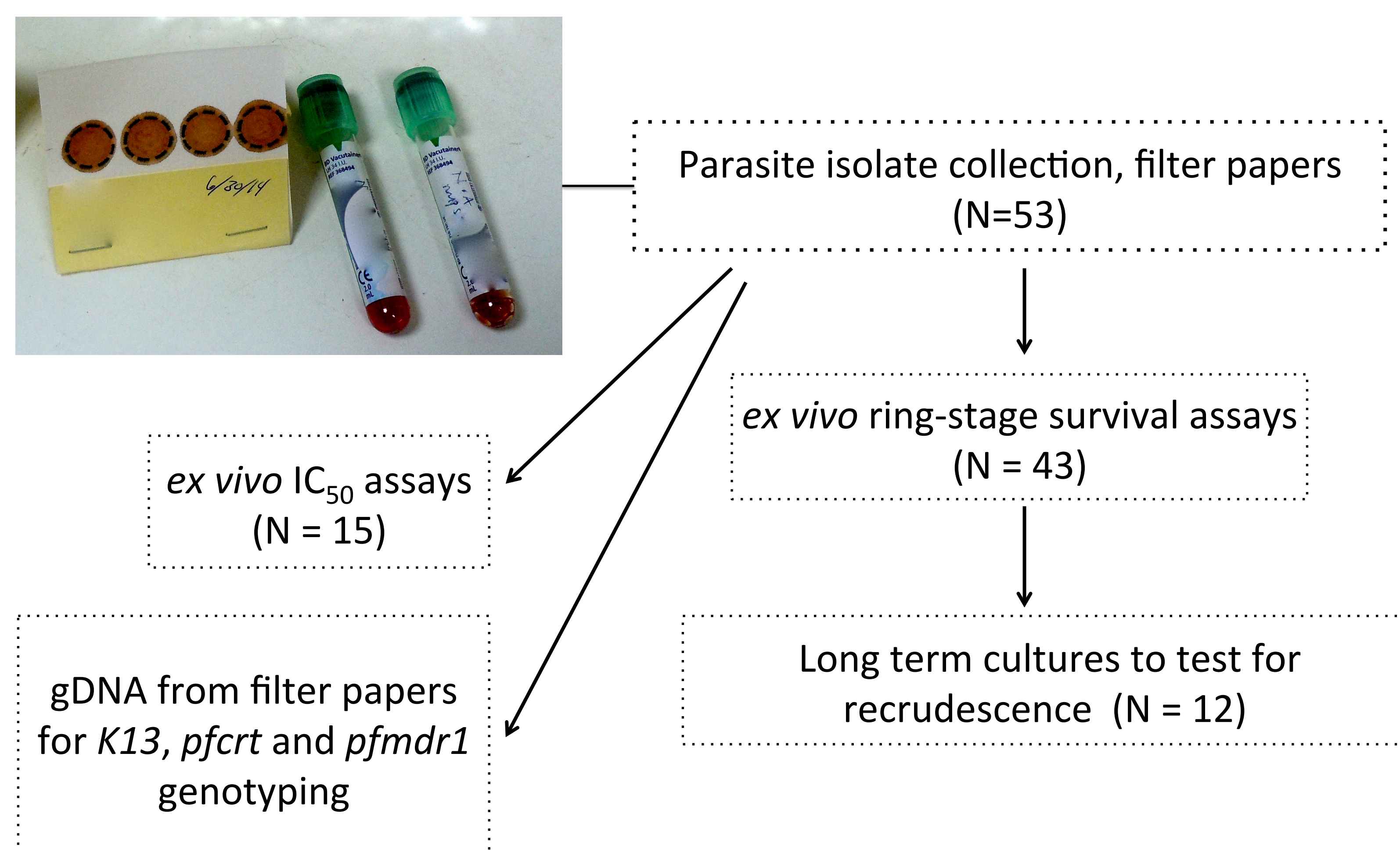
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Introduction

- Artemisinin-based combination therapy is now standard treatment for falciparum malaria. However, this regimen is threatened by resistance to artemisinins, manifest as delayed clearance of parasitemia after therapy, in southeast Asia¹.
- Artemisinin resistance in southeast Asia is associated with increased parasitemias in culture, compared to those in sensitive parasites, 72 hours after a 6 hour pulse with 700 nM dihydroartemisinin (DHA), and with propeller domain polymorphisms in the *Plasmodium falciparum* kelch (*K13*; PF3D7_1343700) gene^{1,2}.
- Given that artemether/lumefantrine has been adopted as standard therapy for malaria within the last decade in Uganda, we characterized artemisinin sensitivity in fresh *P. falciparum* isolates from Kampala using *ex vivo* ring-stage survival and IC₅₀ assays. We also assessed the *K13* gene for polymorphisms.

Methods



53 fresh *P. falciparum* isolates were collected from patients diagnosed with malaria from May-August 2014, at Mulago Hospital, Kampala.

Parasite IC₅₀s to DHA were determined by a standard 72 h *ex vivo* microplate assay using HRP2 detection³.

Parasite susceptibility to DHA was assessed in the *ex vivo* ring-stage survival assay as described². Survival rates were expressed as the proportion of parasites in the 6 h, 700 nM DHA-pulsed cultures relative to DMSO controls, at the end of the 72 hour assay. Twelve cultures exposed to DHA were allowed to grow for 30 d to test for recrudescence.

K13 propeller-encoding domains (codons 440-726) were dideoxy sequenced. Polymorphisms in *pfprt* and *pfmdr1* were assessed with multiplex ligase detection reaction-fluorescent microsphere assays as previously described⁴.

Results

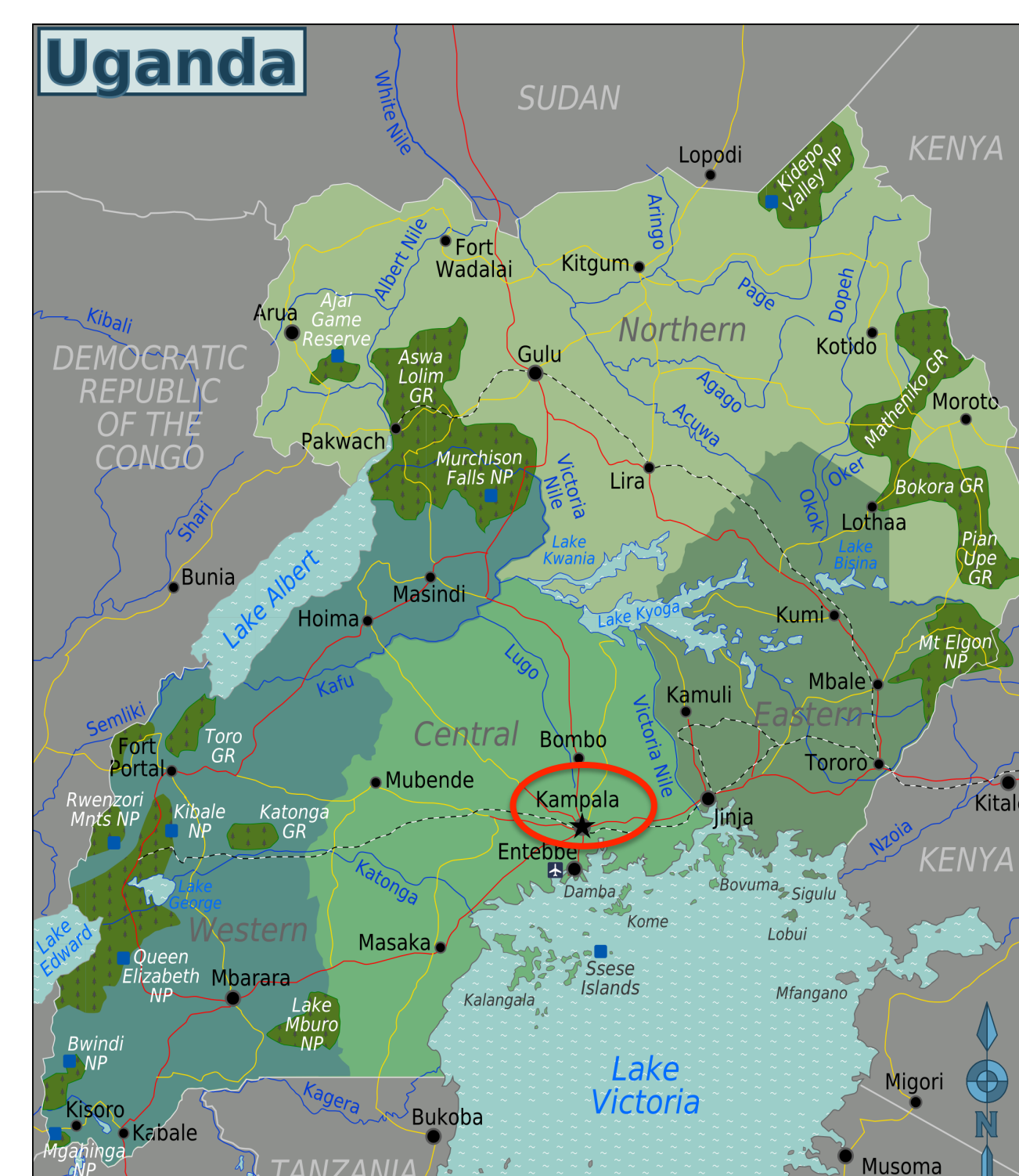


Fig. 1. Study site - Kampala, UG.

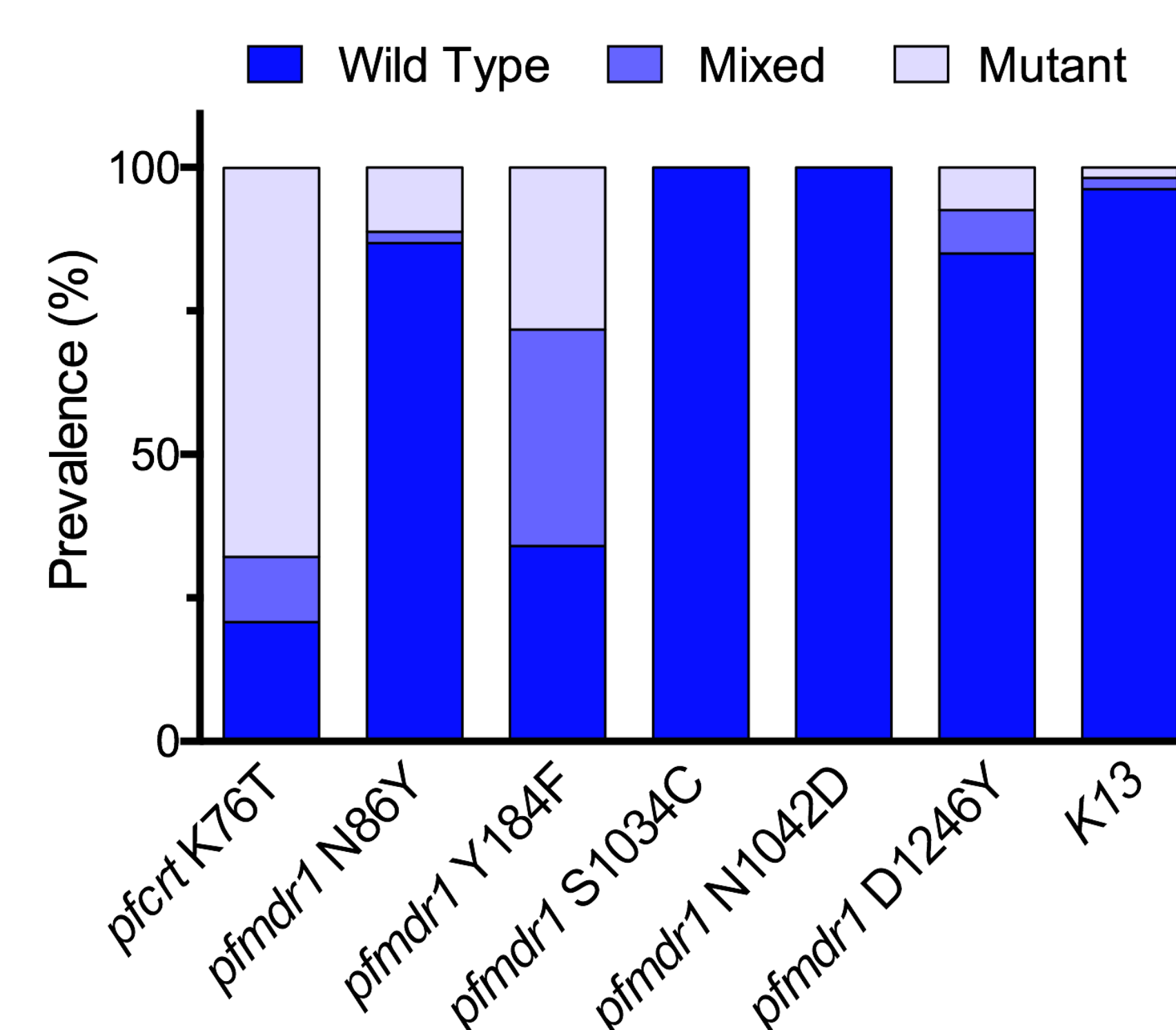


Fig. 3. Prevalences of wild-type, mixed, and mutant sequences at the indicated positions for all 53 isolates.

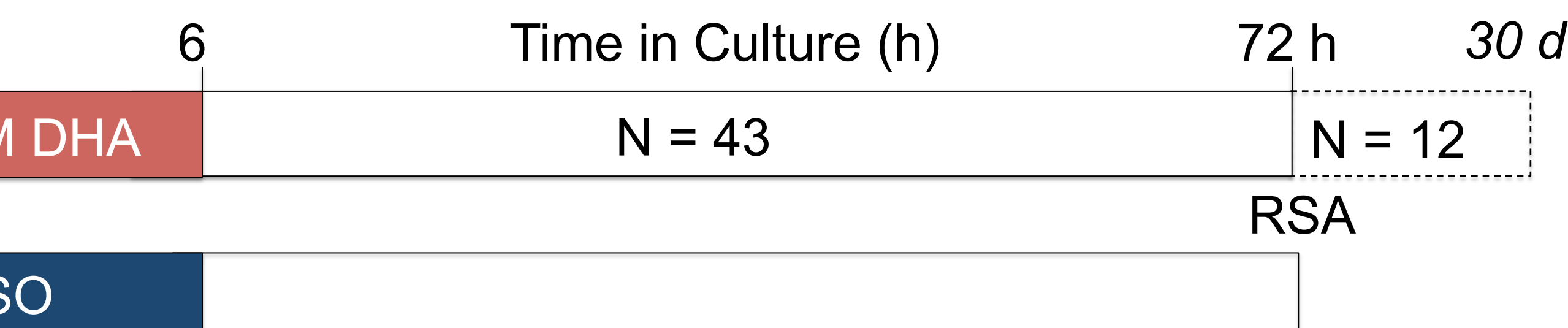


Fig. 2. Ring-stage survival assay.

- Ring-stage survival was 0% in 40/43 cultures
- Ring-stage survival ranged 0.7 - 1.9% in 3/43 cultures
- No association with survival and SNPs in *K13*, *pfprt* or *pfmdr1*
- Parasites reemerged from 10/12 long-term cultures after DHA pulse

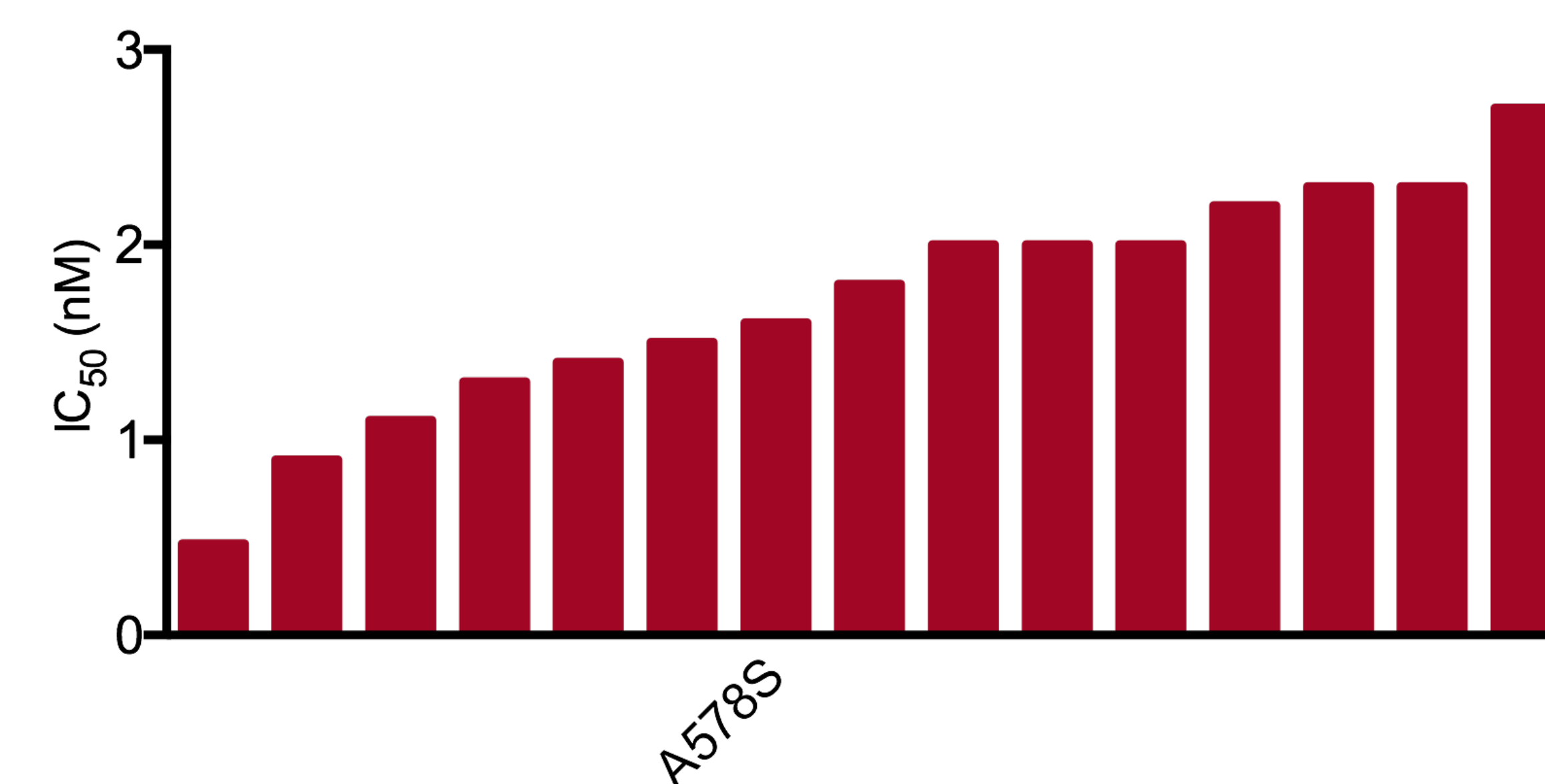


Fig. 4. *Ex vivo* IC₅₀ values for DHA from 15 isolates. The geometric mean IC₅₀ value was 1.6 nM.



Fig. 5. Two kelch 13 (*K13*) polymorphisms were detected from our samples. Numbered boxes indicate the six blades comprising the propeller domain of the kelch 13 protein.

Summary and Conclusions

K13 mutations were found in 2/53 parasite isolates from Kampala, but were not mutations associated with resistance in SE Asia.

RSA and IC₅₀ data showed that parasites remain highly sensitive to DHA *in vitro*.

The results of this study, as well as findings from other studies⁵, suggest that artemisinin resistance is not yet a problem in Uganda. The polymorphic nature of *K13* in Africa⁶ and altered ACT partner drug sensitivity in Uganda⁴ indicate the continued need for surveillance of ACT efficacy in the region.

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