MACEPA PATH
Malaria Control and Elimination Partnership in Africa

LB-5123

Government of the Republic of Zambia

PET-PCR method for supporting malaria elimination infection identification as part of a field trial in mass drug administration in southern Zambia

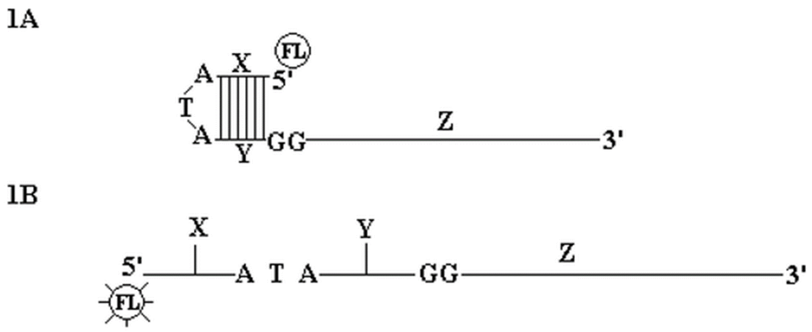
Mulenga Mwenda[1], Sandra Chishimba[1], Sampa Pal[1], Lindsay Yokobe[1], Conceptor Mulube[1], Brenda Mambwe[1] ,Moonga Hawela[2], Busiku Hamainza[2], Anthony Yeta[2], John M Miller[1], Thomas Eisele[3], Travis Porter[3], Adam Bennet[4], Daniel Bridges[1]

[1]PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia; [2]Ministry of Health, National Malaria Elimination Centre, Lusaka, Zambia; [3]Tulane University, New Orleans, USA; [4]University of California, San Francisco, USA

Background

- The Zambia National Malaria Elimination Centre (NMEC) is evaluating the effectiveness of mass drug administration (MDA) or focal MDA (fMDA) campaigns,[1] combined with good vector control and reactive case detection, as part of a comprehensive package of interventions for achieving malaria elimination in Southern Zambia.
- The identification and effective treatment of sub-patent infections[2] with or without a diagnostic[3] tool will be critical in achieving malaria elimination.
- PCR has a lower limit of detection (LOD) than any clinical diagnostic and is capable of identifying sub-patent infections.
- A multiplex real-time-PCR assay for the detection of *Plasmodium genus* and *P. falciparum* has been developed, using self-quenching photo-induced electron transfer (PET) fluorogenic primers (Figure 1).
- This study aimed to identify additional sub-patent infections below the LOD of the HRP2-based RDTs used to screen a cohort of individuals followed longitudinally in a randomized control trial.

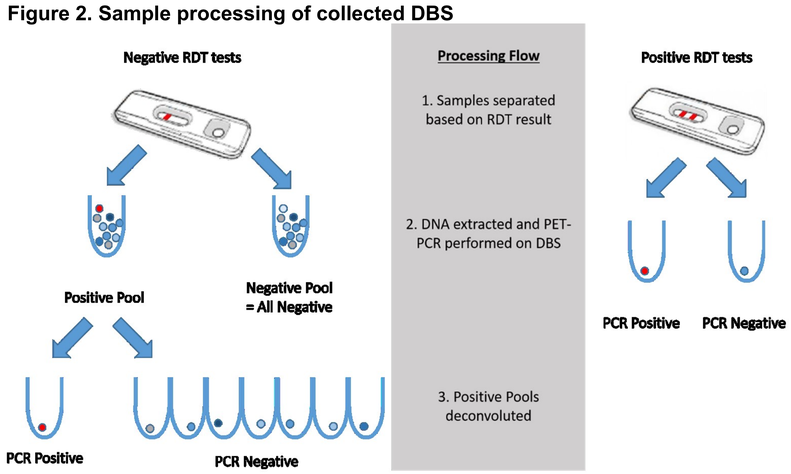
Figure 1. PET-PCR primer design



Methods

- DNA was extracted using QIAamp DNA mini kits from dried blood spots (DBS) collected from the cohort between December 2014 and May 2016.
- RDT-positive or negative samples with very limited blood were extracted individually, while RDT-negative samples were extracted in pools of 10 to conserve resources.[4]
- Extracted DNA was then analyzed by PET-PCR.[5]
- Samples with a CP (crossing point) value <40 were considered positive. Positive pools were deconvoluted by re-extracting DBS individually and reanalyzed (Figure 2).

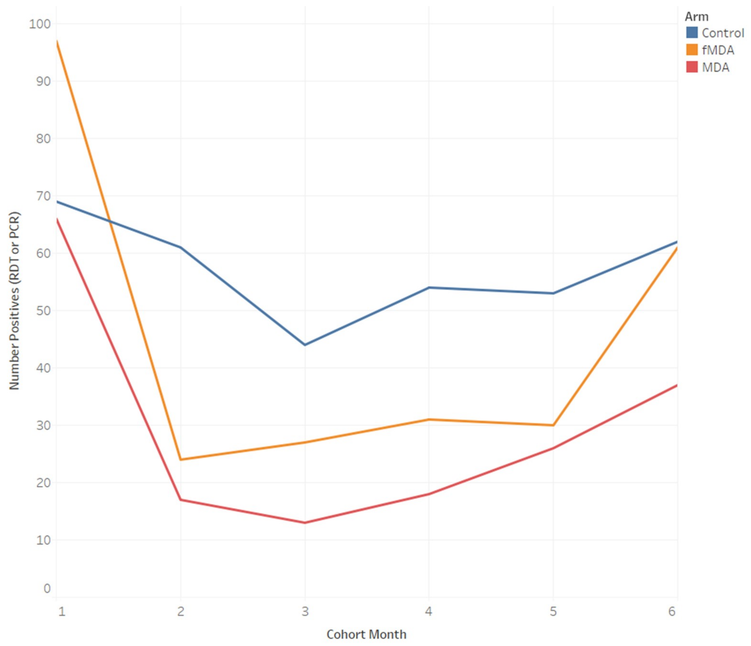
Figure 2. Sample processing of collected DBS



Results

- Approximately 34,000 cohort DBS were collected over the 18-month study period. RDT positivity was around 8% across all arms at baseline, but declined rapidly within the first few months. The MDA arm showed the greatest decline down to 1.8% by month.

Figure 3. Number of positive samples by RDT or PCR over the first 6 months of the study by arm



- 10,218 RDT-negative and 69 RDT-positive DBS were assayed by PET-PCR representing the first 6 months of the samples collected (Figure 3) of which:
  - 10,039 were PCR-negative.
  - 248 were PCR-positive.
- A number of samples showed discordance between the PCR and RDT results (Table 1).

Table 1. Agreement / discordance between PCR and RDT results

	PCR-positive	PCR-negative
RDT-positive	39	30
RDT-negative	189	10,029

- The NPR (below) demonstrates that a negative RDT is highly predictive of a true negative. Unfortunately the PPV shows that a positive RDT is only correct half the time:

$$Negative\ Predictive\ Value = \frac{10{,}029}{10{,}059} = 99.7\%$$

$$Positive\ Predictive\ Value = \frac{39}{69} = 56.5\%$$

- These values translate into a low sensitivity, but high specificity RDT test.

Results continued

$$Sensitivity = \frac{39}{218} = 17.5\%$$

$$Specificity = \frac{10{,}029}{10{,}218} = 98.2\%$$

- No significant additional trends were observed in the data when looking by health facility, arm, or other available factor.

Conclusions

- Some RDT-negative samples were found to be PCR-positive, this group may merit further evaluation to determine if there is evidence of ongoing transmission from these individuals in their household or immediate neighborhood.
- The limits of detection of RDTs and PET-PCR is reported to be around 100 parasites/µl and 3.2 parasites/µl respectively, thus the RDT-negative/PCR-positive samples could have parasite densities between these two limits.
- As expected, low PPV was observed because many of the RDT-positive individuals are likely to have been recently treated and cleared of parasites, and thus PCR-negative.

Next steps

- Complete sample analysis through to the end of the study period.
- Perform genotyping analysis on all PCR-positive samples to assess spatial and temporal relationships between samples as well as assess transmission intensity through multiplicity of infection.

Acknowledgments

- Thanks to all the volunteers who enrolled in the study, as well as government staff at district, provincial, health facility, and community level.

References

1. Eisele, T. P. *et al.* Assessing the effectiveness of household-level focal mass drug administration and community-wide mass drug administration for reducing malaria parasite infection prevalence and incidence in Southern Province, Zambia: study protocol for a community randomized controlled trial. *Trials* **16**, 347 (2015).

2. Okell, L. C. *et al.* Factors determining the occurrence of submicroscopic malaria infections and their relevance for control. *Nat Commun* **3**, 1237 (2012).

3. Wu, L. *et al.* Comparison of diagnostics for the detection of asymptomatic Plasmodium falciparum infections to inform control and elimination strategies. *Nature* **528**, S86–S93 (2015).

4. Hsiang, M. S. *et al.* PCR-based pooling of dried blood spots for detection of malaria parasites: optimization and application to a cohort of Ugandan children. *J Clin Microbiol* **48**, 3539–3543 (2010).

5. Lucchi, N. W. *et al.* Molecular diagnosis of malaria by photo-induced electron transfer fluorogenic primers: PET-PCR. *PloS one* **8**, e56677 (2013).