

## 1. Background to Malaria Institute at Macha (MIAM)

Born by Macha Mission Hospital ...  
Research to fight malaria and find new drugs (1989 -)

MHMRI registered with GOZ 1994  
“..malaria research: drug trials, prevention and control.”

1997 MMRI registered non-profit, USA  
Vector research, incidence studies  
New office block & laboratory space (2001)

2003 MOU (Macha Mission Hospital, MMRI, GOZ, JHMRI)  
“to develop a malaria field research and training centre”  
-new lab. & office facilities, birth of MIAM

MIAM official opening, Jan. 2005

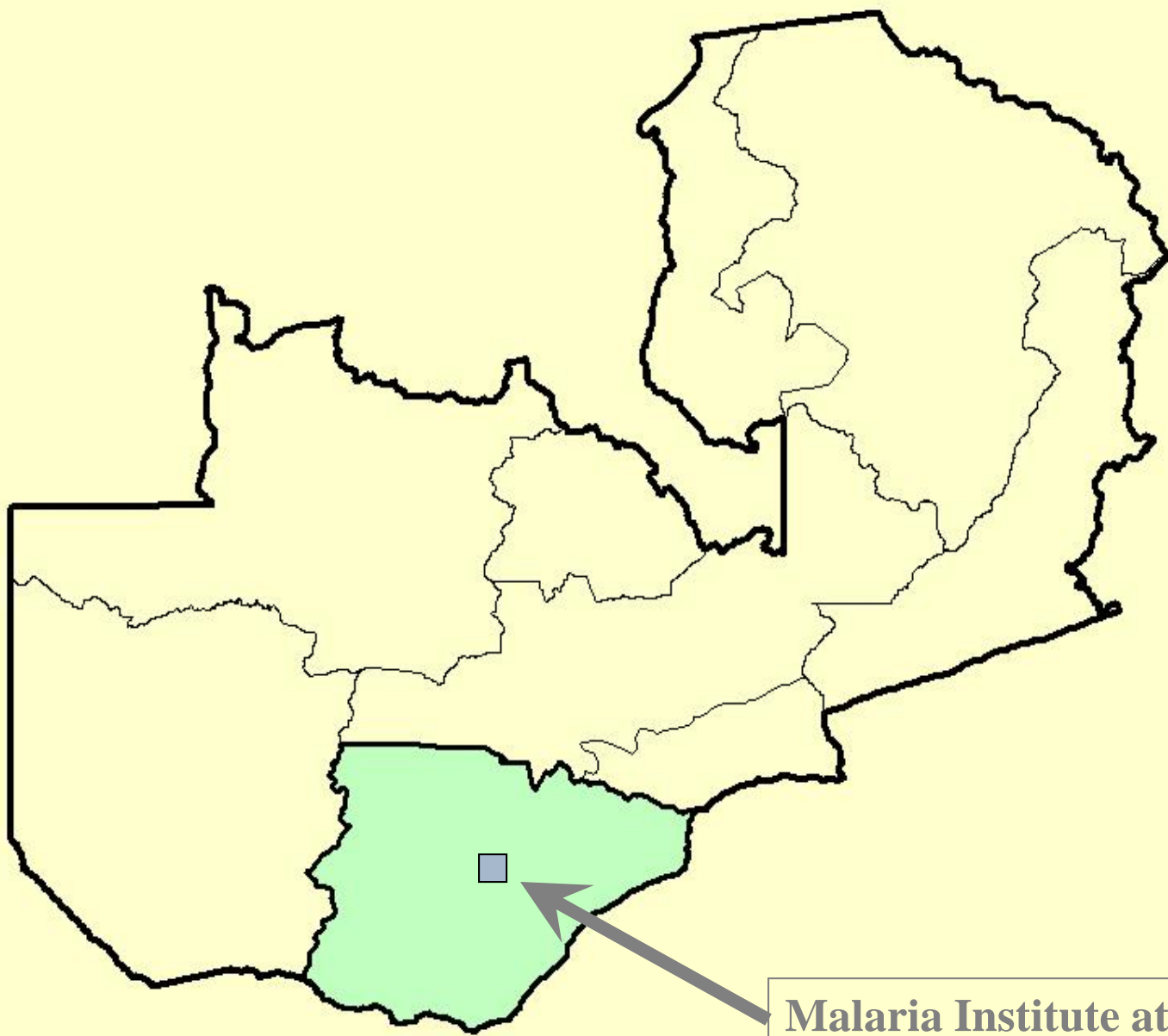




southern africa

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**Malaria Institute at Macha**



## 2. Brief Overview of Current Research at MIAM

Area		FNDP	MDG
Malaria	Epidemiology, transmission, drug resistance	Pgme 2, BHCP Pgme 3, Mal prevent. contrl.	6
	Non-invasive diagnostics	Pgme 2, 3	6
	Malaria elimination	Pgme 3	1, 4, 5, 6
	Surveillance systems	Pgme 3	1, 4, 5, 6
	Entomology	Pgme 2, 3	6
HIV/TB	ART, drug resistance, treatment, prevention	Pgme 4, HIV, AIDS, STIs Pgme 5, TB contr.	1, 4, 5 6

### MGDs

- \* Goal 1: Eradicate extreme poverty and hunger
- \* Goal 2: Achieve universal primary education
- \* Goal 3: Promote gender equality and empower women
- \* Goal 4: Reduce child mortality
- \* Goal 5: Improve maternal health
- \* Goal 6: Combat HIV/AIDS, malaria and other diseases
- \* Goal 7: Ensure environmental sustainability
- \* Goal 8: Develop a Global Partnership for Development

### FNDP (Zambia Fifth National Development Plan)

- \* Programme 2: Basic health care package (BHCP)
- \* Programme 3: Malaria prevention and control
- \* Programme 4: HIV, AIDS, STI
- \* Programme 5: TB control



# Development of Saliva Test for Malaria

## Background

- ❑ New hope for curbing the malaria scourge
  - RBM, public-private partnerships
  - Global scale-up of effective malaria intervention ACT's, ITN's, IRS
  - Possible interruption of transmission
- ❑ Formidable disease resilience necessitates:
  - efficient **epidemiological surveillance**
  - resurgence/drug resistance
  - accurate screening for ***asymptomatic reservoirs*** essential



# Current *P. falciparum* screening constraints

## Necessitates drawing blood

- Finger-prick
- Venipuncture

## Limitations

- requires use of needles or sharps in remote settings
  - Adequately trained personnel –not readily available
    - VHW level
    - Home level
  - Biohazard, accidental infection risk
- certain communities: blood taboos, beliefs etc
- Repeated testing -drug/vaccine efficacy trials/monitoring
- Low access to potential reservoirs for research/control programmes

Important need for non-invasive detection



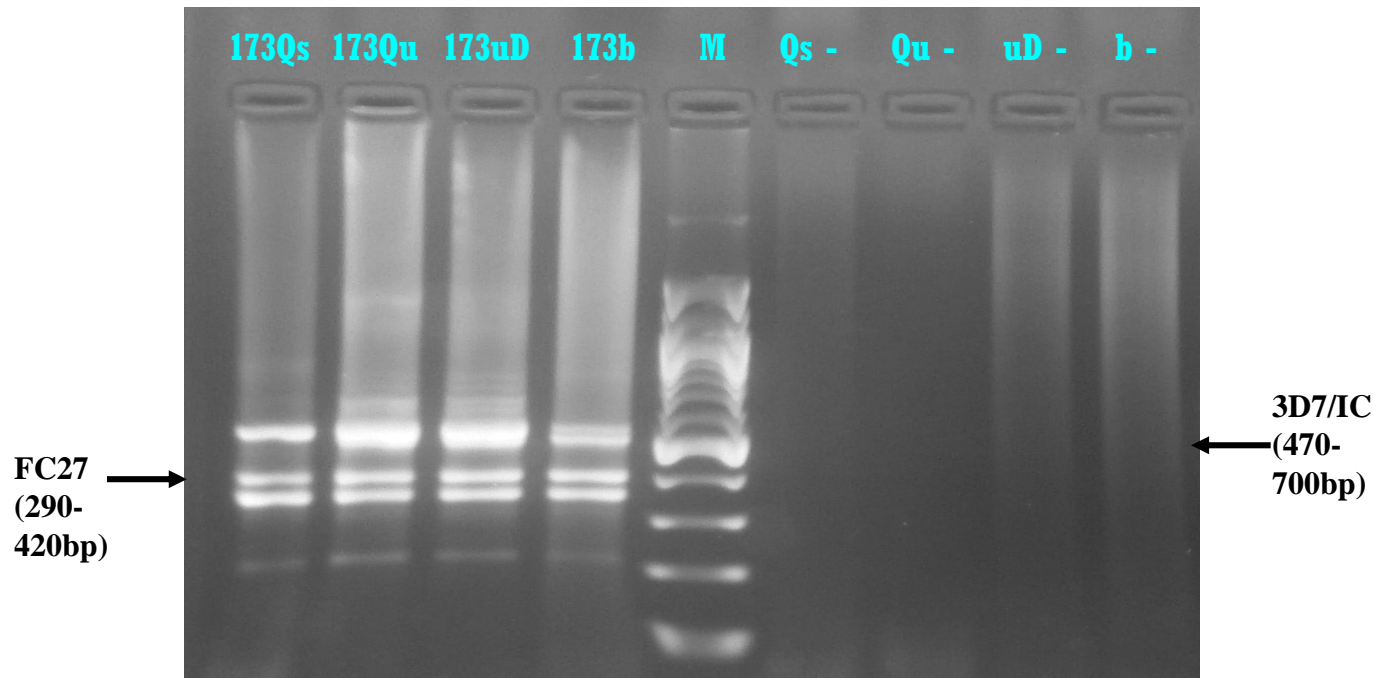
## Why saliva screening?

- Non-invasive, simpler, safer
- Greater community participation/co-operation
- Greater access to subclinical reservoirs for research/control programmes
- Strengthen research/surveillance
- Reduced sample collection cost/workload



## DNA Detection:

*Is the infection in saliva the same as in blood sample?*



*Plasmodium falciparum* MSP2 amplicon from saliva (173Qs), urine (173Qu, 173u) and blood (173b) samples of patient 173. Qs and Qu denote saliva and urine samples extracted by Qiagen commercial kit, while uD denotes whole urine sample extracted by the Chelex method. Qs-, Qu-, uD- and b-, denote amplicon from corresponding extracts of saliva, urine and blood provided by thick film negative healthy control. 3D7, amplicon from positive control laboratory standard. Extraction of urine replicate sample 173u was performed on 20.02.06, while Qiagen extractions were carried out on 09.02.06. Identical MSP2 alleles are apparent in amplicon from urine, saliva and blood samples of patient 173, as compared to 3D7 lab standard.



## DNA Detection:

*Is the infection in saliva the same as in blood sample?*

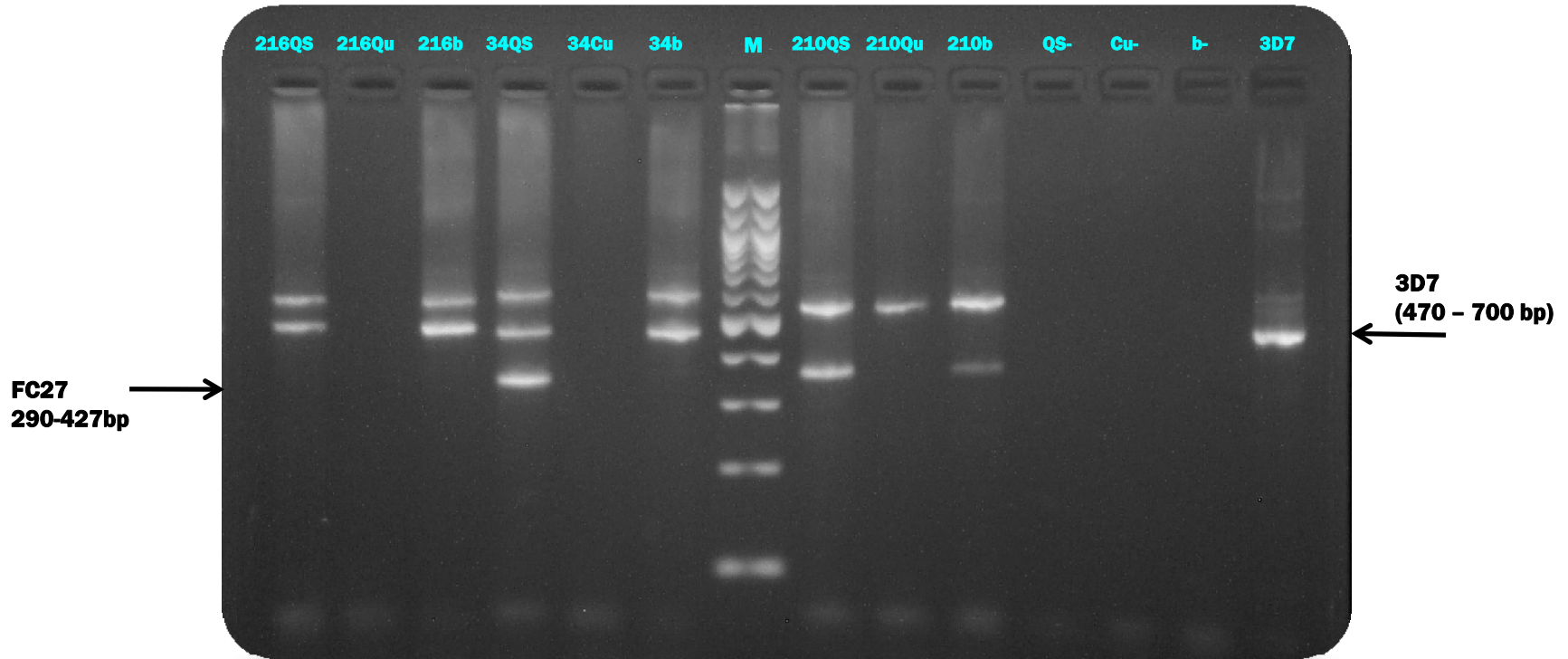


Figure 1 Amplicon from saliva, urine and blood extracts of field samples 216, 34 and 210. Matching MSP2 alleles are seen in saliva (Qs) and blood (b) amplicon from each individual. In contrast, between-patient polymorphic differences are evident, reflecting diverse infections. Urine samples from these patients did not amplify, except that of 210 (210Qu). Qs, Qu denote Qiagen saliva and urine extracts, respectively, by crude lysate approach; Cu denotes Qiagen urine extracts, by cultured animal cells protocol; Du denotes Chelex direct extraction on whole urine. Qs-, Cu-, b- were corresponding extracts of saliva, urine and blood samples from healthy negative control, while 3D7 was positive control laboratory standard



## Is *P. falciparum* DNA detection in saliva repeatable?

### ❑ Yes, by range of genomic primers and extraction methods, machines...

MSP2 -chr 2	✓ Chelex on filter paper
PfDHFR - chr 4	✓ Whatman FTA classic card
PfDHPS -chr 8	✓ Whatman 903
Pfmdr1 -chr 5	✓ Qiagen DNEasy kit
Pfcrt -chr 7	✓ Oragene kit
TA81 -chr 5	✓ Polyethylene glycol (PEG)
18S rRNA gene (chr 1, 5, 7, 11)	

### ❑ Yes, extracted at different time points with different methods

### ❑ Yes, other investigators, using Real-time platform:

- ❑ Nwakanma *et al* (2007) *Am J Trop Med Hyg* **77** (Suppl.): 233.
- ❑ Nwakanma *et al* (2009) *J Infect Dis* **199** (11) 1567-74.



# What are the determinants of amplicon yield?

FACTOR	SUMMARY
Extraction method	Higher amplicon with Qiagen than Chelex Urine: 2.24X higher Saliva: 2.25X higher
Sample type	Saliva extracts: 1.6 fold higher amplicon yield than urine
Parasite density	For each unit increase in log parasite density: 1.82-fold increase in amplicon yield
Primer set	U1-U4 (370bp, 229bp) 18.5X more likely to amplify than FC27 (750bp, 290-420bp)



## Conclusions

- ❖ *P. falciparum* infection detectable using saliva samples
- ❖ Infection detected in saliva identical to that found in blood
- ❖ Sound prospects of oral-based malaria testing

## Questions/Future Work

- Peak season surveys and assay optimizations
- What is the source of *P. falciparum* material in the saliva?
- Does saliva secretion gland matter?
- NYU collaboration

