Recent Experience of Investigational Parasite Detection Methods in Controlled Human Malaria Infection Studies

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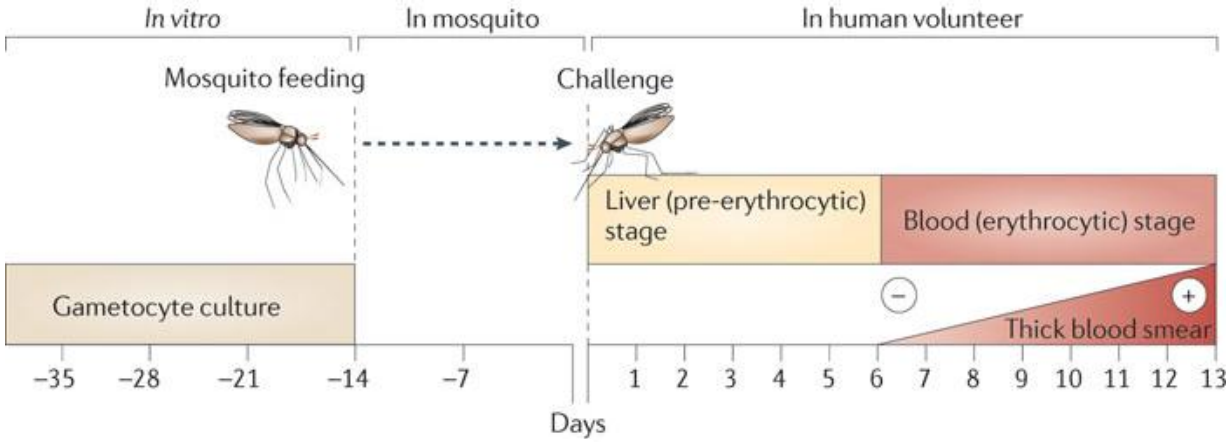
Disclosures

- Biofire Defense (consulting)
- Novartis (clinical trial support)

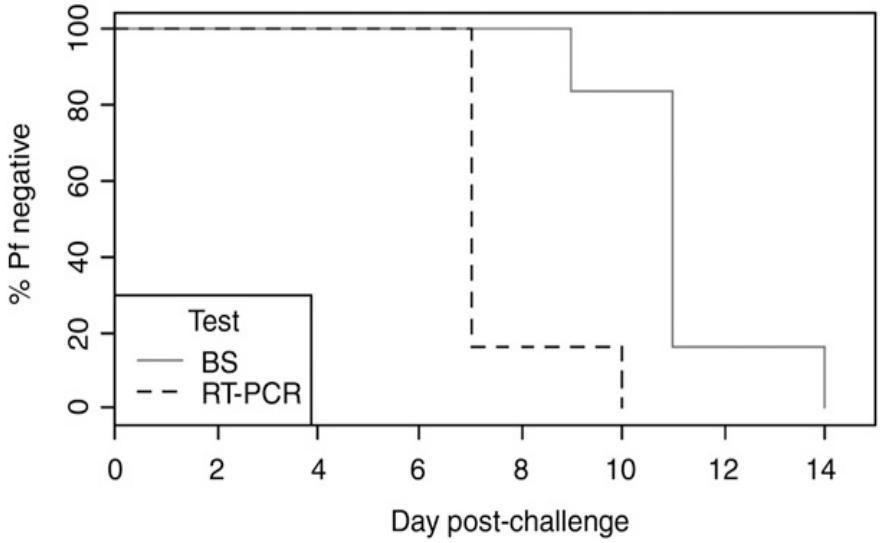
Outline

- *Plasmodium* 18S rRNA/rDNA
- Characteristics of NATs at major CHMI sites
- NAT biomarker kinetics in different CHMI study designs
- NAT-based rescue treatment thresholds
- Appropriate sampling frequency
- Recrudescence vs. gametocytemia

NAT-based approaches accelerate infection detection.



Seattle Biomed MC-001 Demo Trial by Pf 18S rRNA qRT-PCR assay

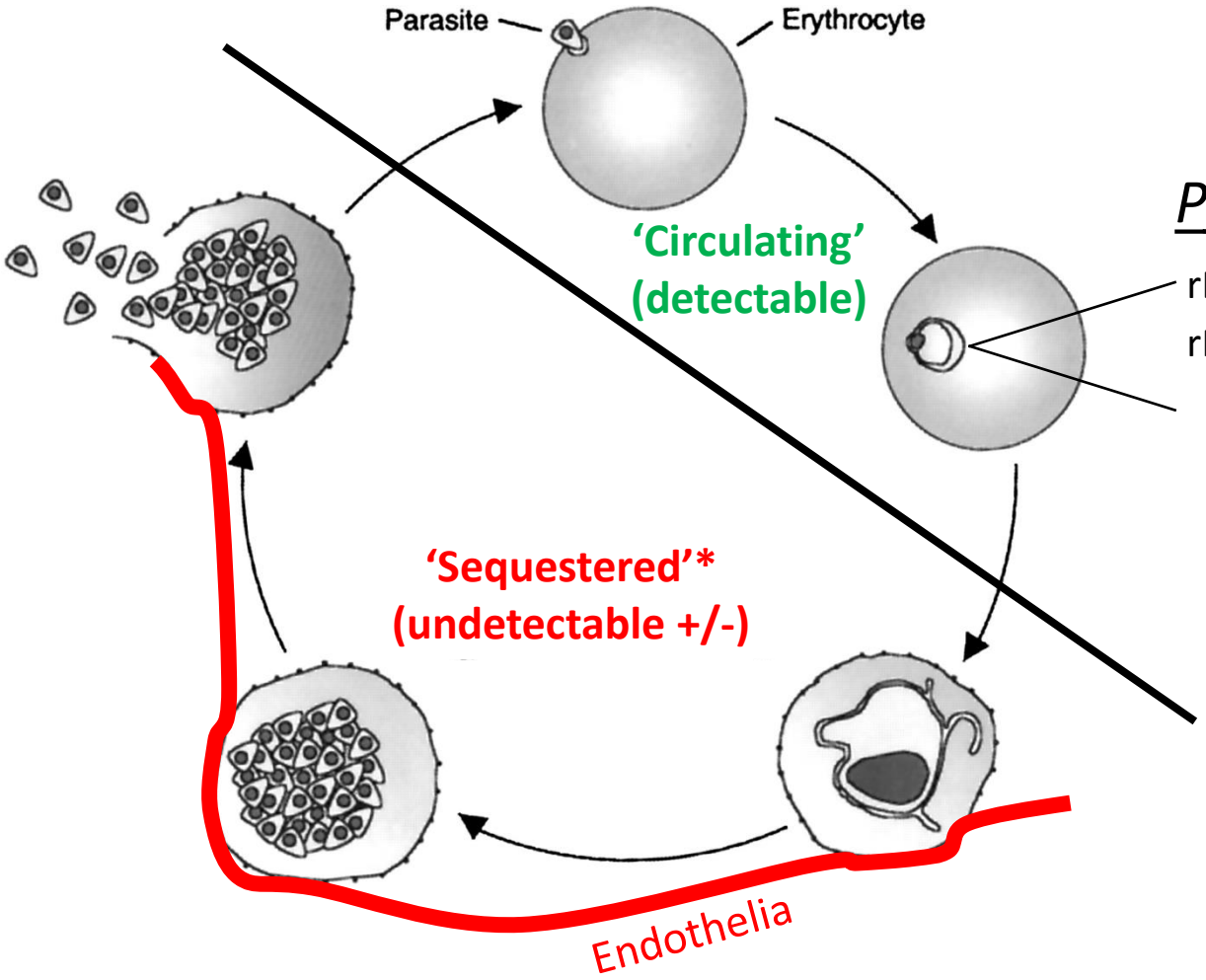


Top: Sauerwein et al. 2011. *Nature Reviews Immunology* 11:57; Bottom: Murphy et al. 2012. *AJTMH* 86:383

Detection of *Plasmodium* nucleic acids

- Extraction from whole blood
  - DNA *or* RNA *or* Total nucleic acid
- Reverse transcription or cDNA synth (if applicable)
- DNase treatment (if pfs25/pvs25 testing)
- Real-time PCR (usually with hydrolysis/Taqman probes)
  - Quantitative *or* Qualitative
  - Most assays target *Plasmodium* 18S rRNA/rDNA
    - A few non-CHMI labs target multi-copy non-18S rRNA targets or mitochondrial cytochrome oxidase subunit 1, etc.

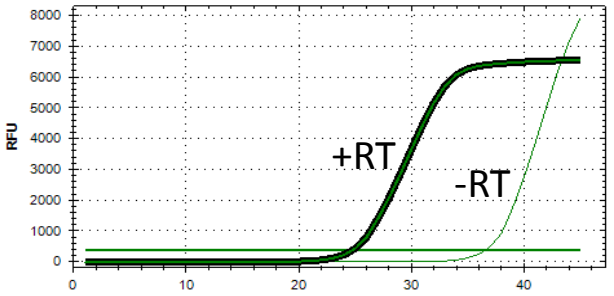
Plasmodium 18S rRNA/rDNA is the most common molecular biomarker in CHMI studies.



Pf 18S rRNA/rDNA

rDNA: 2 A-type and 2 S-type

rRNA: 3,500-10,000 A-type rRNAs



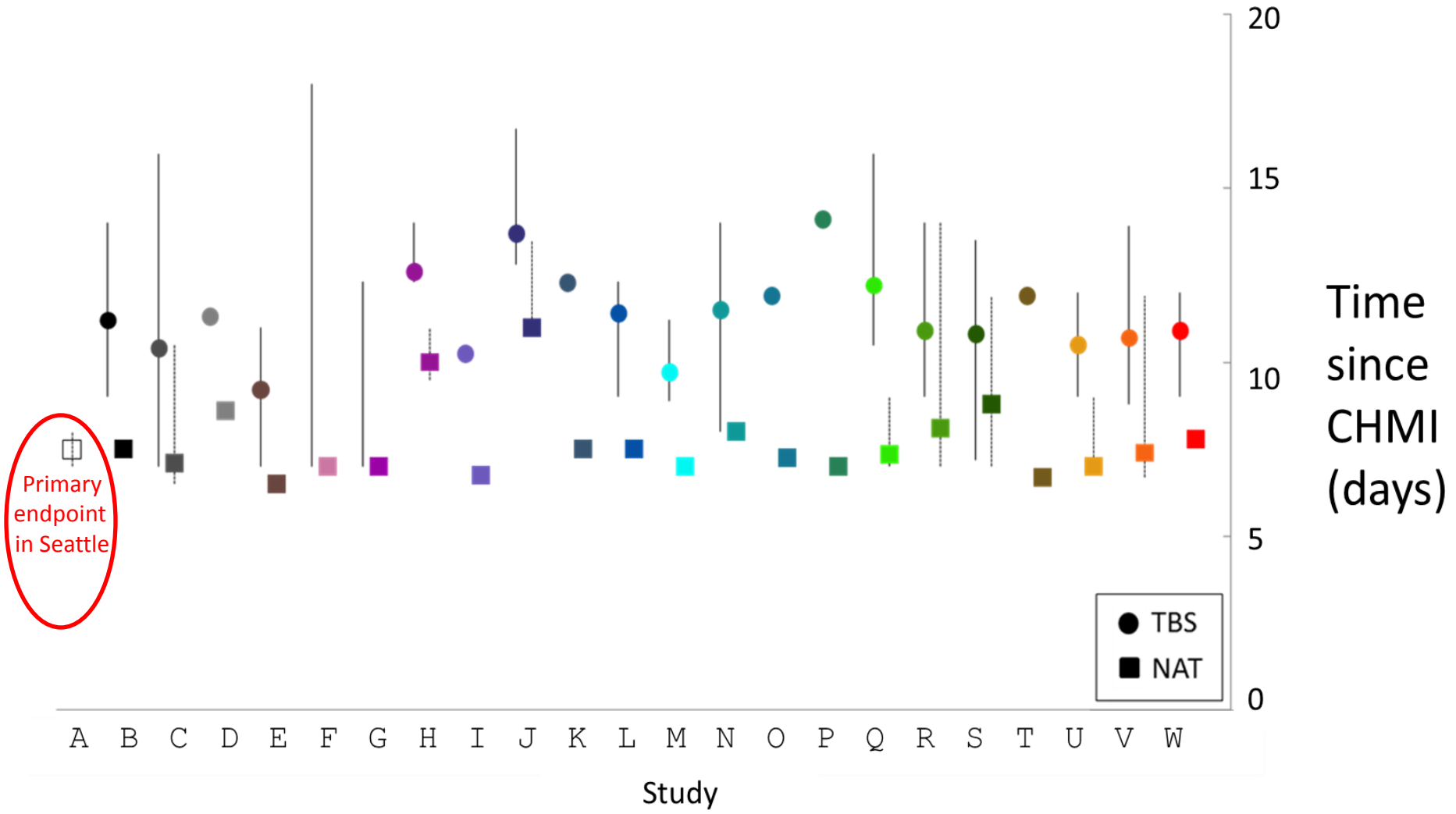
**P. falciparum* as shown

Pv/Po/Pm/Pk do not sequester

Gametocytes also express 18S rRNA (not shown)

Adapted from Kirk. 2001. *Physiol Rev* 81(2):495-537.

P. falciparum 18S rRNA/rDNA NATs are positive 2-5 days earlier than TBS.



PMID #s: A: unpublished; B: 25405724 & 22403305; C: unpublished; D: 25889522; E: Unpublished; F: 19641203; G: 21514658; H: 22615322; I: 23149582 & 25776749; J: 22701640; K: 25070995 & 25776749; L: 18231580; M: 23089736; N: 23089736; O: 25336730; P: 25627033; Q: 25566206 & 25627033; R: 23457473; S: 21903775; T: 23929949; U: 25066459; V: 19390585; W: 23874828; X: 21042404; Y: Unpublished

Performance characteristics of NATs at major CHMI sites

Plasmodium 18S rRNA/rDNA NATs

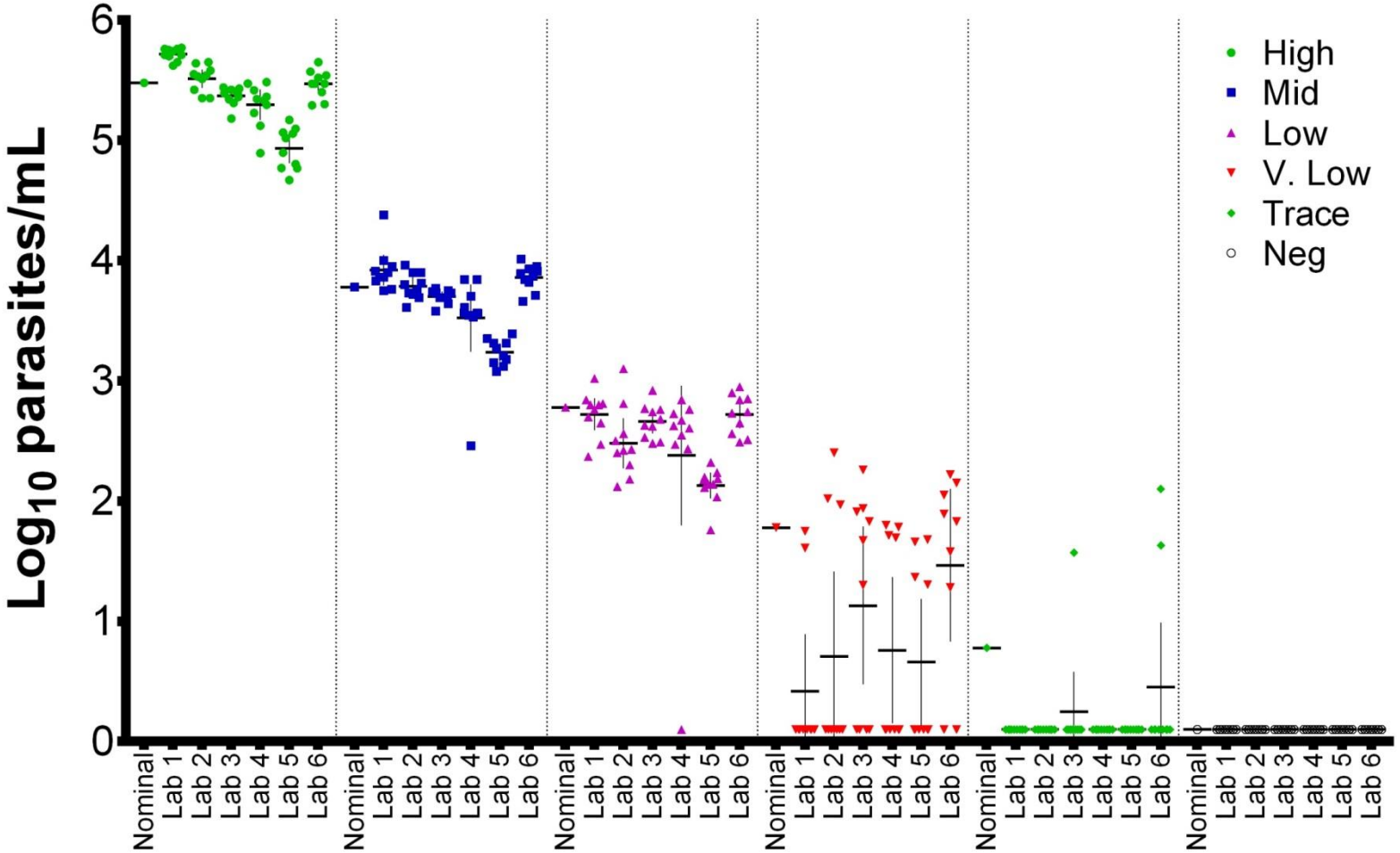
CHMI site	18S target		Extraction	Analytical Sensitivity (est. para/mL)	Volume (μL)	Ref.
	rRNA	rDNA				
UW/Seattle MCTC	A	A	Abbott m2000 sp	20	50 (into LB)	1
U. Maryland		S	Manual QIAamp DNA Mini Kit	20	500	2
VRC/NIH Clin Ctr		A	bioMerieux easyMag	500	200 (into LB)	3
Jenner/Oxford		S	Qiagen QIAsymphony	10	500 (filtered)	4
RUMC/Nijmegen		S	Roche MagNA Pure	20	500	5
QIPD/QIMR/Brisbane		S	Manual QIAamp DNA Mini kit	64	250 (pRBC)	6
Tübingen	A/S	A/S	Qiagen QIAsymphony	5	500	7

References: [1]Murphy et al 2012. *J Clin Micro* 50:4128; [2]Lyke et al 2010 *PLoS One* 5:e13490; [3]Seder et al 2013 *Science* 341:1359; [4]Sheehy et al 2013 *PLoS One* 8:e65960; [5]Schats, Bijker et al 2015 *PLoS One* 10:e0124243; [6]Rockett et al 2011 *Malar J* 10:1; [7]Unpublished (not yet included in EQA study)

A, A-type 18S rRNA; S, S-type 18S rRNA; LB, bioMerieux NucliSENS lysis buffer; Prim, primary; Sec, secondary

Summary data current as of June 29, 2016

Comparable EQA performance amongst sites



EQA sample results were plotted (log_{10} parasites/mL) for participating laboratories (bars = mean +/- 95% confidence interval). Nominal (expected) values were High (300,000 parasites/mL); Mid (6,000 parasites/mL); Low (600 parasites/mL); Very Low (60 parasites/mL), Trace (6 parasites/mL) and Negative (no parasites;). Samples with no parasites detected were plotted as 0.1 log_{10} parasites/mL. *Lab 2 quantities were generated by regression of C_T values to expected EQA values and are provided to visualize variation and qualitative agreement. Full data with the exception of Lab 6 data are available in Murphy et al. 2014. PLoS One 9(5): e97398.

NAT biomarker kinetics in different CHMI study designs

CHMI by different routes, different stages?

- Mosquito bite vs. intravenous sporozoites:
  - Does not change the duration of LS
  - No indication for NAT testing on Days 0-5
  - No difference in NAT kinetics
- Sporozoite vs. iRBC challenge:
  - NAT positivity depends on assay LoD and parasite density.
  - Sporozoite challenge: sufficient iRBCs usually present by Day 7-8
  - iRBC challenge: sufficient iRBCs usually present by Day 4

LoD Considerations

ROUTE 1: 5 mosquitoes

(# sporozoites/mosquito)

% of sporozoites injected

ROUTE 2:
3500 spz by DVI

% that successfully invade

~$3x10^4$ merozoites/hepatocyte
x # of infected hepatocytes
= Max. # iRBCs upon emergence

Day (spz)	Tot. Para./Total RBCs	= Est. para/mL (%)
1-6	Undetectable (10 iHep)	= 0 (0.0%)
6.5	$3x10^5/2.25x10^{13}$	= 60 (0.000001%)
8.5	$3x10^6/2.25x10^{13}$	= 600 (0.00001%)
10.5	$3x10^7/2.25x10^{13}$	= 6000 (0.0001%)

Day (iRBC)	Tot. Para./Total RBCs	= Est. para/mL (%)
0	$1.8x10^3$/2.25E13	= <1 (0.000000007%)
2	$1.8x10^4/2.25x10^{13}$	= 3.6 (0.00000007%)
4	$1.8x10^5/2.25x10^{13}$	= 36 (0.0000007%)
6	$1.8x10^6/2.25x10^{13}$	= 360 (0.000007%)
8	$1.8x10^7/2.25x10^{13}$	= 3600 (0.00007%)

ROUTE 3:
Infected RBCs by needle inoculation
$1.8x10^3$ iRBCs

~10-fold rise/48 hr starting D6.5 (spz) or D0 (iRBC)

NAT positive at D6.5-9 post-spz or D4 post-iRBC

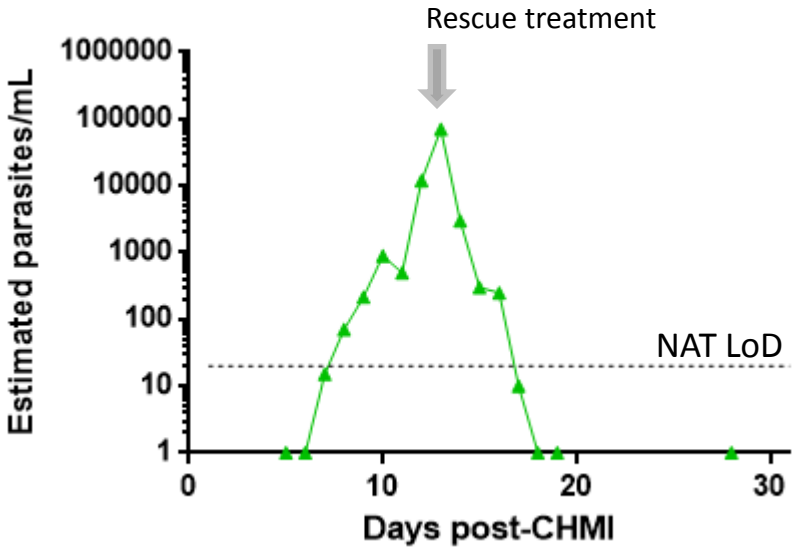
TBS positive at D10-13 post-spz or D9-11 post-iRBC

Positive result if NAT LoD is 10-100 est. para/mL.

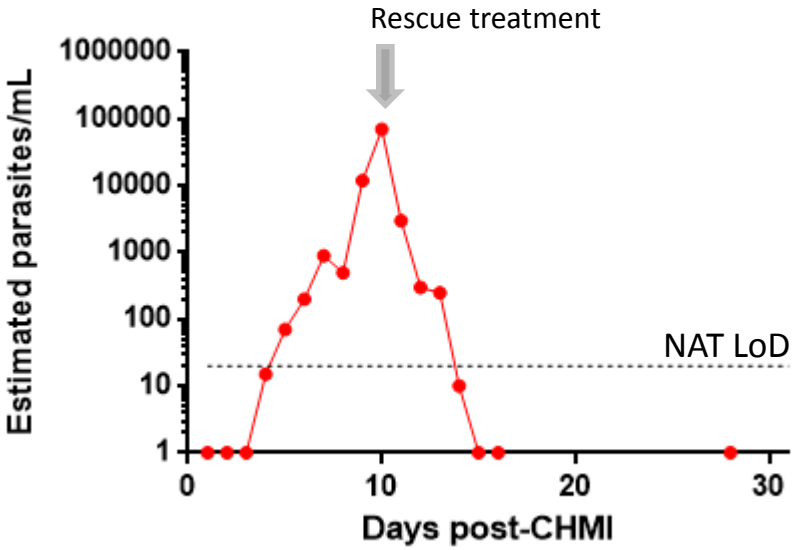
Based on CHMI data from Andrews et al 2005 *AJTMH*; Bejon et al 2005 *JID*; McConkey et al 2003 *Nat Med*; Walther et al 2005. *Vaccine*. Roestenberg et al 2009 *NEJM* 361:468

CHMI using TBS-based rescue

Sporozoite inoculum



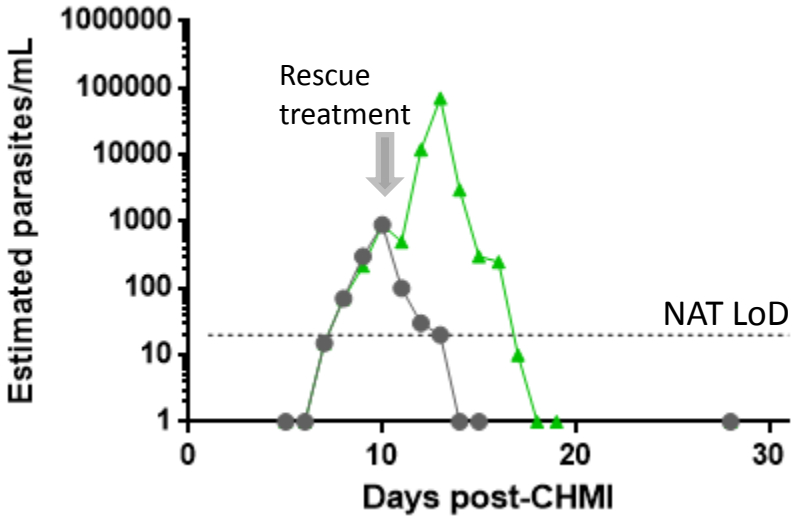
iRBC inoculum



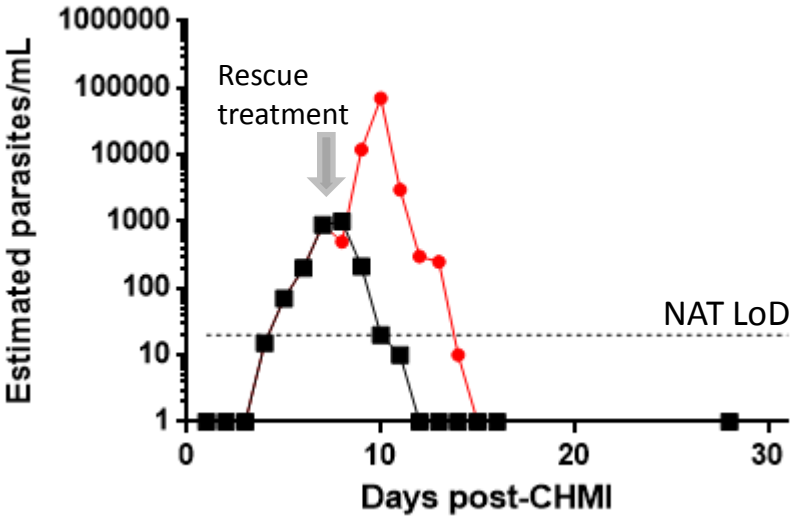
Inoculum:	5 mosquito bites or 3500 DVI	$1.8x10^3$ *P. falciparum*-infected RBCs
Time to positivity if naïve/no drug treatment:		
NAT(+)	Usually by D7-8	Usually by D4
TBS(+)	Usually by D10-13	Usually by D9-11
Rescue Rx	Upon TBS positivity	Upon TBS positivity

CHMI using NAT-based rescue

Sporozoite inoculum



iRBC inoculum



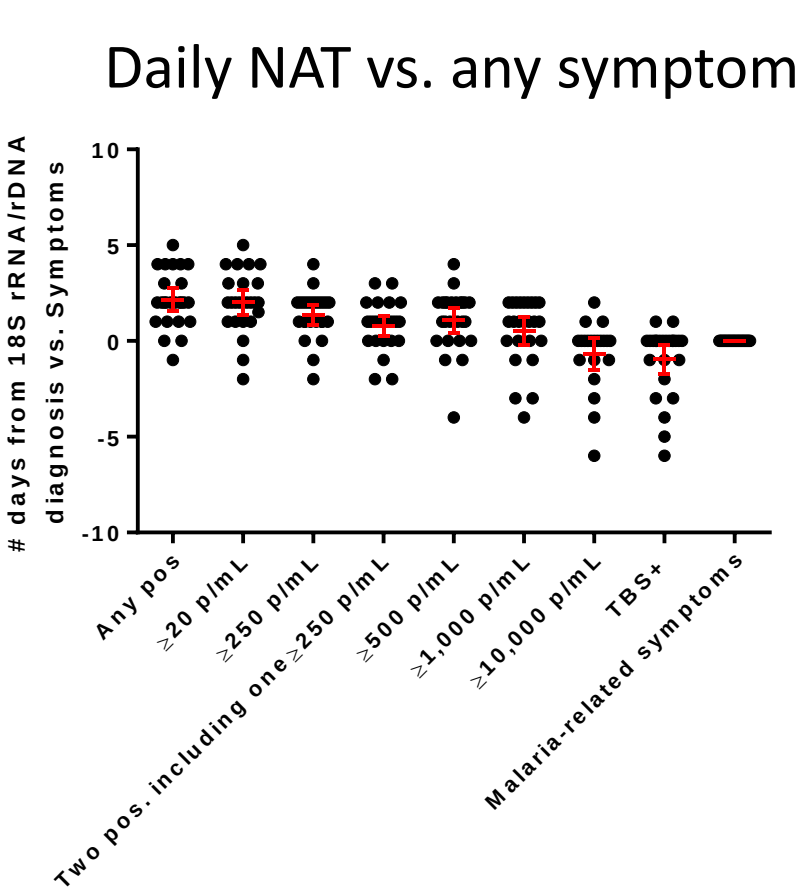
Inoculum:	5 mosquito bites or 3500 DVI	1.8×10^3 *P. falciparum*-infected RBCs
Time to positivity if naïve/no drug treatment:		
NAT(+)	Usually by D7-8	Usually by D4
TBS(+)	Usually TBS-negative	Usually TBS-negative
Rescue Rx	Usually by D8-10	Usually by Day 7-9

Study-specific NAT rescue thresholds

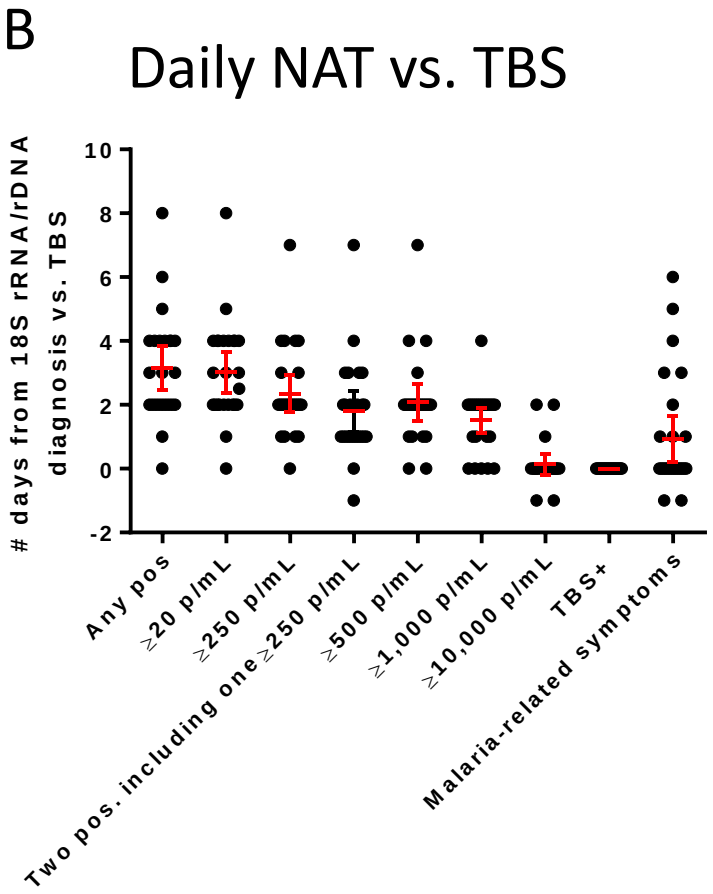
- **PROPHYLAXIS** at liver and/or RBC stage
  - Goal: Trigger rescue treatment; minimize symptoms
  - Rescue threshold range ~100-500 est. p/mL
    - Seattle MCTC/UW: 250 est. para/mL (daily testing)
    - RUMC/Nijmegen: 100 est. para/mL (daily testing)
    - VRC: Two positive results (LoD ~500 est. para/mL)
    - Some centers still rely on TBS for safety endpoint (>$2x10^4$/mL)
- **RADICAL CURE** at RBC stage
  - Goal: generate safe/adequate RBC-stage infection, initiate experimental dosing and monitor for clearance/recrudescence
  - Higher threshold than for liver stage prophylactic studies
    - QIMR: 800-1,000 est. para/mL (Pasay et al. 2016 *JID* doi: 10.1093/infdis/jiw128)

Defining a rescue treatment threshold

A



B



Time (days) from positive NAT at the indicated threshold (x-axes) to malaria-related symptoms (A) or TBS positivity (B). Example data from once daily testing in a closed Seattle-based study. 'Any positive' indicates all positives including unquantifiable low positive results. Symptoms include all protocol-defined malaria-associated symptoms including headache, fever, chills, abdominal pain, myalgia, low back pain and nausea. TBS served as the primary study endpoint. Red bars, mean +/- 95%CI.

Rescue treatment threshold modeling

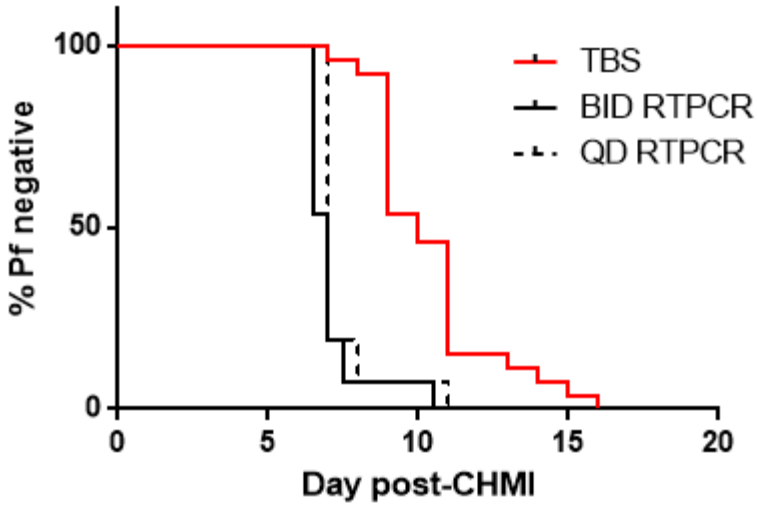
p values for Thresholds compared to:	TBS positivity	Any symptom
Any positive RT-PCR including Low Positives	****	****
First positive RT-PCR ≥20 p/mL	****	****
First positive RT-PCR ≥250 p/mL	****	**
First positive RT-PCR ≥500 p/mL	****	0.06
Two positive RT-PCR including one ≥250 p/mL	***	0.28
First positive RT-PCR ≥1,000 p/mL	**	0.73
First positive RT-PCR ≥10,000 p/mL	0.99	0.52
TBS positive	NA	0.13
Malaria-related symptom onset	0.13	NA

**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (one-way ANOVA)

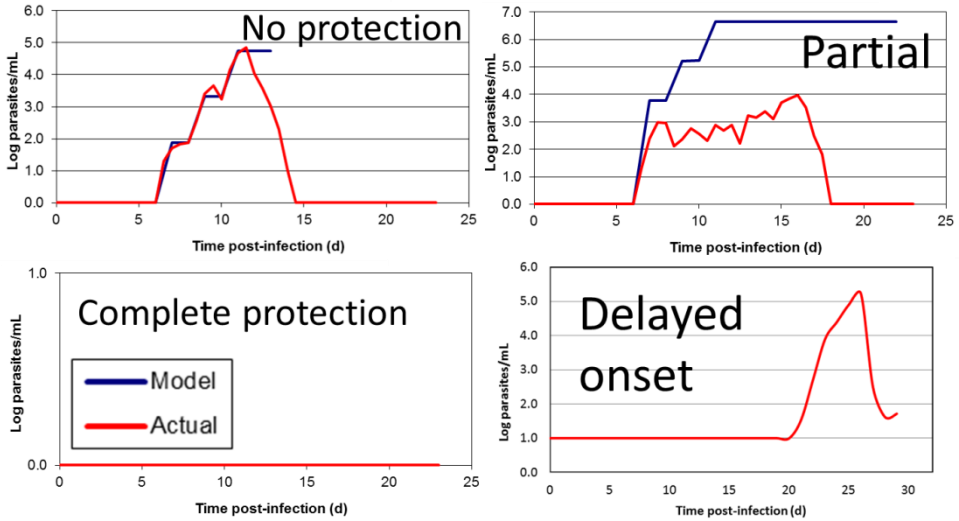
What is the appropriate sampling frequency?

Daily testing is suitable for infection detection...

Infection detection post-sporozoite CHMI



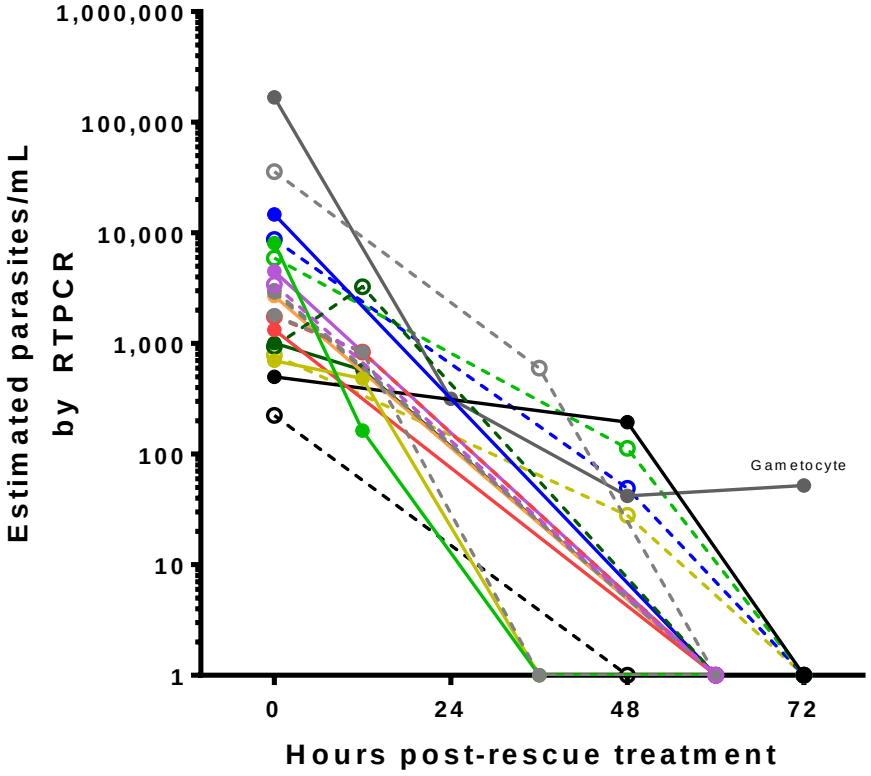
Models that estimate liver burden rely on the maximum estimated density on the first day of positivity.

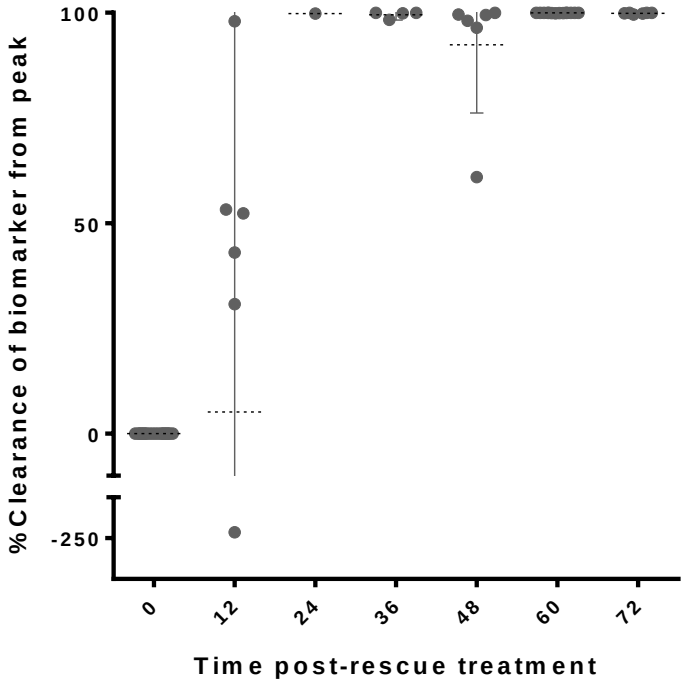


Some centers increase sampling on D7-8.

Left: Seattle MCTC/CIDR, Right: example model calculations based on Hermsen (2004 *AJTMH*)

Biomarker clearance is rapid following rescue treatment with FDA-approved drugs (atovaquone-proguanil shown).





20 subjects, Seattle MCTC

More frequent sampling is used for modeling clearance in experimental radical cure studies.

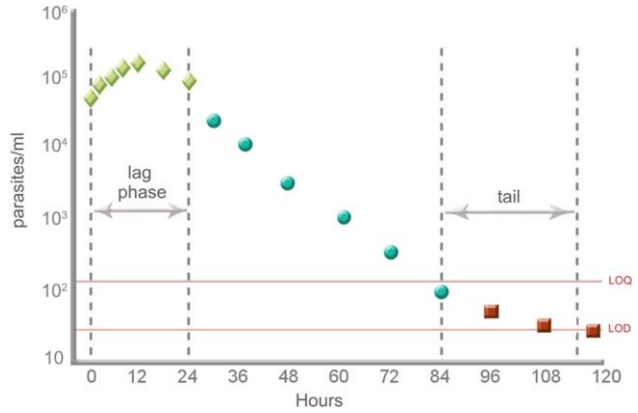
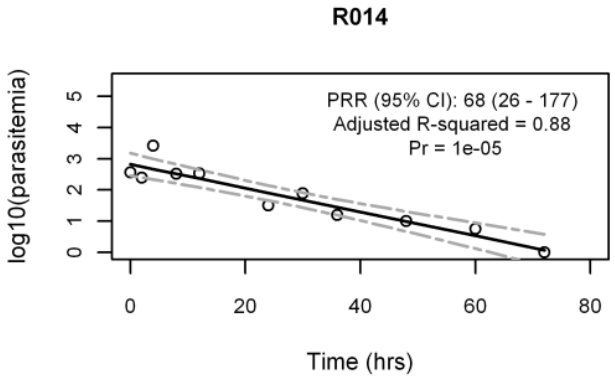
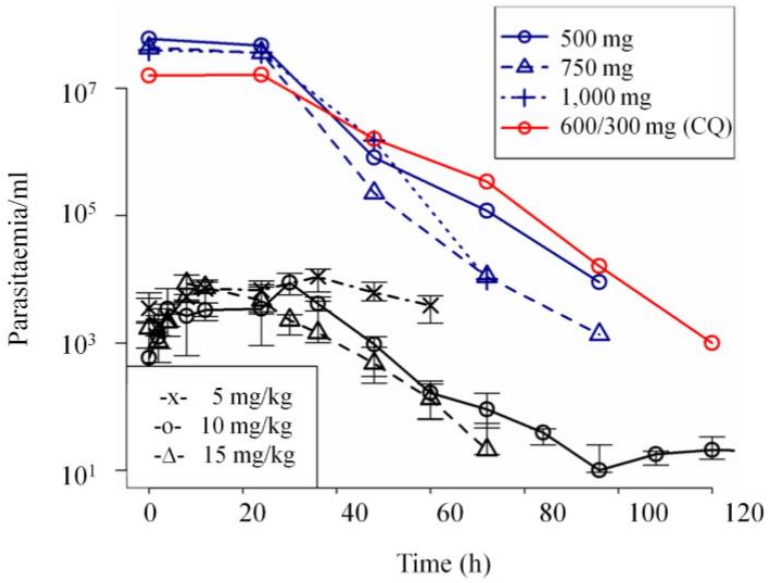


FIG 1 Effect of lag phase and tail exclusion on the calculation of the clearance rate constant. (Modified from Flegg et al. with permission of the author.)



Modeling radical cure in IBSM



Parasitemia in mefloquine treated, *Plasmodium*-infected volunteers (this study) or malaria patients. Black lines: this study (mefloquine). Blue lines: patients treated in earlier studies with mefloquine. Red lines: chloroquine (CQ: 600/300 mg) (28, 29).

Left: Marquart et al 2015 *AAC* 59:4249; Right: McCarthy et al 2016 AAC (doi:10.1128/AAC.02883-1)

Recrudescence vs. gametocytemia

The Journal of Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America

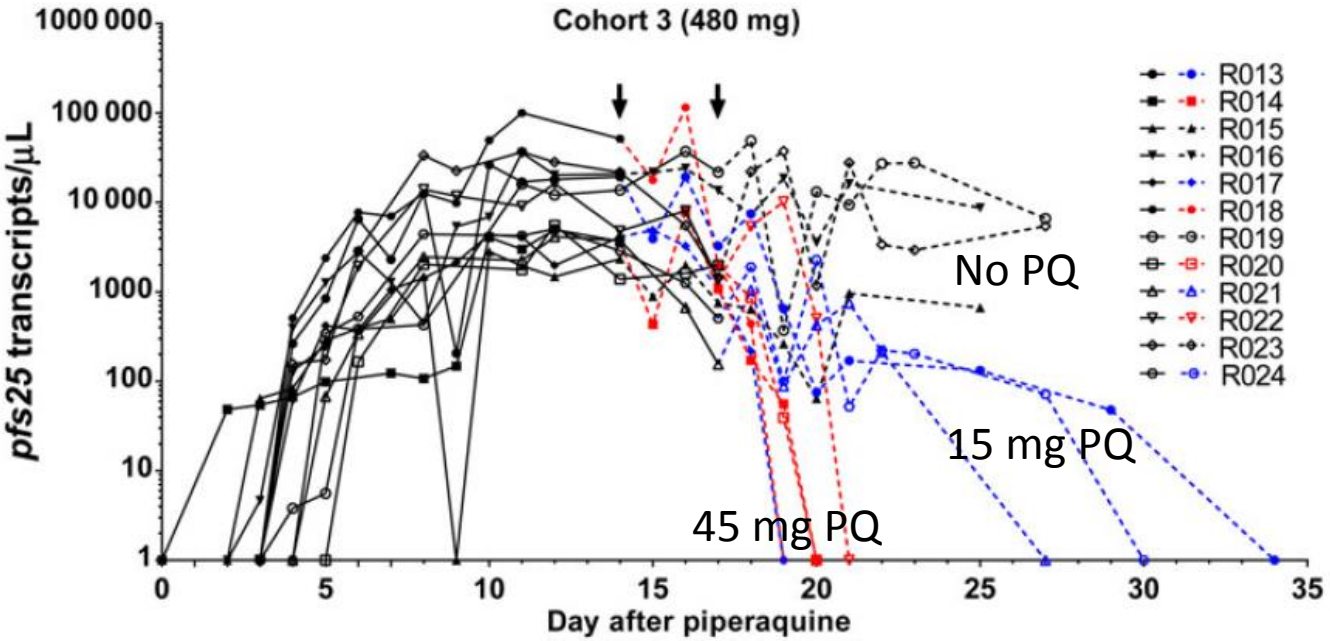
hivma hiv medicine association

OXFORD

Piperaquine Monotherapy of Drug-Susceptible *Plasmodium falciparum* Infection Results in Rapid Clearance of Parasitemia but Is Followed by the Appearance of Gametocytemia

Cielo J. Pasay,[1] Rebecca Rockett,[2,4] Silvana Sekuloski,[1] Paul Griffin,[1,3,5,6] Louise Marquart,[1] Christopher Peatey,[7] Claire Y. T. Wang,[4] Peter O'Rourke,[1] Suzanne Elliott,[5] Mark Baker,[8] Jörg J. Möhrle,[9] and James S. McCarthy[1,3,5]

[1]QIMR Berghofer Medical Research Institute, [2]School of Chemistry and Molecular Biosciences, [3]School of Medicine, University of Queensland, [4]Queensland Pediatric Infectious Diseases Laboratory, [5]Q-Pharm, [6]Department of Infectious Diseases, Mater Health Services and Mater Research Institute, and [7]Australian Army Malaria Institute, Brisbane; [8]Medicaments pour Tous, Rolle, and [9]Medicines for Malaria Venture, Geneva, Switzerland

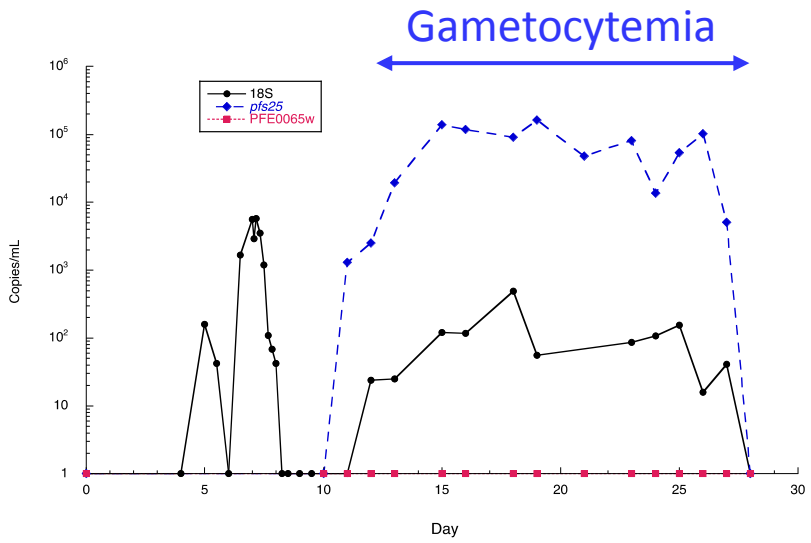


Pasay et al. 2016. *JID* 214:105

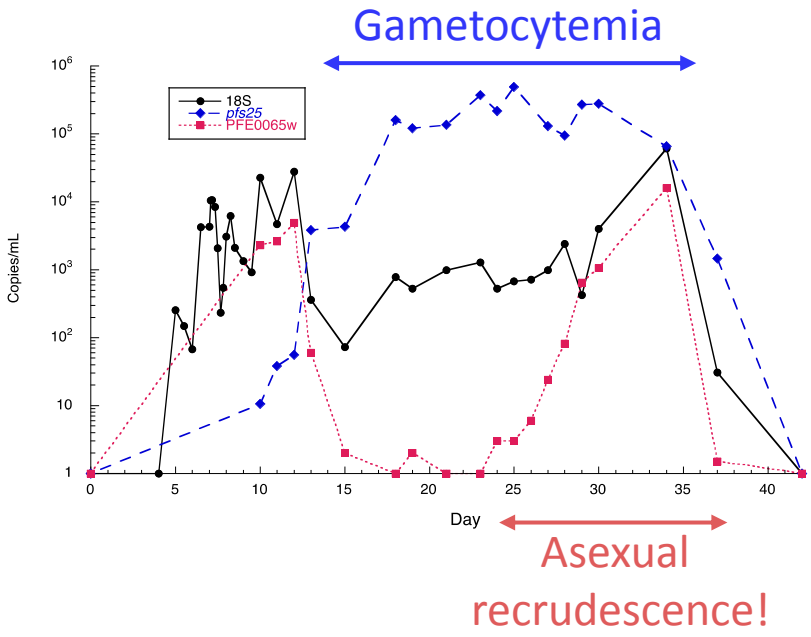
NAT-based differentiation between gametocytemia & recrudescence

- *Plasmodium* 18S rRNA/rDNA (positive in asexual and gametocyte stages)
- Gametocyte-specific mRNA (e.g., Pfs25, Pfs230 and Pvs25 or other mRNA targets)
- Ring-stage specific mRNA PFE0065w (Joice et al 2013 *PloS Comput Biol* 9:e1003392)

Example A



Example B



Pasay et al JID 2016 (Slide provided by J. McCarthy - QIMR)

Standards, Calibrators & EQA

- Standards (run controls)
  - Infected whole blood (no commercial source)
- Calibrators
  - Plasmids encoding full-length (A or S) (several labs) or hybrid plasmid (5'S + 3'A-type 18S rRNA genes) (UW)
  - Full-length *Pf* 18S rRNA as custom Armored RNA (UW)
- External quality assurance
  - WHO EQA scheme for malaria NAT in development

Summary

- Most common target: *Pf* 18S rRNA/rDNA
- NATs in use at most CHMI centers with increasing use for primary safety and/or efficacy endpoints
- Useful in sporozoite and iRBC CHMI with rescue thresholds as major difference
- Ongoing issues: recrudescence vs. gametocytes; harmonization; standards; calibrators; EQA

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- CFAR Retrovirology Core

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- Ryan Jensen
- Elizabeth Duke

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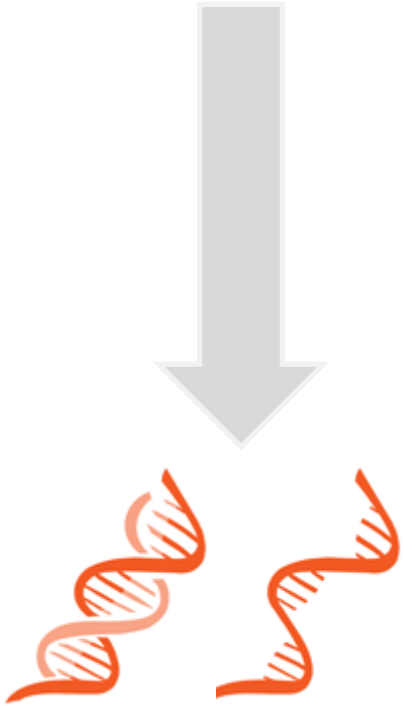
- PATH BIOME
- Clinical trial support (NIH, DoD, MMV, Novartis, CIDR)



Top: Photo by Peter Ginter, New York Times, 3/4/08