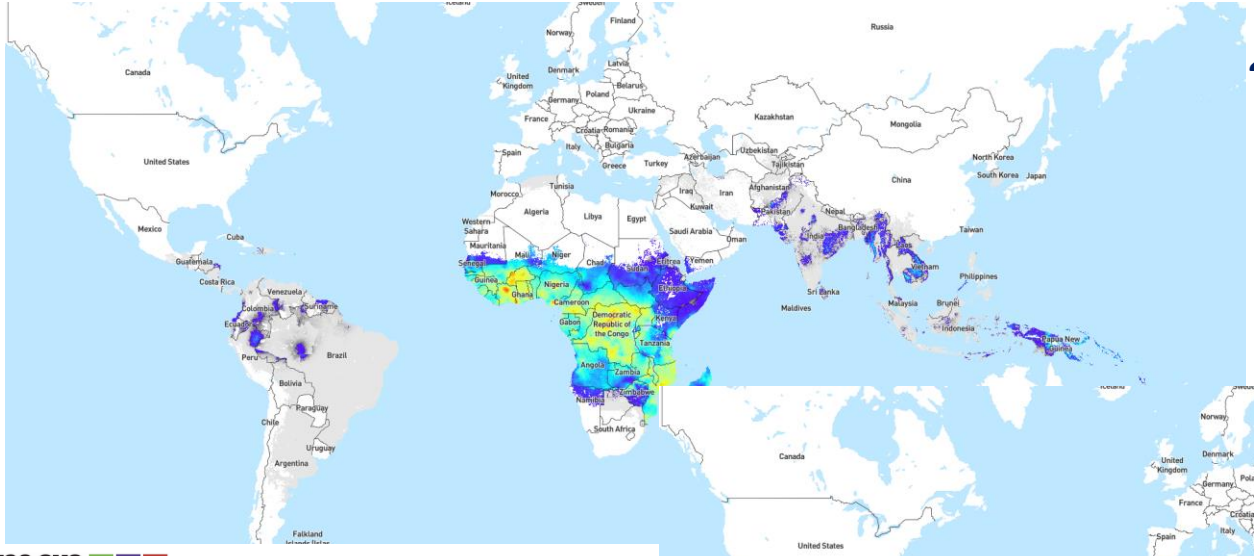
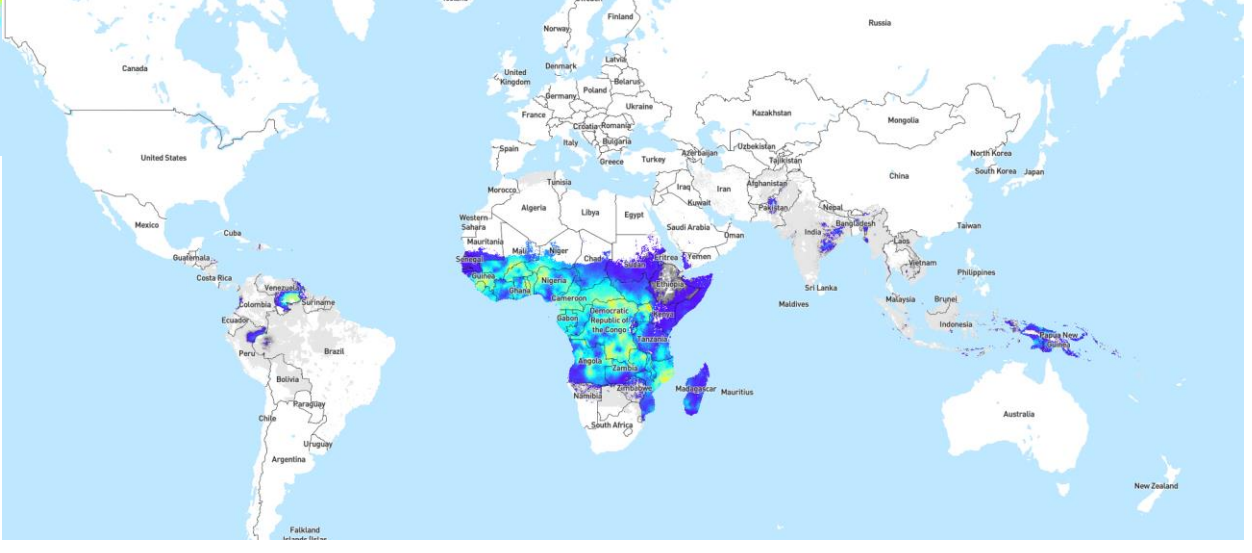
Malaria diagnostics: Progress, possibilities and priorities

Manipal. August 2019

David Bell

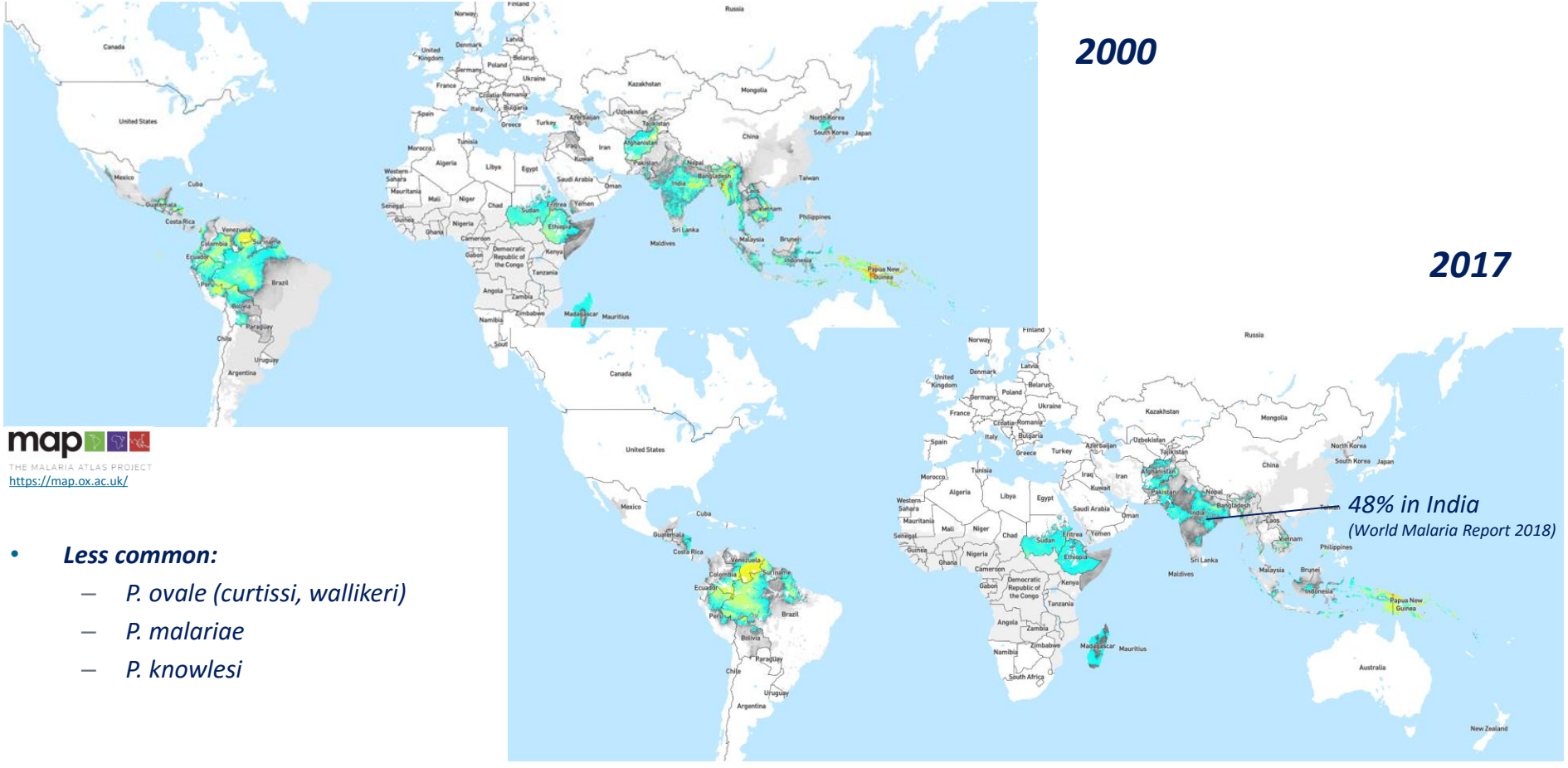
Malaria distribution and progress since 2000 – *P. falciparum*





map
THE MALARIA ATLAS PROJECT
https://map.ox.ac.uk/

Malaria distribution and progress since 2000 – *P. vivax*

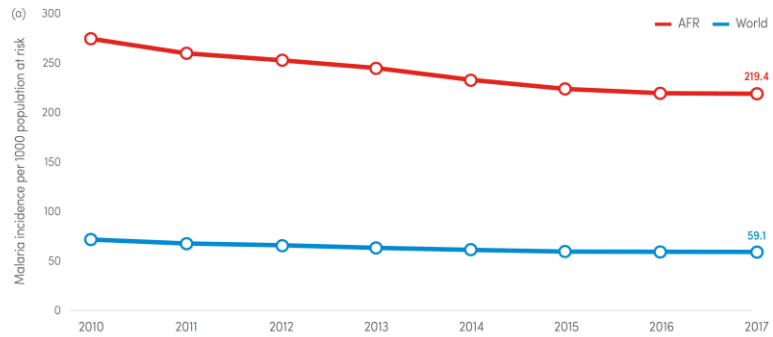


- ***Less common:***
  - *P. ovale (curtissi, wallikeri)*
  - *P. malariae*
  - *P. knowlesi*

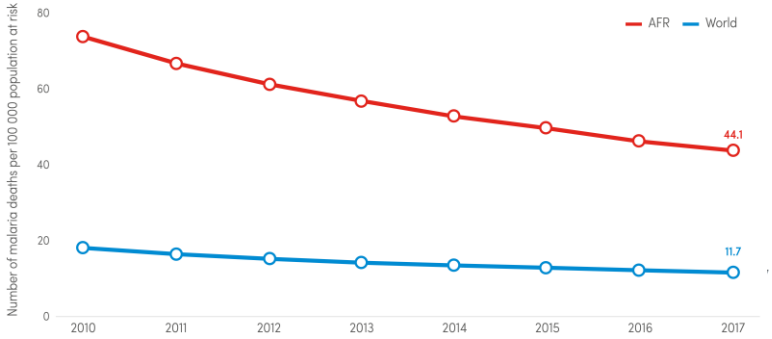
But progress is slowed, or halted…….Global malaria trends 2010 - 2017

Global malaria trends: cases per 1000, deaths per 100K of pop'n at risk

Trends in malaria case incidence rate (cases per 1000 population at risk), globally and by WHO region, 2010–2017 The WHO European Region has reported zero indigenous cases since 2015. *Source: WHO estimates.*



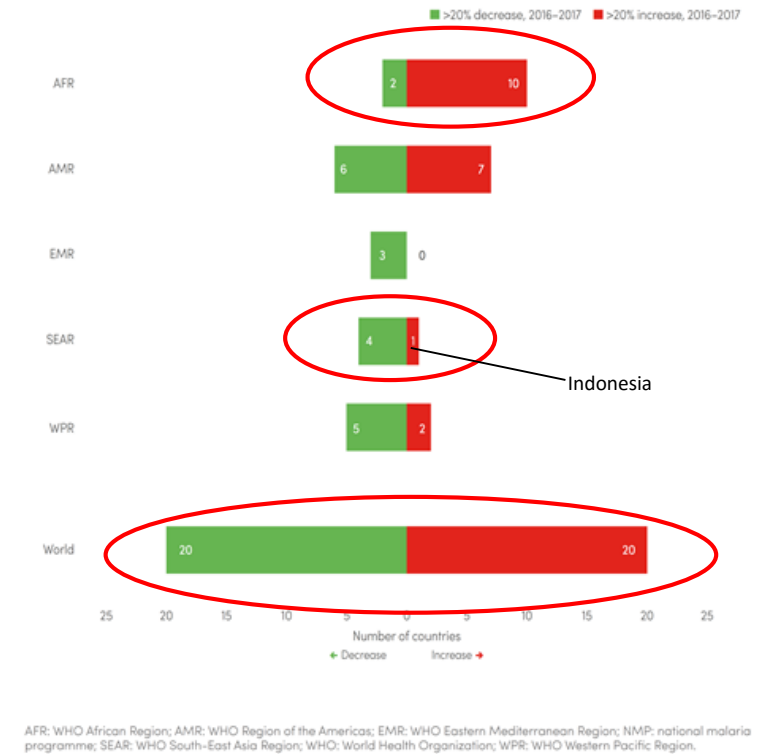
Trends in malaria mortality rate (deaths per 100 000 population at risk), globally and in the WHO African Region, 2010–2017 *Source: WHO estimates.*



World malaria Report 2018 (World Health Organization)

Countries with large changes in malaria case rate

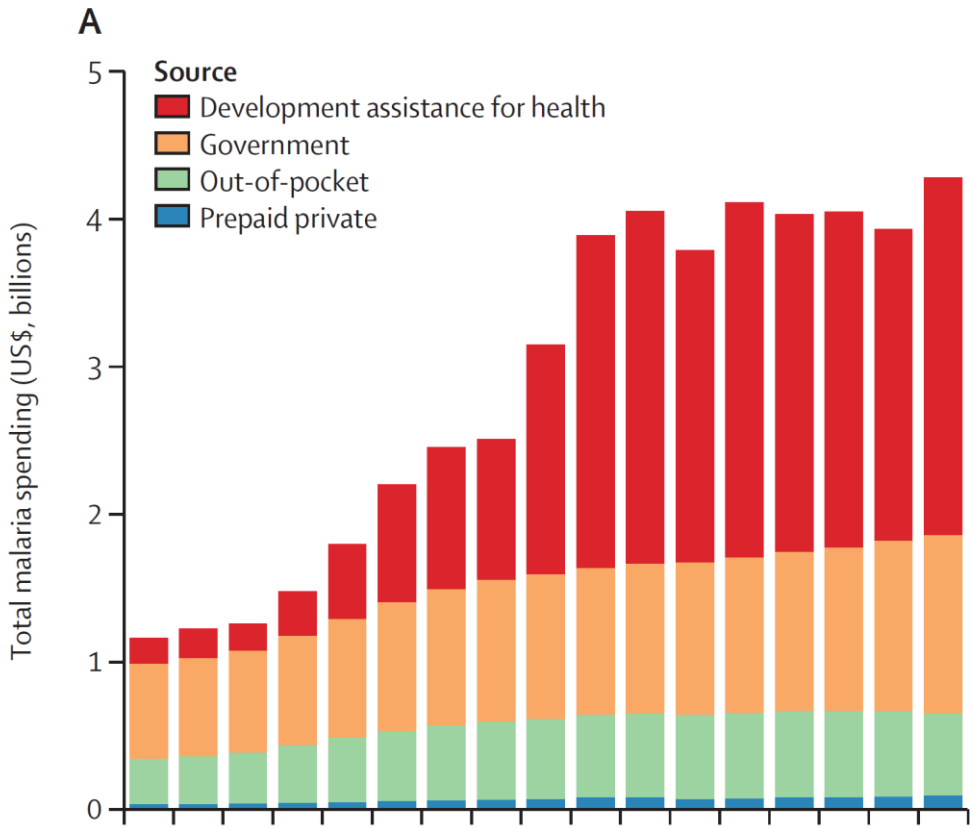
Number of countries and areas where a reduction (green) or an increase (red) of more than 20% i malaria cases has occurred between 2016 and 2017, by WHO region *Sources: NMP reports and WH estimates.*



AFR: WHO African Region; AMR: WHO Region of the Americas; EMR: WHO Eastern Mediterranean Region; NMP: national malaria programme; SEAR: WHO South-East Asia Region; WHO: World Health Organization; WPR: WHO Western Pacific Region.

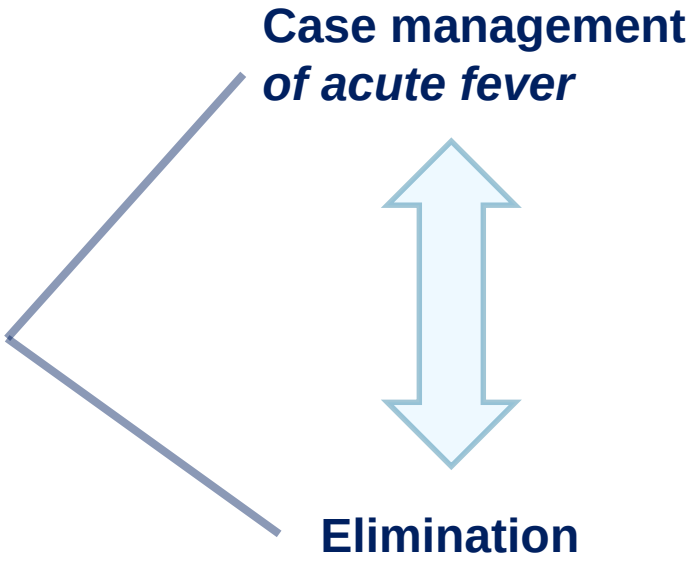
World malaria Report 2018 (World Health Organization)

…And funding is fragile…



Haakenstad A, et. Al.,. Tracking spending on malaria by source in 106 countries, 2000-16: an economic modelling study. *Lancet Infect Dis*. 2019 Jul;19(7):703-716.

Malaria diagnosis – Why?



Diagnosis of disease

Distinguish malaria-related fever from other causes:

Direct use of anti-malarial drugs

Identify non-malaria cases requiring further investigation

Gather data on malaria incidence

Planning and targeting of interventions (assumes a decent HIS)

Detection of parasites

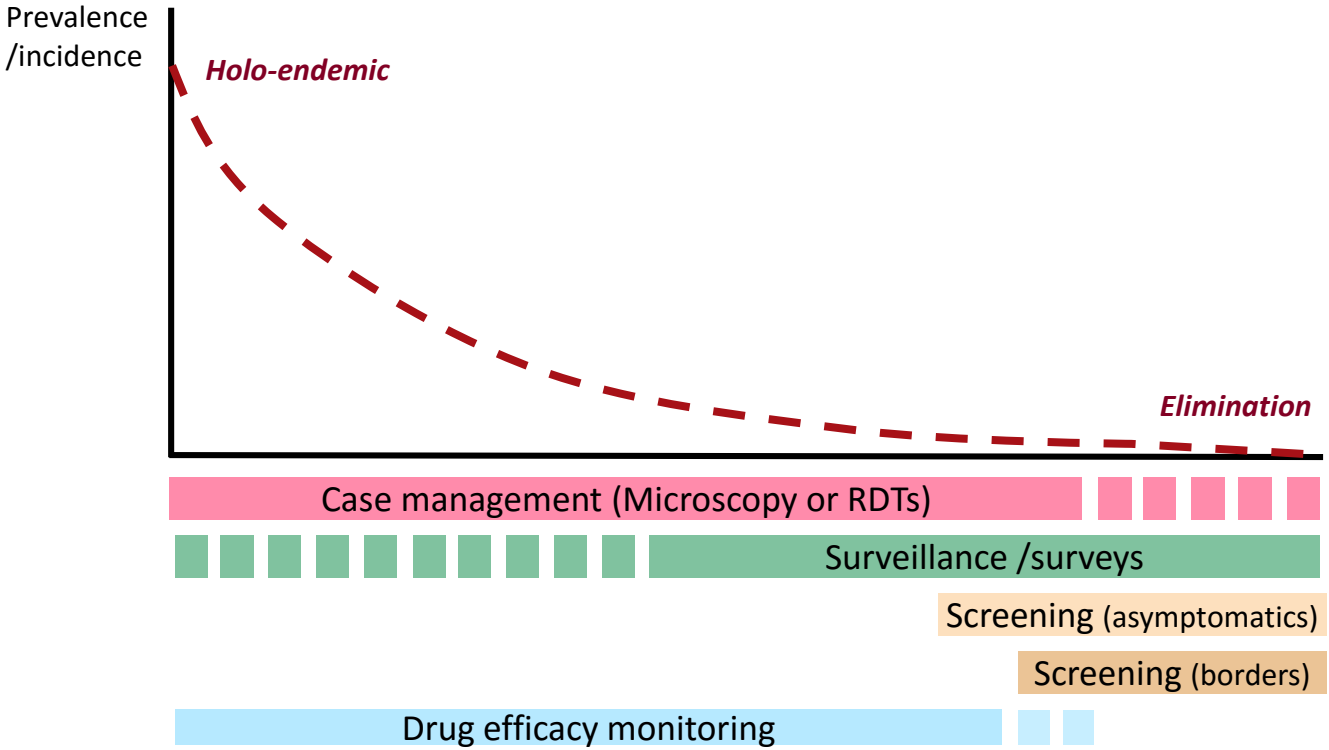
Parasite-screening tests

Detect parasitaemia for treatment, to prevent onward transmission

Detection of transmission

Population screening tests

Malaria detection and malaria prevalence



Why distinguish between these classes of diagnostics?

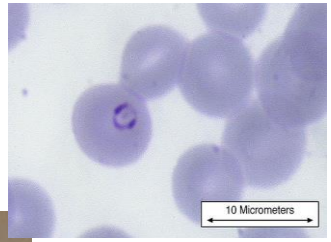
Diagnosis of disease , Parasite detection

Implications:

- **Different product specifications**
- **Different implementation challenges**
- **Different funders /funding mechanisms**
- **Different willingness-to-pay**

Current tests for case management

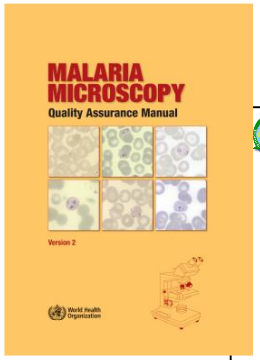
Light microscopy

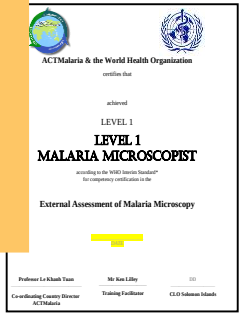


~200M diagnoses /year
Quantify parasites
Species differentiation
Useful for other diseases…

But…

- **Highly technician dependent**
- **Highly variable threshold of detection**
- **Not so cheap to do it well**
- ***Slow for Point-of-Care***

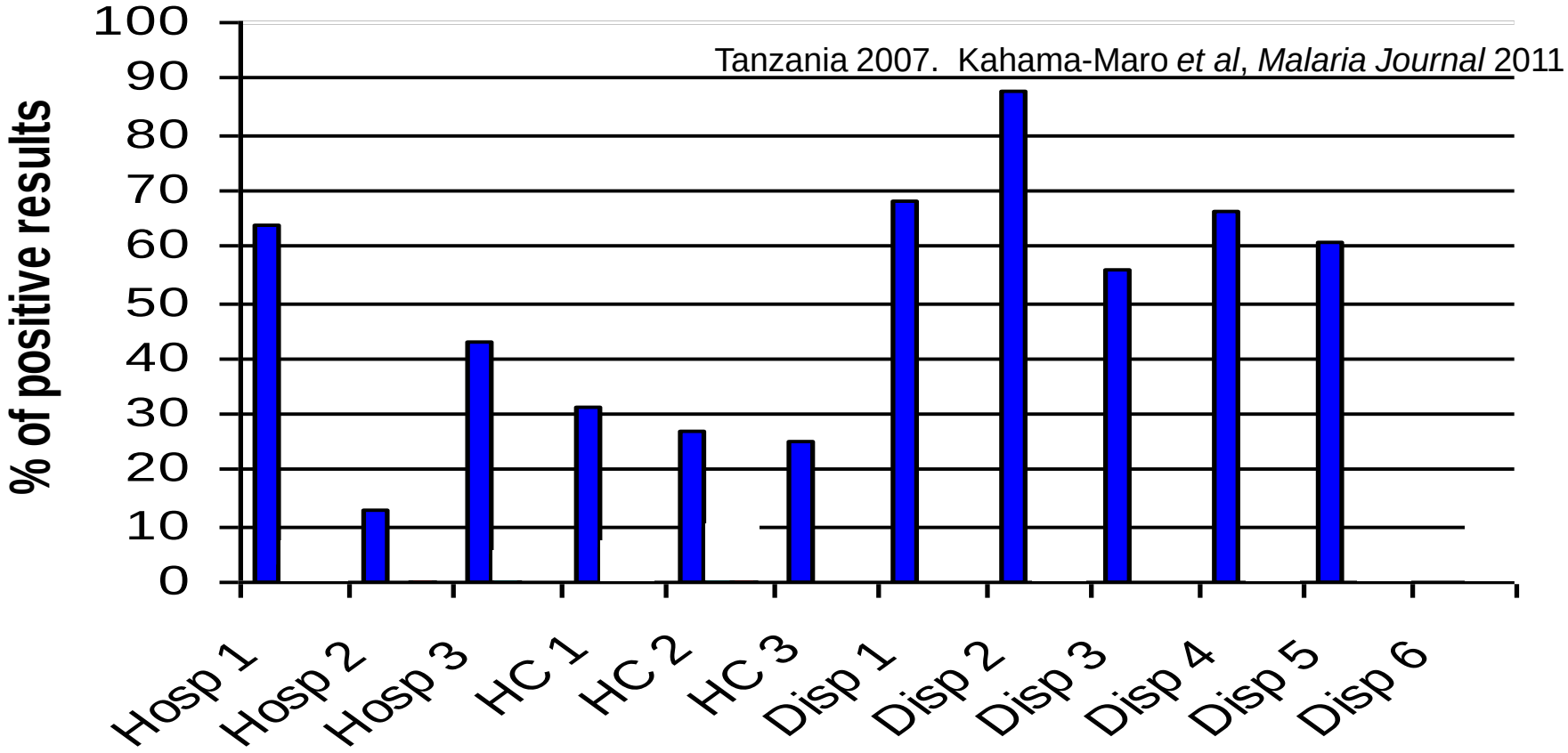


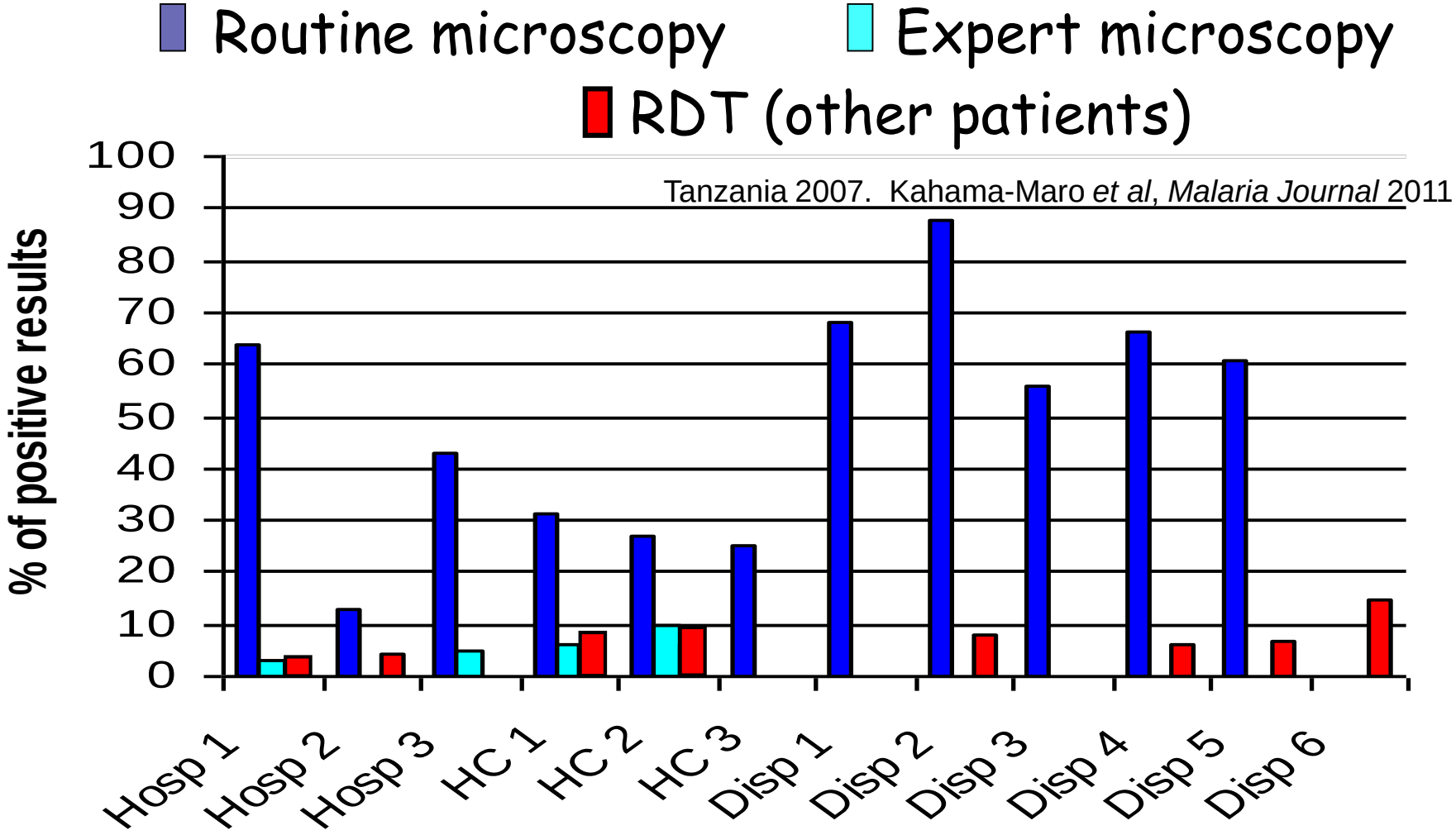


QA

Go digital

Routine microscopy




Routine microscopy
Expert microscopy
RDT (other patients)
Tanzania 2007. Kahama-Maro et al, Malaria Journal 2011
% of positive results
100
90
80
70
60
50
40
30
20
10
0
Hosp 1
Hosp 2
Hosp 3
HC 1
HC 2
HC 3
Disp 1
Disp 2
Disp 3
Disp 4
Disp 5
Disp 6

Factors influencing microscopy quality

Selection

Training

Assessment

Competency

Supervision
Cross -checking

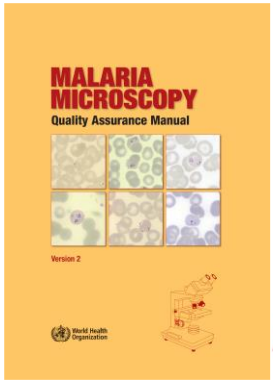
Equipment/
reagents

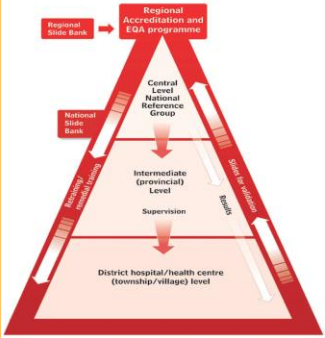
Support network
Slide /results
delivery

Work environment
/accuracy

Performance /
accuracy

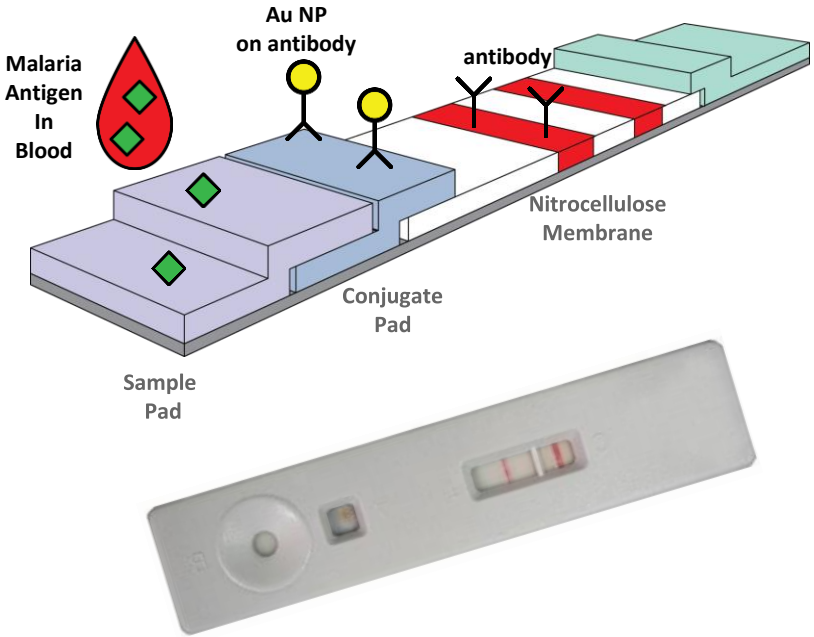
Good microsocpy is not cheap!





Commercially-available Rapid Diagnostic Tests

~300M diagnoses /year



Target antigens

Histidine-rich protein 2 (HRP2

P. falciparum only

Antigen persists in circulation after treatment

Plasmodium Lactate dehydrogenase (pLDH)

Pan/specific or species-specific

Clears rapidly after treatment

(Aldolase)

Formats:

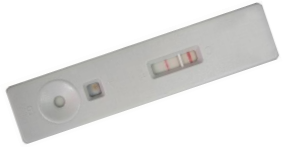
Pf-only

Pf/pan

Pf/Pv

Other

Maintaining diagnostic quality



Supply chain management ***Transport and storage***

Manufacture
-ISO13485 etc

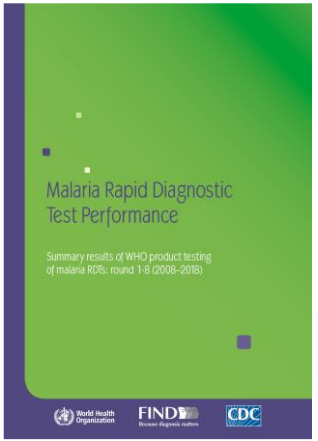
End users
-Appropriate training and instructions
-Management of positive and negative results
-Monitoring of commodity supply and disease rates

Stage 1: Pre-qualification /Product testing
Evaluate product performance, manufacture quality

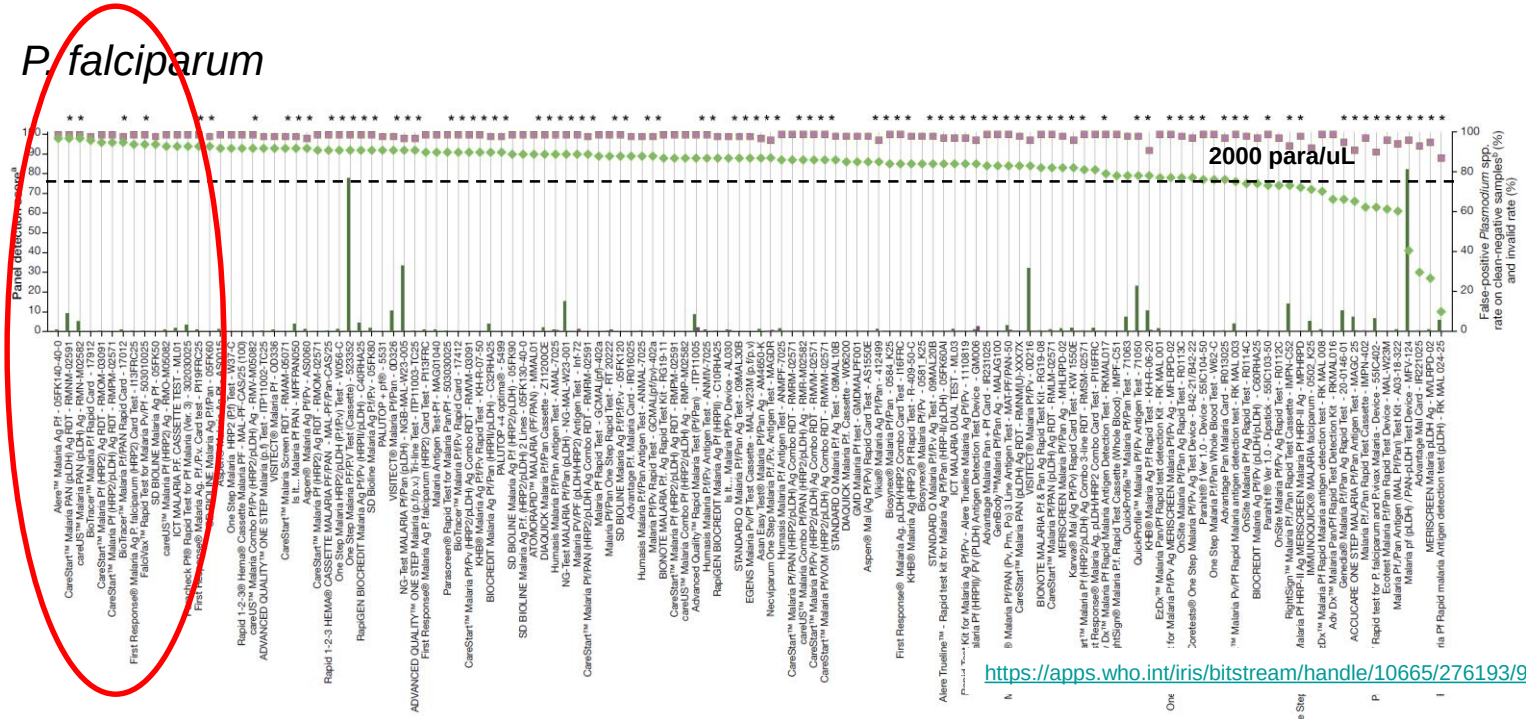
Stage 2: Testing manufactured lots within country (India - NIMR)
Confirm product quality on arrival.
Test samples from field

WHO Malaria RDT Product Testing: Rounds 1-8 results

(227 unique products)



P. falciparum



https://apps.who.int/iris/bitstream/10665/276193/9789241514958-eng.pdf?ua=1

Are these tests sensitive enough?

Is it safe to withhold anti-malarial treatment from RDT-negative febrile children in high-prevalence *P. falciparum* areas?

Yeboah-Antwi K, et al. *PLoS Med* 2010. Zambia (*ICT Malaria Pf)*

963 children with fever – 698 RDT negative, no anti-malarials **Mortality: 2 (non-malarial)**

Faucher JF, et al. *Malar J* 2010. Benin (*Paracheck)*

677 children with fever - 281 RDT negative , no antimalarials (~61% ABs)
3 episodes of malaria noted in 14 days follow-up. **No mortality**

D'Acremont V,. *Clin Infect Dis* 2010. Tanzania (*ParaHit f)*

1000 children with fever, 601 RDT negative , no antimalarials
3 episodes of parasitaemia noted later. **Mortality: 2 (non-malarial causes)**

Mtve G et al. *Malar J* 2011. Tanzania (*ParaHit f)*

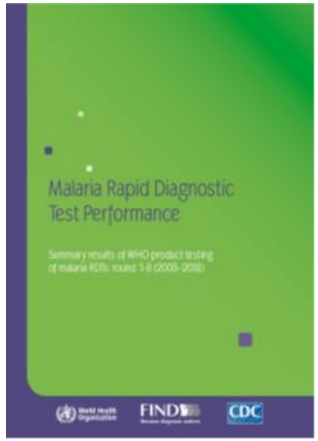
965 children with fever, 807 RDT negative , no antimalarials (~various other)
6 episodes of parasitaemia noted later (all PCR neg at enrolment). **No mortality**

Senn N et al. *Clin Infect Dis* 2011. Papua New Guinea (*ICT Malaria Combo)*

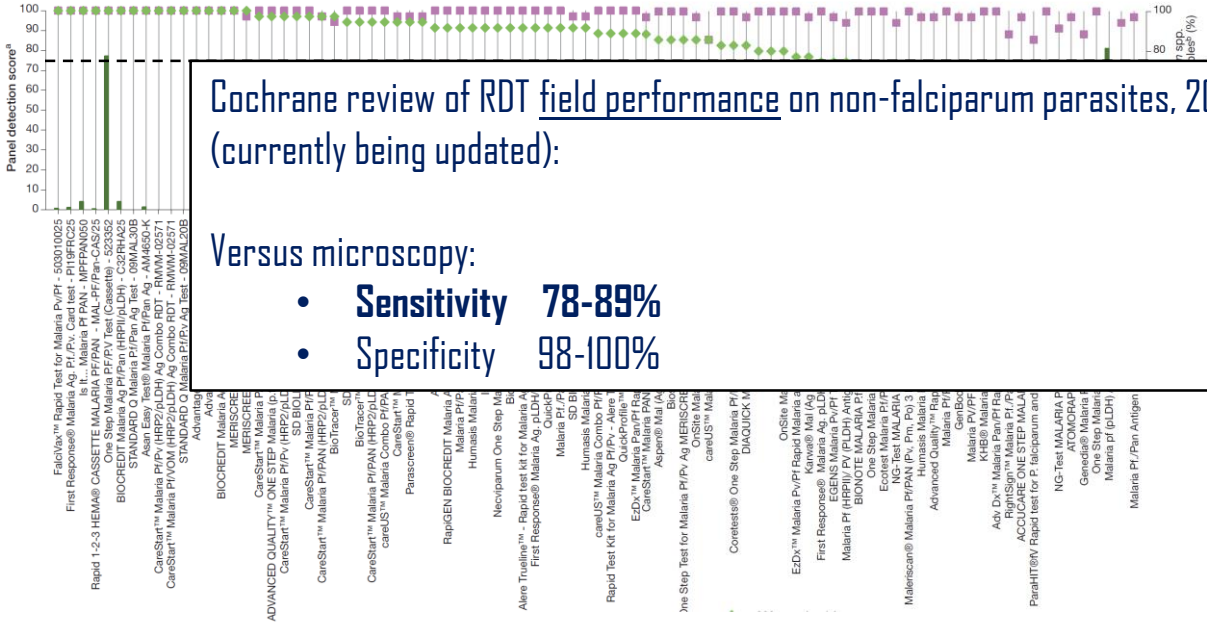
5670 childhood fevers, 3942 RDT negative , no antimalarials
29 episodes of parasitaemia noted later. **Mortality: 2 (non-malarial causes)**

WHO Malaria RDT Product Testing: Rounds 1-8 results

(227 unique products)



P. vivax



Cochrane review of RDT field performance on non-falciparum parasites, 2014 (currently being updated):

Versus microscopy:

- **Sensitivity 78-89%**
- Specificity 98-100%

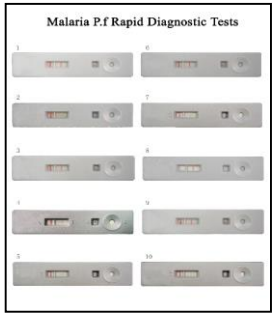
parasites/µL
0 parasites/µL
e-positive *Plasmodium* spp. rate (%)
lid rate (%)

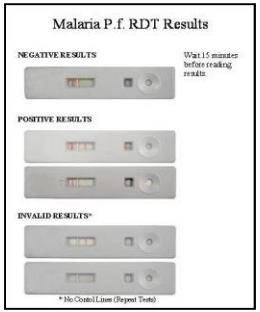
Translating lab performance into clinical performance

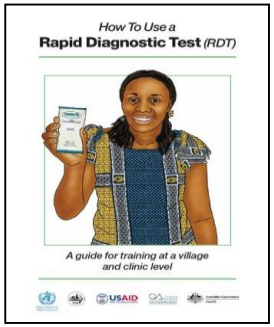
Impact of commercial pressures and realities of routine logistics and use.

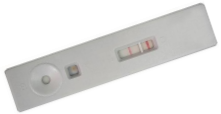
Photo courtesy of Helen Counihan, Malaria Consortium

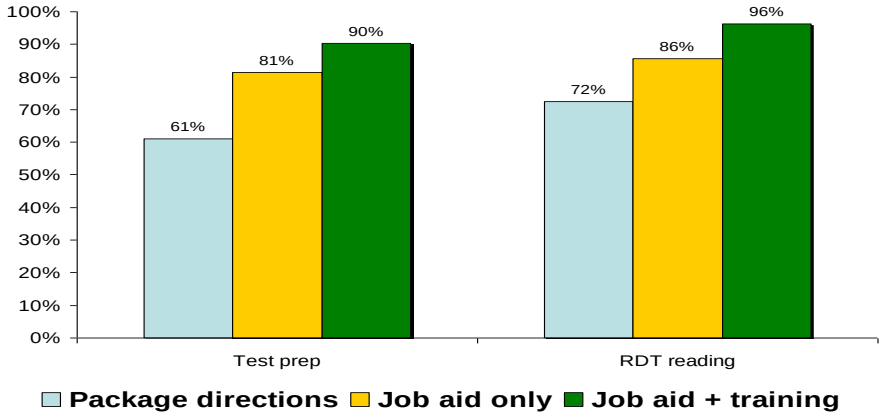
Improving user performance











Zambia MoH, URC, WHO, TDR, FIND, Malaria Consortium

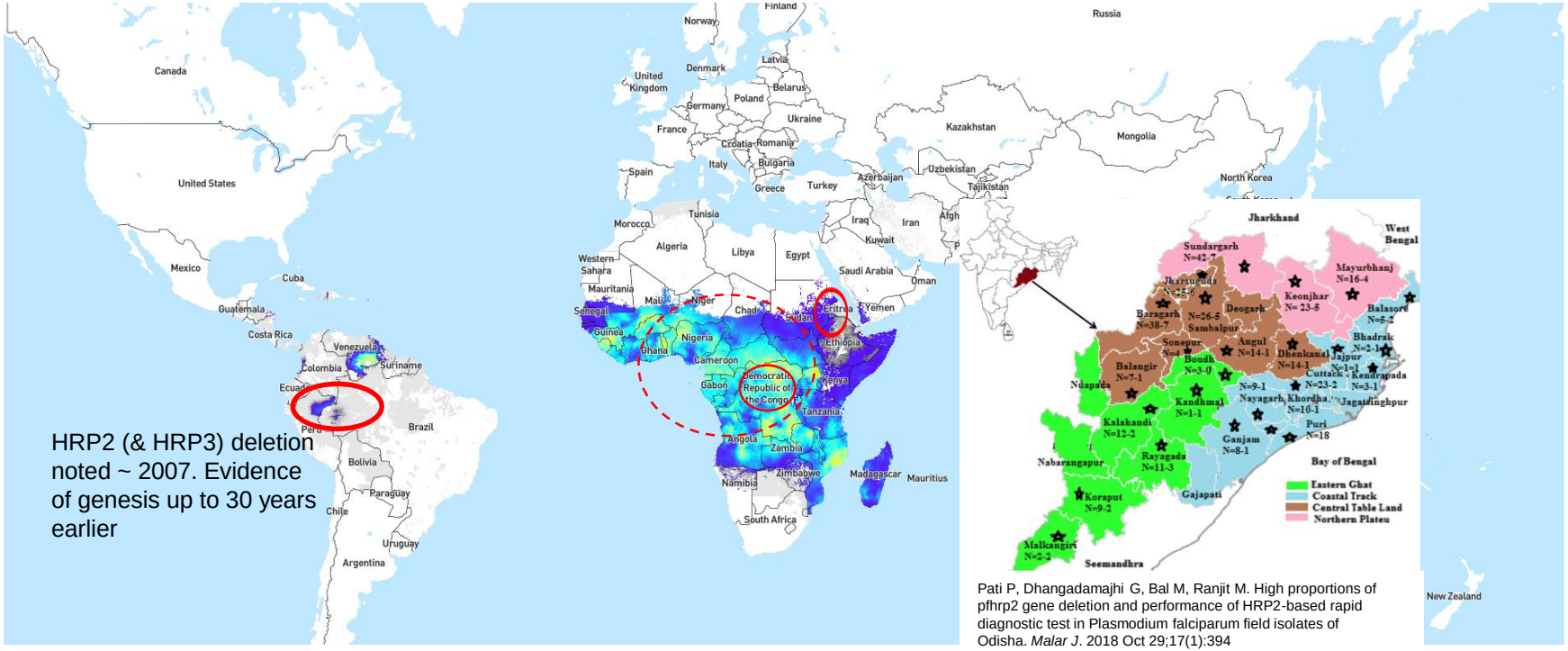
Case management - Threats and challenges…..

Threats and challenges…..
Vivax management

- *P. vivax* tests remain insufficiently sensitive
- No tests exist for liver stage (latent) *P. vivax* infection
- 14 day courses of primaquine (liver stage clearance) are poorly adhered to.
- Therefore, reduction in transmission is greatly retarded by relapse
- Tafenoquine (single dose 8-aminoquinolone) now prequalified but requires G6PD deficiency testing (*single long-acting dose, so ~higher risk of severe haemolysis*)

  …but no good point-of-care G6PD test exist
- We will likely not accelerate *P. vivax* elimination until part or all of this is solved.

Threats and challenges…..
HRP2 deletions



HRP2 (& HRP3) deletion noted ~ 2007. Evidence of genesis up to 30 years earlier

Pati P, Dhangadamajhi G, Bal M, Ranjit M. High proportions of pfhrp2 gene deletion and performance of HRP2-based rapid diagnostic test in Plasmodium falciparum field isolates of Odisha. *Malar J*. 2018 Oct 29;17(1):394

map
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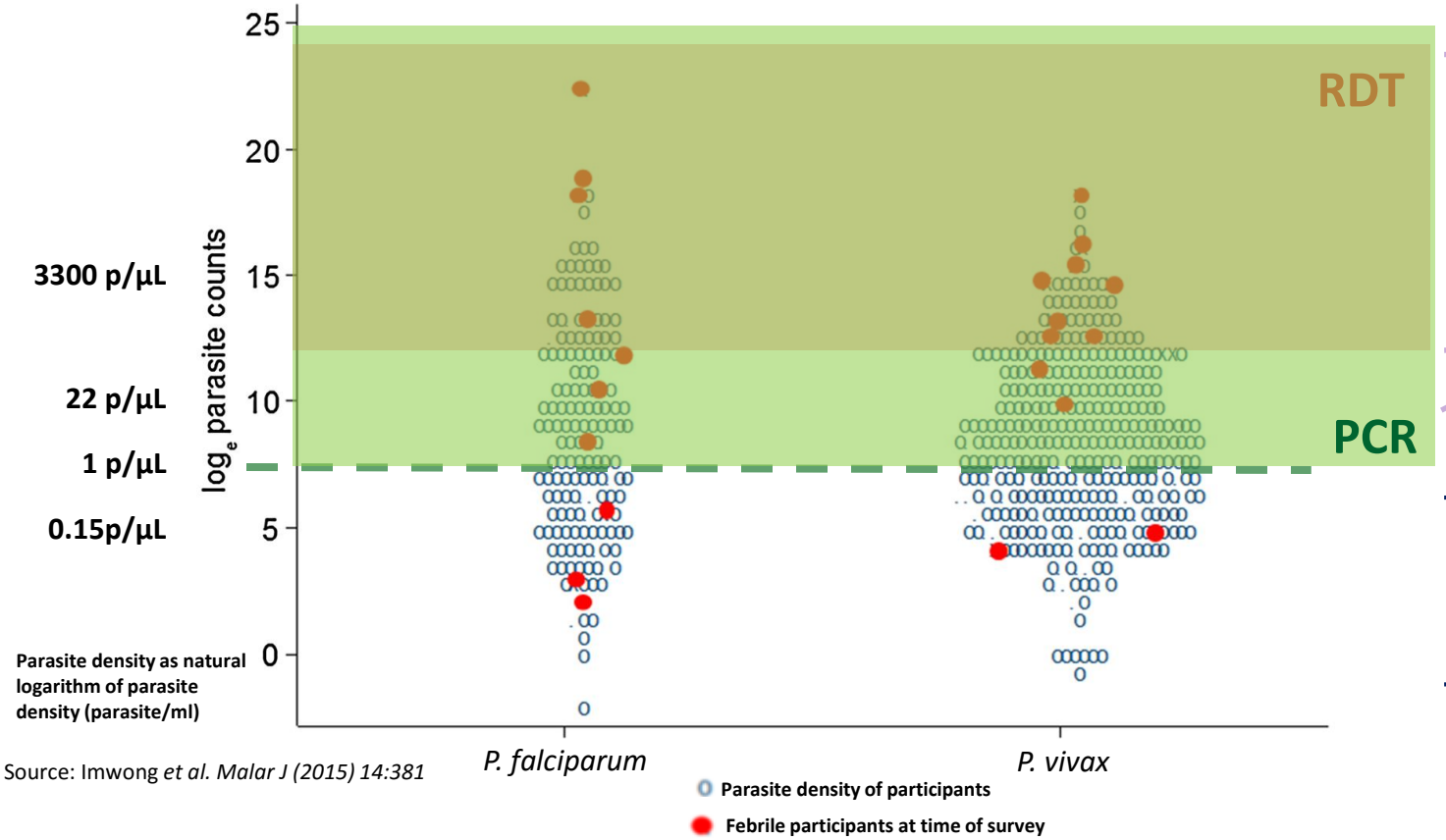
https://map.ox.ac.uk/

Parasitaemia in very low transmission /elimination

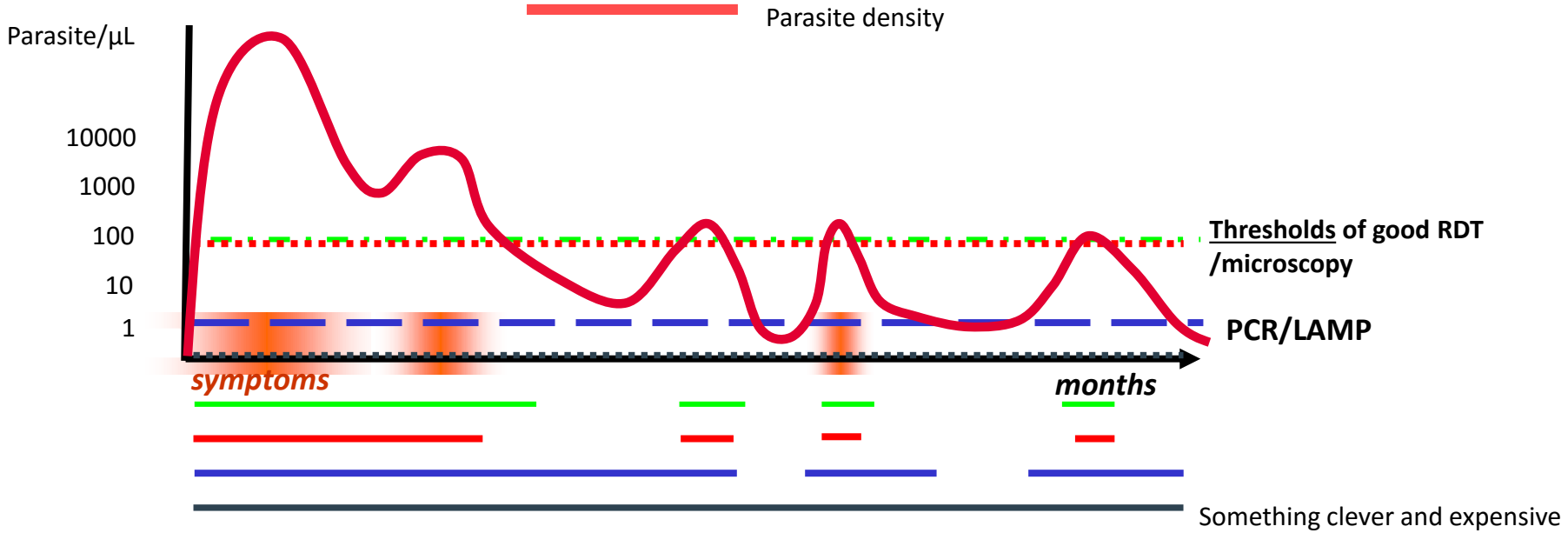
How sensitive do we need to be?

Tradeoff between sensitivity and ability to implement



Source: Imwong *et al. Malar J (2015) 14:381*

Place of POC diagnostic methods in malaria detection…

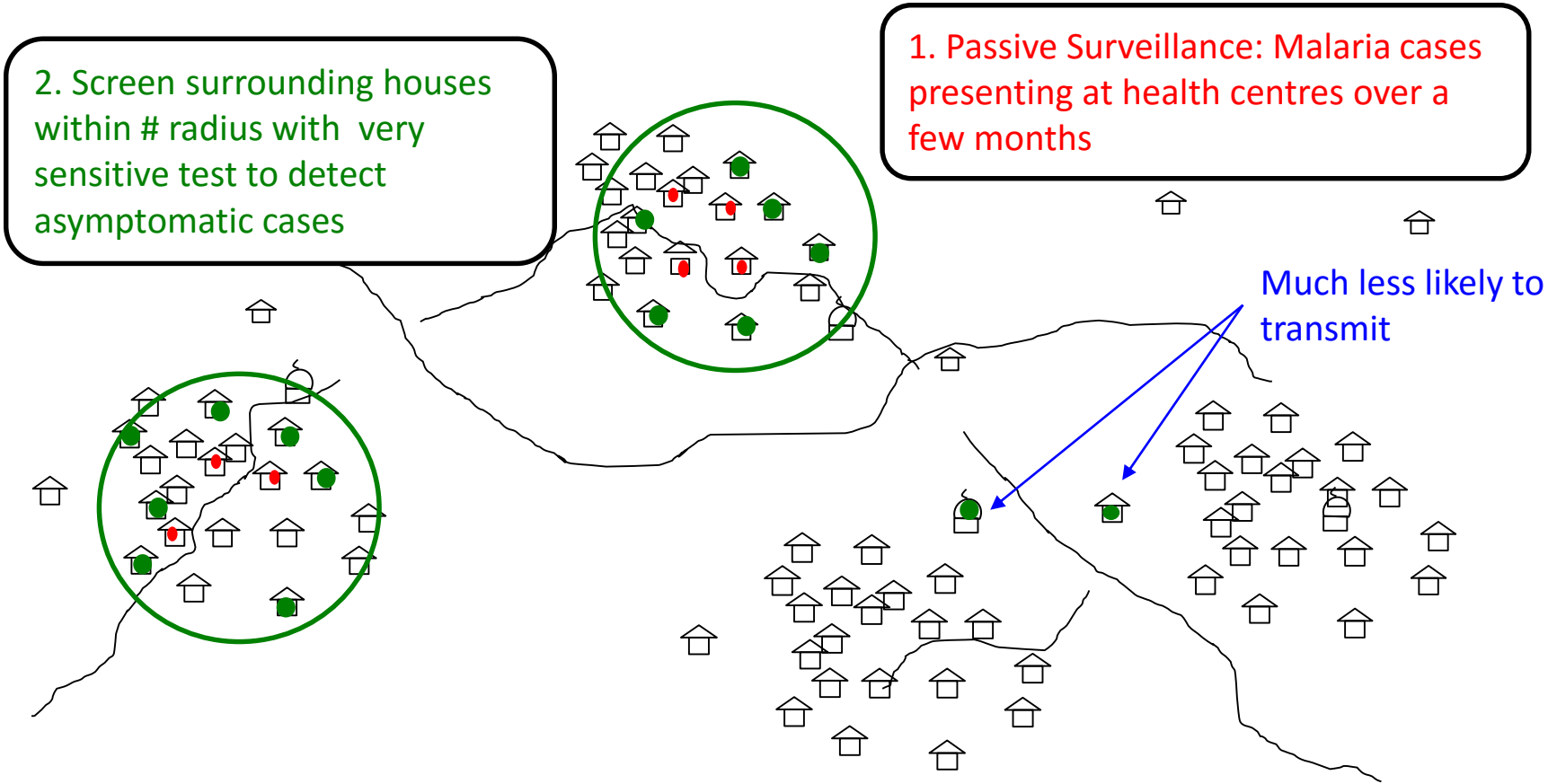


May be possible to transmit infection through all of this period

Derived from observations of controlled infections to induce fever in tertiary syphilis patients, compiled by Collins and Jeffreys, US CDC. Unpublished data.

Focusing screening and treatment (FSAT) in areas with passively-detected foci

robust anthropophilic vector (e.g. *A. gambiae*, *A. farauti*)

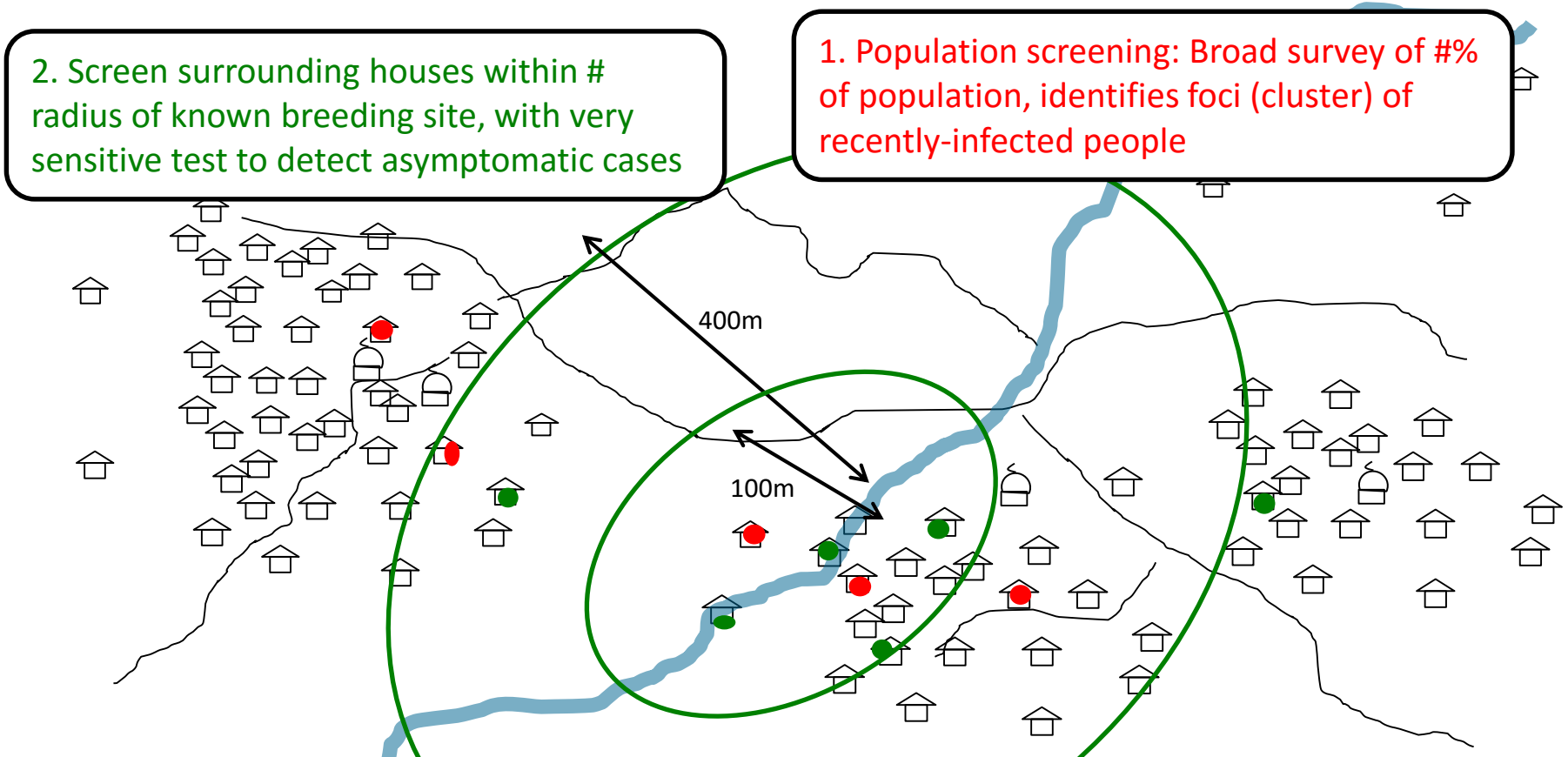


Focal screening (FSAT) around a confined vector breeding site

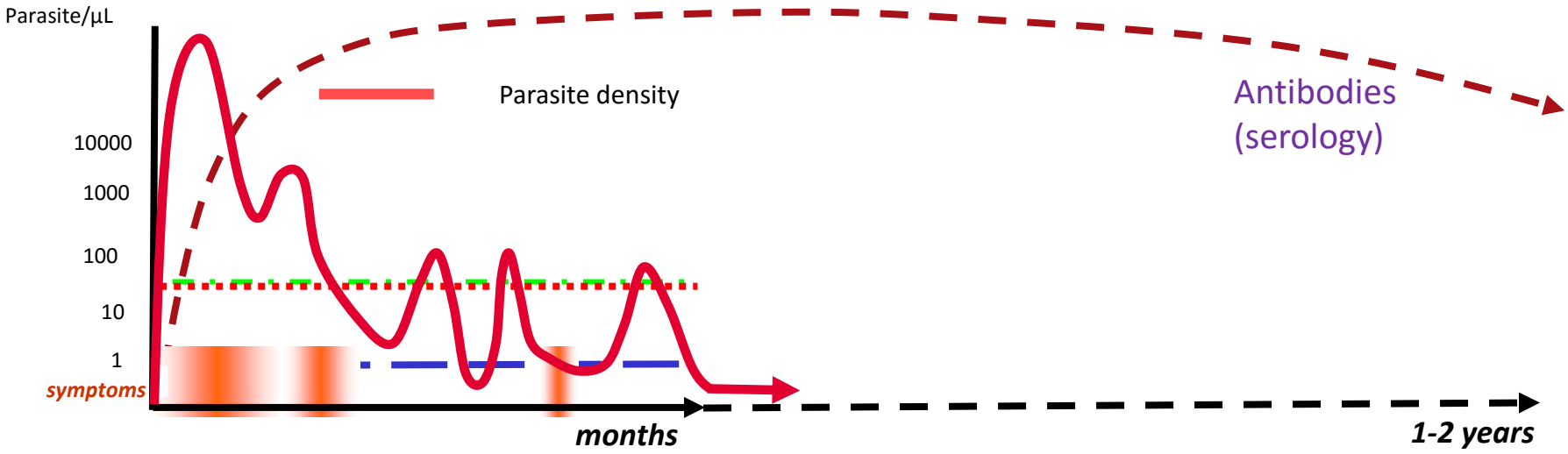
(A flavirostris: Foley et al. TRSTMH, 2003)

2. Screen surrounding houses within # radius of known breeding site, with very sensitive test to detect asymptomatic cases

1. Population screening: Broad survey of #% of population, identifies foci (cluster) of recently-infected people

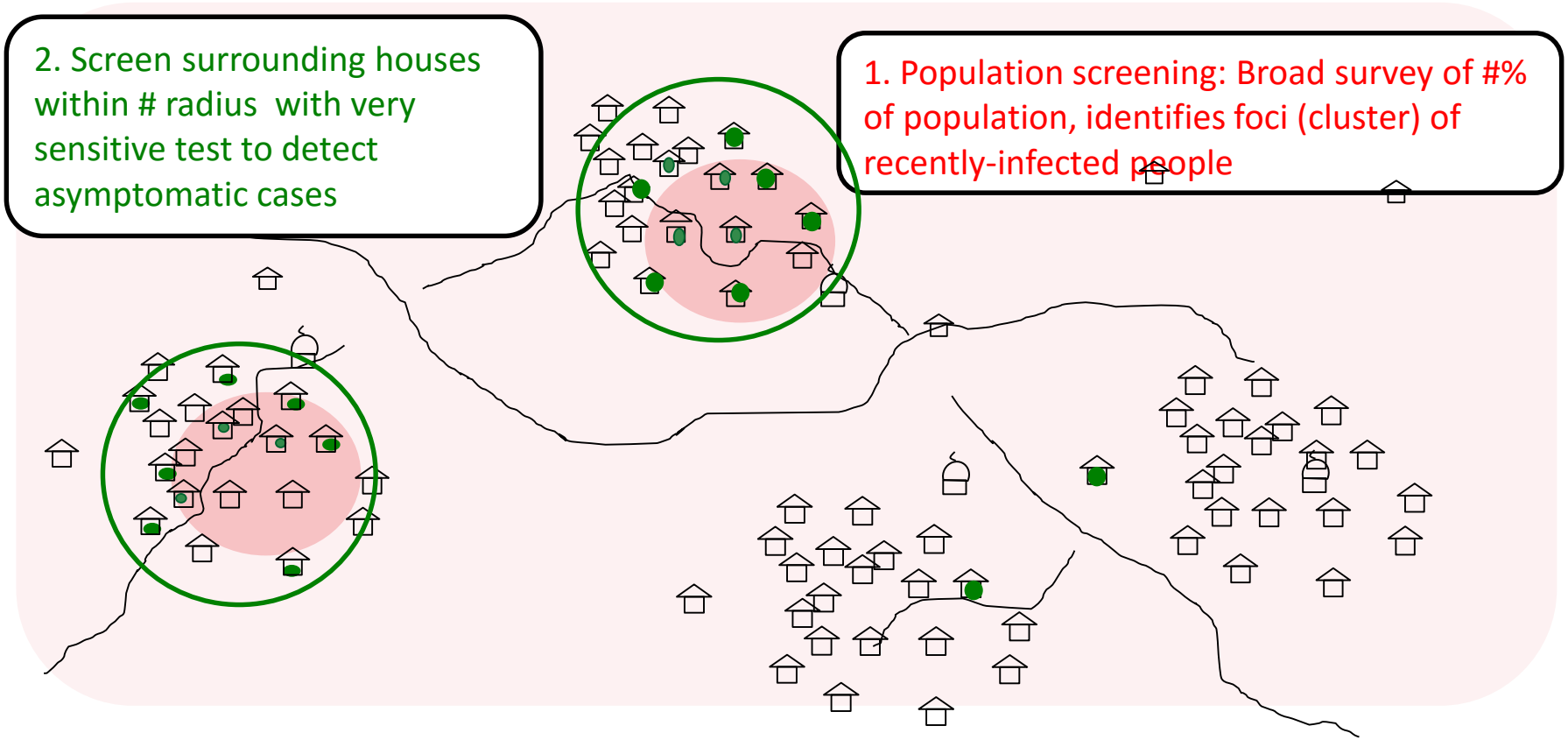


Extending the window of detection of ‘infection’ to identify transmission foci within a broad area



Derived from observations of controlled infections to induce fever in tertiary syphilis patients, compiled by Collins and Jeffreys, US CDC. Unpublished data.

Identifying and managing foci through broad population surveys



Emerging technologies

Detecting very low density infection – Current options

Nucleic acid amplification tests (NAAT):

PCR….	Potentially cassette-based field products in future
	Current PCR techniques applicable if lab close to community (eg. Cambodia)
LAMP…	Capable of near-patient operation with sensitivity similar to PCR

Enhancement of Lateral Flow Assays (RDT)

Improved LFA strip
Sample pre-concentration
Signal enhancement with e-readers

Other:

Haemozoin detection
Digital microscopy, pattern-recognition software,
All those cell-phone Apps…

LAMP

(Loop-mediated isothermal amplification)

Malaria LAMP Assay (Eiken /FIND):

- Need heat block, +/- centrifuge, reaction tubes, bench.
- Results (including sample prep) within 2 hours
- Reading: visual (fluorescence) or turbidimeter

-Sensitivity roughly equivalent to PCR

-98% sensitivity at ≥ 2 para/µL

- Hopkins et al. JID, 2013, Sutherland et al. JID, 2013

(-) (+)

Fresh samples, dry blood spots

Much simper, nearer patient than PCR

Cassette-based PCR

e.g. QuantumDx:

- Cassette-based PCR
-~20 minutes to result
-Disposable cassette in Reader module
-Company was initially concentrating on malaria diagnosis
    -Now pre-cancer, STI etc

Standardizing PCR and reducing costs remains a major issue for malaria research and elimination, but who would pay?

Courtesy of QuantumDx

Enhancement of Lateral Flow Assays (RDT)

e.g. Abbott (Alere) Ultra-sensitive HRP2 RDT

- Some improvement on current RDTs (lower limit of detection)
  - Outcomes vary between different studies
- 20 minutes to result (as per case-management RDTs)
- *P. falciparum* only

Various groups developing more sensitive RDTs for P. vivax (LDH)

Serology tests for P. falciparum and P. vivax transmission detection in development

Accuracy of LM, Pf/Pv RDT, Pf RDT, and HS-RDT for the diagnosis of *P. falciparum* infections in peripheral blood of pregnant women.

		PCR			Value (95% CI)				
		(+)	(-)	Total	Sensitivity	Specificity	PPV	NPV	Kappa
LM	(+)	27	0	27	77.1%	100.0%	100.0%	98.9%	0.9
	(-)	8	702	710	(61.0–87.9)	(99.5–100.0)	(87.5–100.0)	(97.8–99.4)	(0.8–1.0)
Pf/Pv RDT	(+)	27	0	27	77.1%	100.0%	100.0%	98.9%	0.9
	(-)	8	702	710	(61.0–87.9)	(99.5–100.0)	(87.5–100.0)	(97.8–99.4)	(0.8–1.0)
Pf RDT	(+)	29	1	30	82.8%	99.9%	96.7%	99.1%	0.9
	(-)	6	701	707	(67.3–91.9)	(99.2–100.0)	(83.3–99.4)	(98.2–99.6)	(0.8–1.0)
HS-RDT	(+)	30	4	34	85.7%	99.4%	88.2%	99.3%	0.9
	(-)	5	698	703	(70.6–93.7)	(98.5–99.8)	(73.4–95.3)	(98.3–99.7)	(0.8–1.0)

LM (light microscopy); Pf (*P. falciparum*); Pv (*P. vivax*); RDT (rapid diagnostic test); HS-RDT (highly sensitive rapid diagnostic test); nPCR (nested polymerase chain reaction); (+) (positive); (-) (negative); PPV (positive predictive value); NPV (negative predictive value); CI (confidence interval)

https://doi.org/10.1371/journal.pone.0201769.t003

ANTE-NATAL SCREENING: Vásquez AM et al. Performance of a highly sensitive rapid diagnostic test (HS-RDT) for detecting malaria in peripheral and placental blood samples from pregnant women in Colombia. *PLoS One*. 2018 Aug 2;13(8):e0201769.

Haemozoin detection

e.g. Hemex Gazelle malaria assay

- Detects haemozoin in finger-prick blood
- 1 minute from sampling to result
- Similar sensitivity to current RDTs for *P. falciparum*
- Higher sensitivity for *P. vivax*
- High specificity

- *<u>Under trial at Manipal</u>*
- *Uses:*
  - *? Vivax diagnosis*
  - *HRP2-deleted P. falciparum*
  - *Rapid screening*
  - *?*

	Sensitivity	Specificity	Time to Result
RDTs/kits	*79%*	*100%*	*20 min*
Hemex	*98%*	*100%*	*1 min*

Clinical study in Mumbai, India, N=154, Primarily P. vivax, Compared to LM

	Sensitivity	Specificity	Time to Result
Microscopy	*89%*	*100%*	*45 min*
Hemex	*95%*	*100%*	*1 min*

Clinical study in Peru with US Navy, N=118, Compared to PCR*

Hemex Health - unpublished

Various groups evaluating potential for non-invasive Hz detection

Summary

Case management

- Good tools for *P. falciparum*
- May need to re-think vivax diagnosis if to make significant inroads
- But much actual malaria diagnosis remains symptom-based, with parasite-based diagnosis mainly supported by external funding *(highly fragile)*

Elimination

- Screening tools are inadequate, need:
  - Better management systems for routine diagnostic data
  - More sensitive screening tests
  - Population screening tests (monitor transmission)

  …that are affordable and usable within national programs (i.e. sustainable)

Global market for elimination tests remains highly dependent on mood of external funders – not a good prospect for manufacturers

Thank you