CRISPR-based Ultrasensitive Diagnostics for Malaria

LEE ROSE MD MSPH, DE PUIG GUIXE HELENA PHD, DVORIN JEFFREY MD PHD, POLLOCK NIRA MD PHD, COLLINS JAMES PHD

Conflict of Interest Disclosure

- No financial relationships with a commercial entity producing healthcare-related products and/or services.

Global Burden of Malaria

In 2017 there were an estimated 219 million cases with 435,000 deaths.

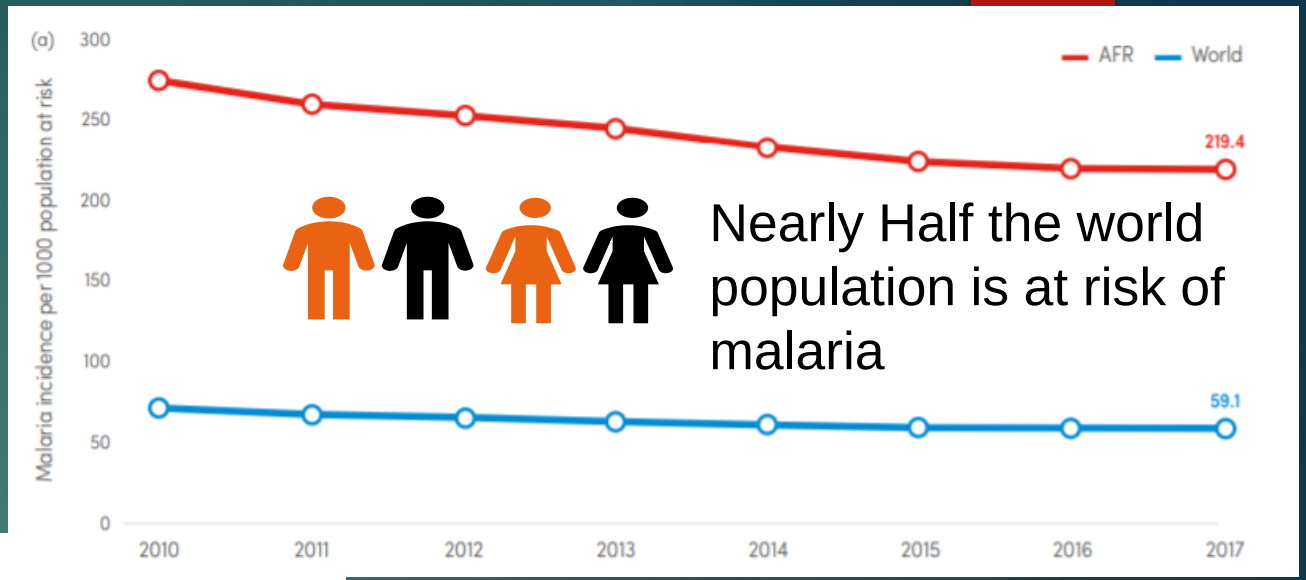
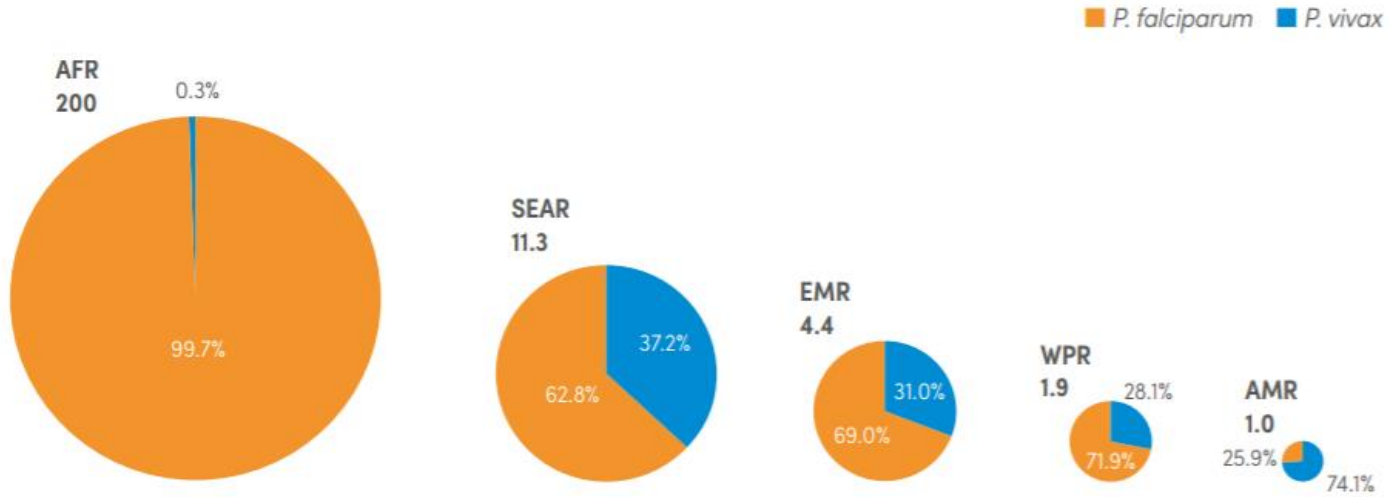


FIG. 6.1.

Estimated malaria cases (millions) by WHO region, 2017 The area of the circles is shown as a percentage of the estimated number of cases in each region. *Source: WHO estimates.*



AFR: WHO African Region; AMR: WHO Region of the Americas; EMR: WHO Eastern Mediterranean Region; *P. falciparum*: *Plasmodium falciparum*; *P. vivax*: *Plasmodium vivax*; SEAR: WHO South-East Asia Region; WHO: World Health Organization; WPR: WHO Western Pacific Region.

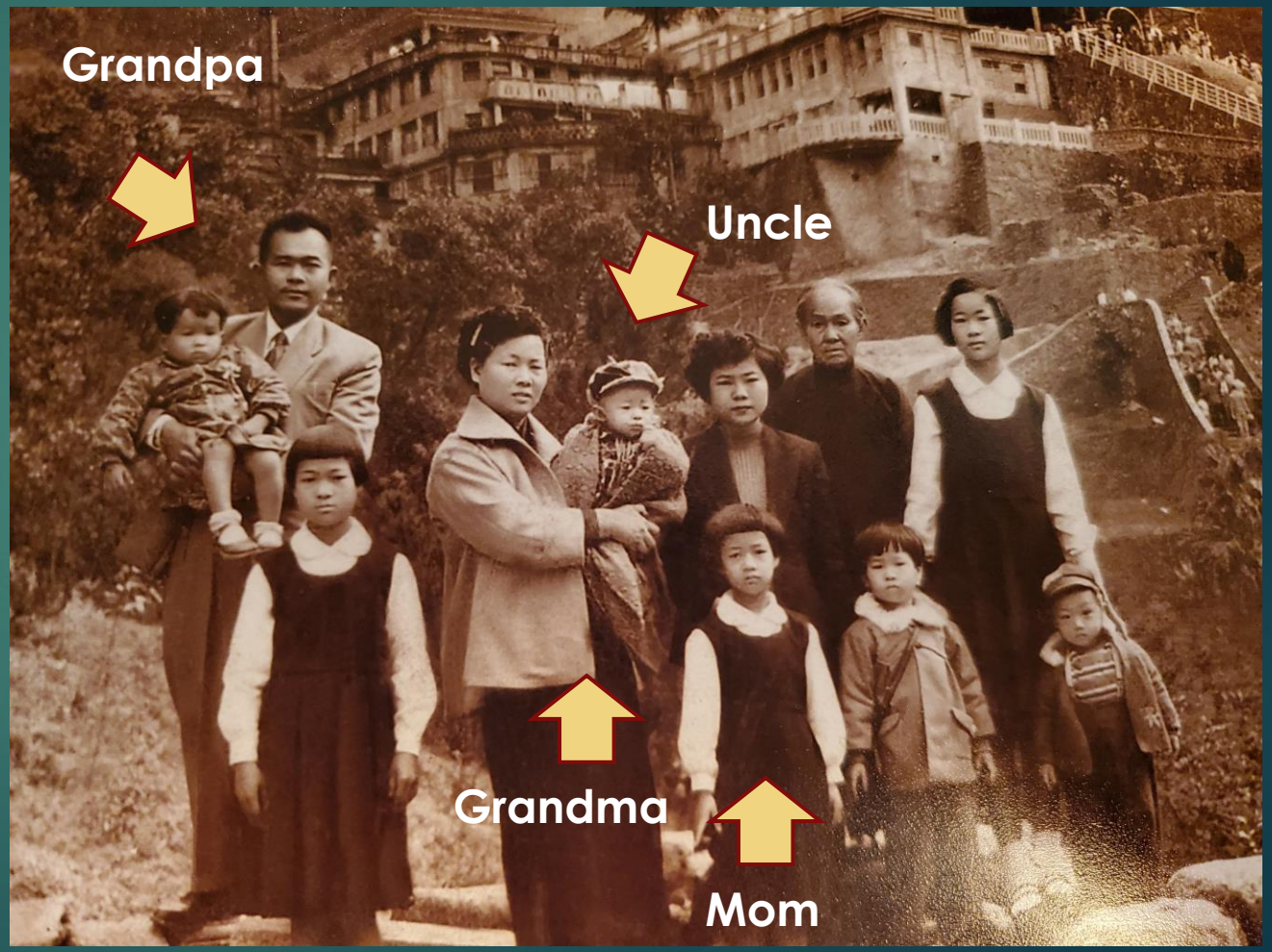
In 2007 the WHO endorsed the goal of Malaria Elimination and Eradication

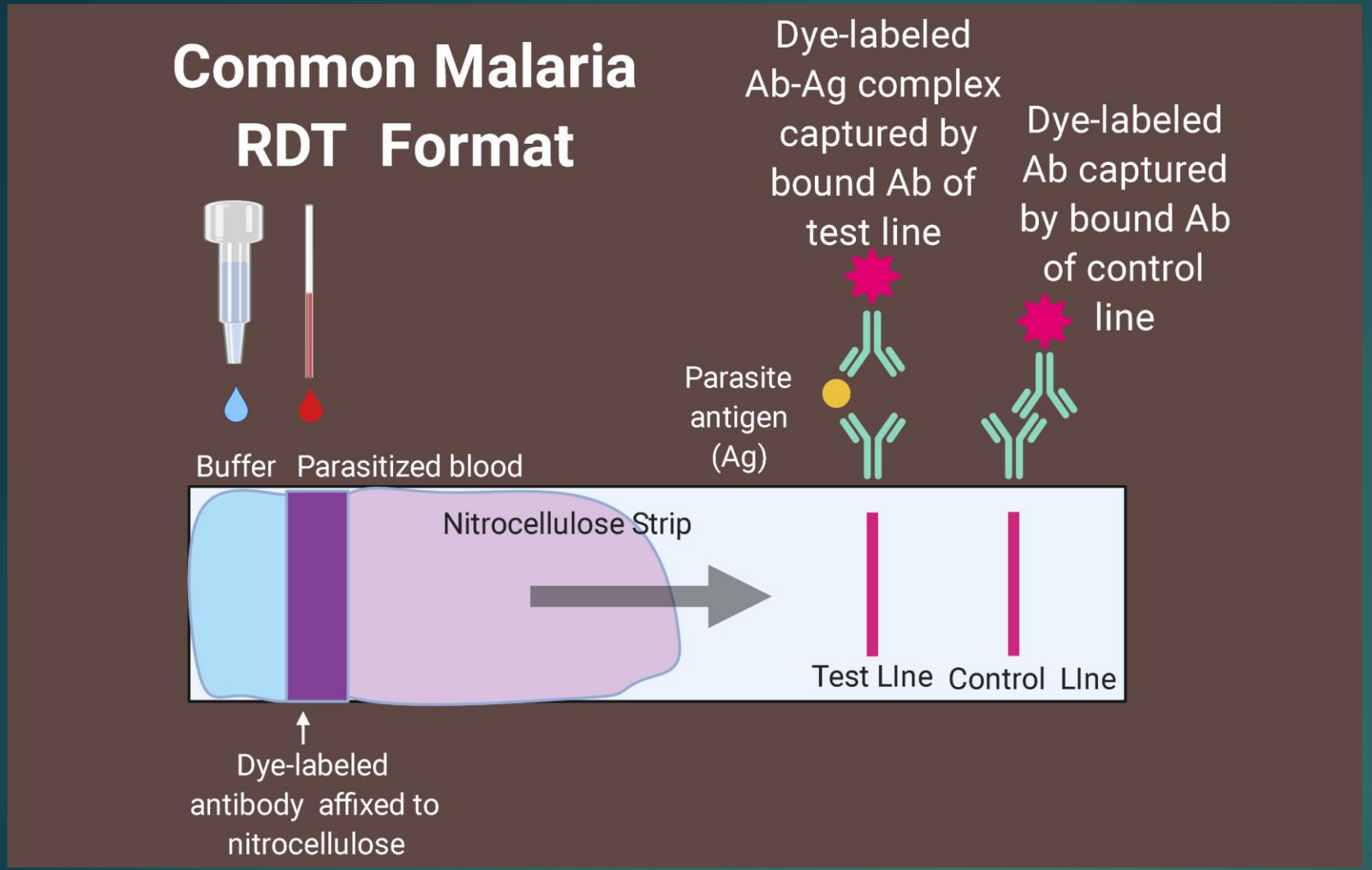
World Health Organization

Children under 5 years are the most vulnerable group affected malaria accounting for 61% of all malaria deaths worldwide.

Every

minutes a child dies of malaria

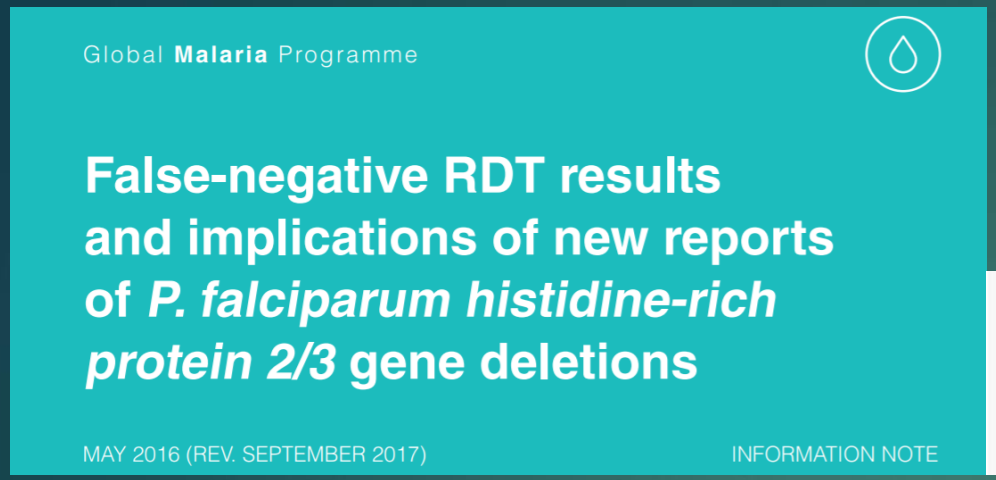




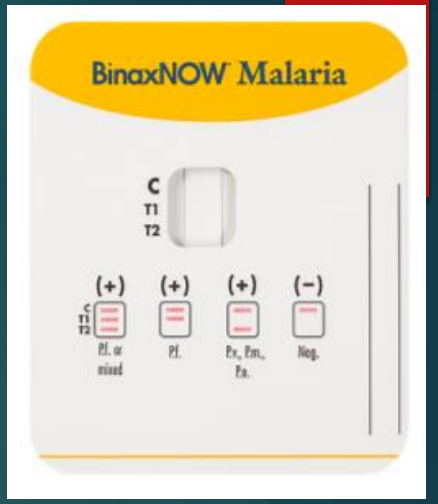
In 2017 there were an estimated 276 million **Rapid Diagnostic Tests (RDTs)** sold globally.

Most RDTS (66%) detected *P. falciparum* only and were supplied to sub-Saharan Africa.

In sub-Saharan Africa, RDTs are becoming the most used method to test for malaria. In 2017, an estimated 75% of malaria tests were conducted using RDTs, up from 40% in 2010.



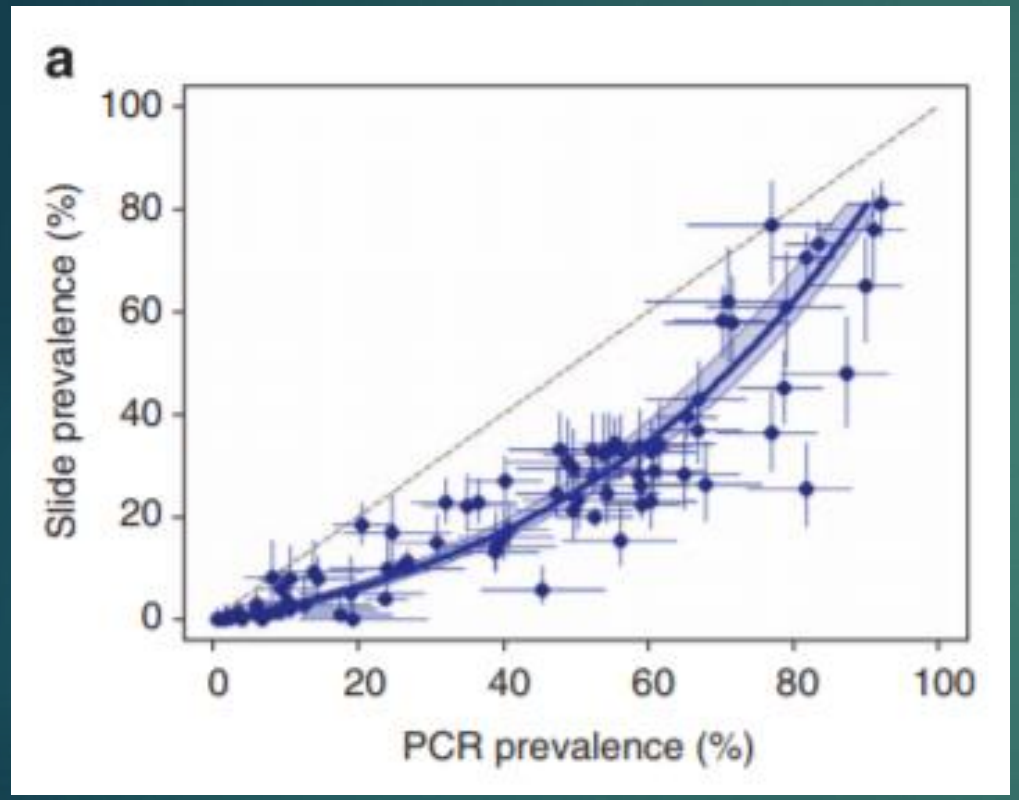
World Health Organization



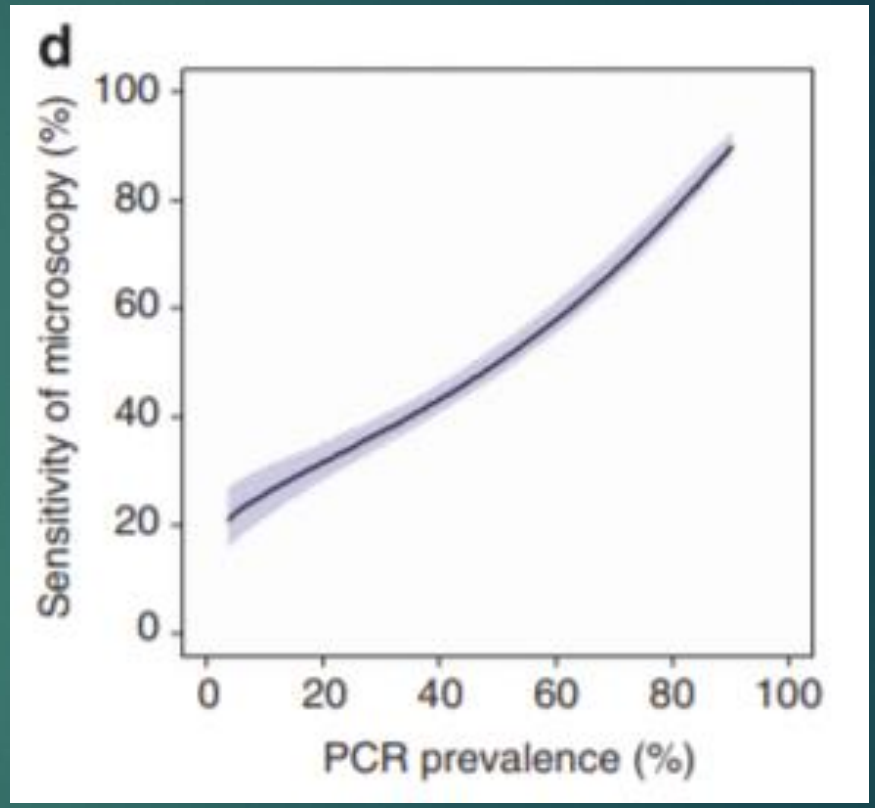
- Most currently available Rapid Diagnostic Tests (RDTs) work by detecting ***P. falciparum* histidine-rich protein II (HRP2) antigen**
- **2010** first confirmed identification of *P. falciparum* parasites with ***pfhrp2/pfhrp3* gene deletions**
  - Significant increase in Peruvian Amazon from 20.7% during 1998-2001 to 40.6% during 2003-2005.
  - Lower prevalence in other countries but HRP2 deletions found in Eritrea, Ghana, Kenya, Rwanda, and India

Parasitemia % Presume MCV 80fL (60fL)	Parasites per microliter	BinaxNOW Sensitivity: *Plasmodium falciparum*	BinaxNOW Specificity: *Plasmodium falciparum*	BinaxNOW Sensitivity: *Plasmodium vivax*	BinaxNOW Specificity: *Plasmodium vivax*
>0.04% (0.03%)	>5000	99.7% (326/327)	94.2% (3297/3500)	93.5% (462/494)	99.8% (2863/2870)
0.008% - 0.04% (0.006% - 0.03%)	1000-5000	99.2% (126/127)		81% (277/344)	
0.0008% - 0.008% (0.0003% - 0.006%)	100-1000	89.2 - 92.6% (33/37 - 25/27)		23.6 - 47.4% (34/144 - 37/78)	
0 – 0.0008% 0 – 0.0006%	0-100	53.9% (21/39)		6.2% (8/129)	

Ultrasensitive Detection Needed for Malaria Eradication



Prevalence data with 95% CI from 86 surveys containing both adults and children, and fitted model (blue line) with 95% CI of the mean (light blue area)



Estimated average sensitivity of microscopy and 95% CI of the mean in all-age surveys according to underlying PCR prevalence

Okell et al. Nature communications. 2012 Dec 4;3:1237

World Health Organization

WHO Evidence Review Group on Malaria Diagnosis in Low Transmission Settings

WHO Headquarters, Geneva, 16-18 December 2013

Malaria Policy Advisory Committee Meeting
12-14 March 2014, WHO HQ, Geneva
Session 10

The following preferred product characteristics for new technologies were discussed at the meeting:

- An ability to detect parasitaemia of ≤2 parasites/µl.
- Need for a sample volume of not more than 50µl blood.
- An assay that is not instrument specific.
- Flexibility in power supply.
- An ability to detect malaria parasites at genus level and then conduct species differentiation on positive samples.
- Results should ideally be available within 16 hours (same working day or early on the following day for patients providing samples just before closing hours), with a maximum waiting time of 24 hours for results.
- The assay should allow processing of 48 samples/person/platform/day.
- Reagents should be stable at 4°C for a minimum of one year, and at room temperature for a minimum of six months.

So what's needed for future malaria diagnostics?

High Density Infections	**Medium Density Infections**	**Low Density Infections**
Plasmodium falciparum	*Plasmodium falciparum*	*Plasmodium falciparum*
Non-falciparum malaria (***P. vivax, ovale***, *malariae, knowlesi*)	Non-falciparum malaria (***P. vivax, ovale***, *malariae, knowlesi*)	Non-falciparum malaria (***P. vivax, ovale***, *malariae, knowlesi*)

RESEARCH ARTICLE

Field Evaluation of a High Throughput Loop Mediated Isothermal Amplification Test for the Detection of Asymptomatic *Plasmodium* Infections in Zanzibar

Berit Aydin-Schmidt[1,2]*, Ulrika Morris[1], Xavier C. Ding[3], Irina Jovel[1], Mwinyi I. Msellem[4], Daniel Bergman[1], Atiqul Islam[1], Abdullah S. Ali[4], Spencer Polley[5], Iveth J. Gonzalez[3], Andreas Mårtensson[6], Anders Björkman[1]

1 Centre for Malaria Research, Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, **2** Unit of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden, **3** Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland, **4** Zanzibar Malaria Elimination Programme, Ministry of Health, Zanzibar, Tanzania, **5** Department of Clinical Parasitology, Hospital for Tropical Diseases, University College London Hospitals, NHS Foundation Trust, London, United Kingdom, **6** Department of Women's and Children's Health, International Maternal and Child Health (IMCH), Uppsala University, Uppsala, Sweden

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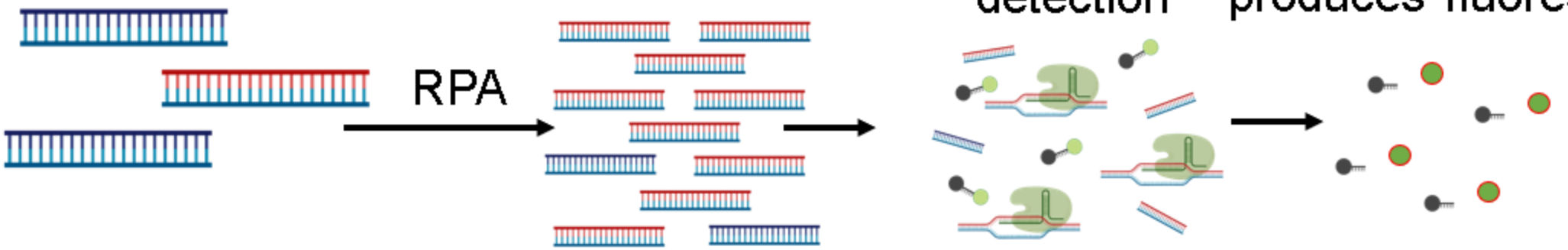
Table 3. Diagnostic accuracy of Malaria pan HTP-LAMP compared to PCR for 3008 field samples.

	PCR +	PCR -	Total
HTP-LAMP +	20	2	22
HTP-LAMP -	29	2957	2986
Total	49	2959	3008
	p < 0.001*		
Sensitivity	40.8% (95%CI 27.0–55.8%)		
Specificity	99.9% (95%CI 99.8–100%)		
Positive predictive value	90.9% (95%CI 70.8–98.9%)		
Negative predictive value	99.0% (95%CI 98.6–99.3%)		

* by McNemar´s test.

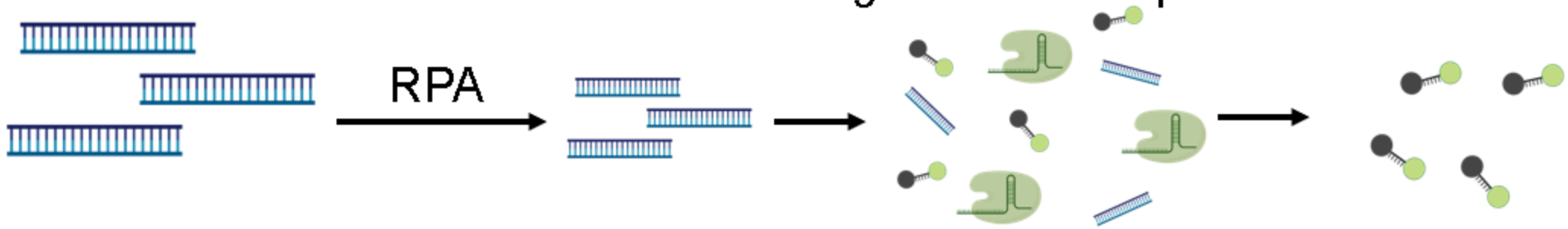
doi:10.1371/journal.pone.0169037.t003

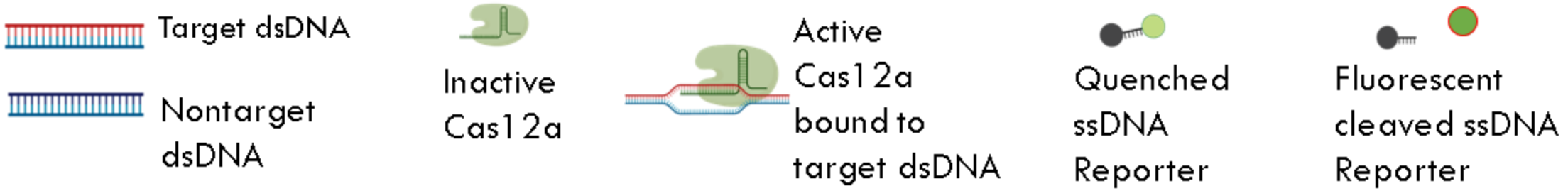
Target *Plasmodium* DNA present



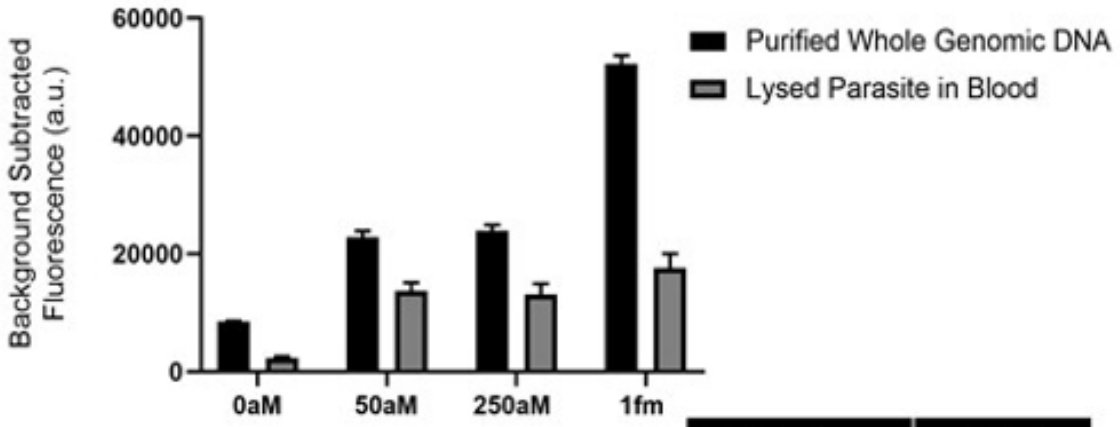
No Target DNA present

Un-activated Cas12a results in no collateral cleavage of ssDNA reporter and releases no signal

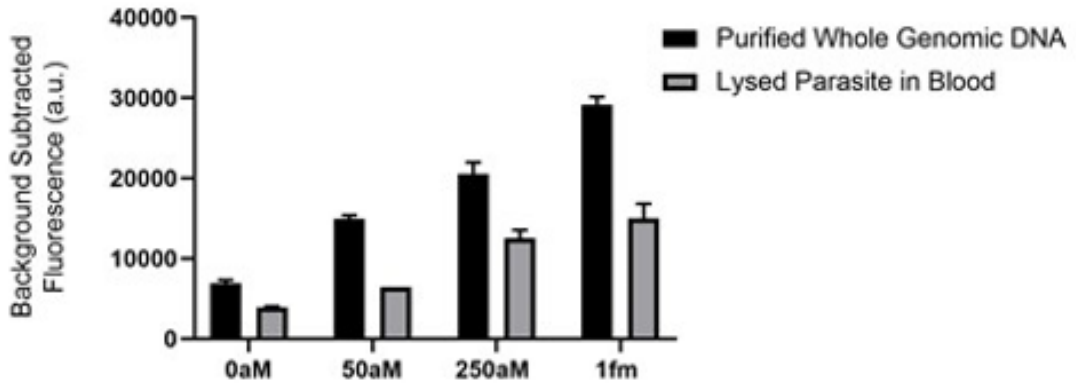




a

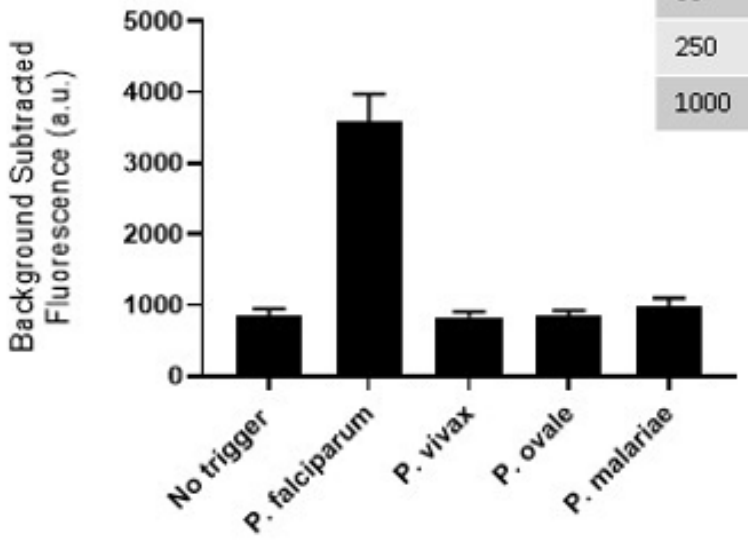


b

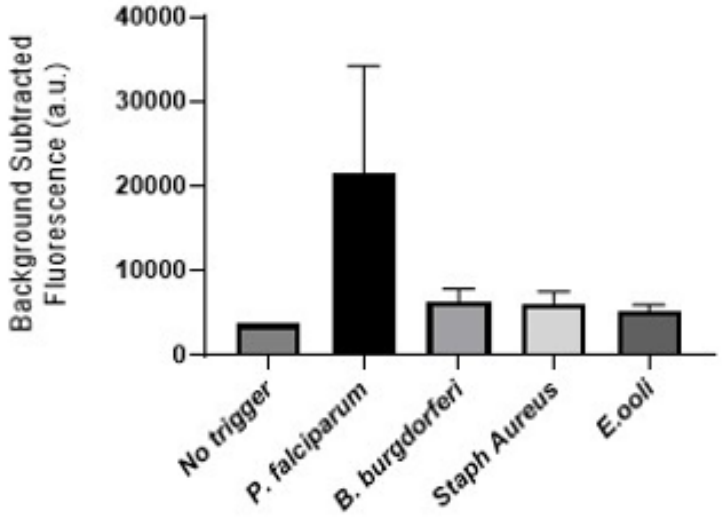


attomolar concentration	parasites per µL
50	2
250	10
1000	40

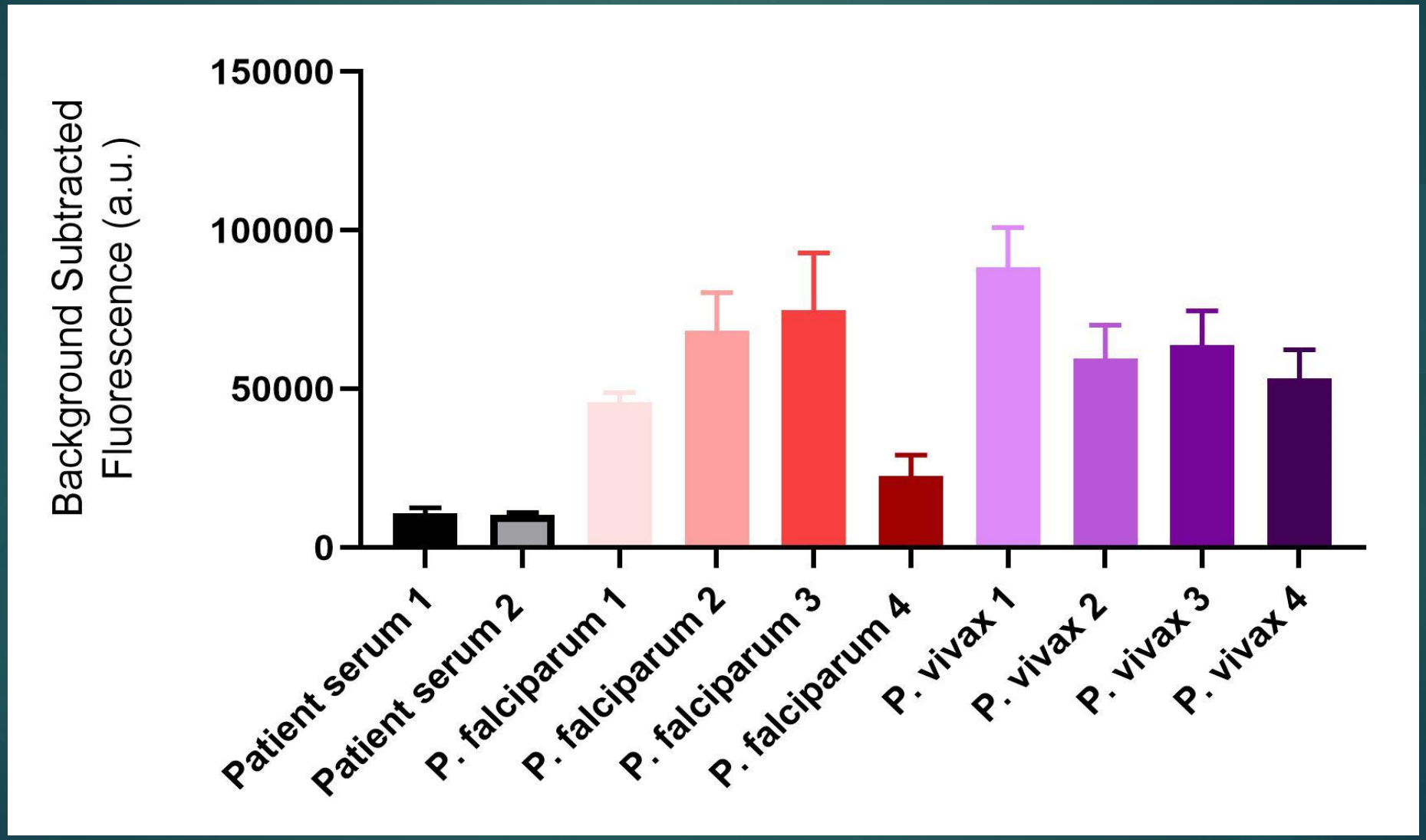
c



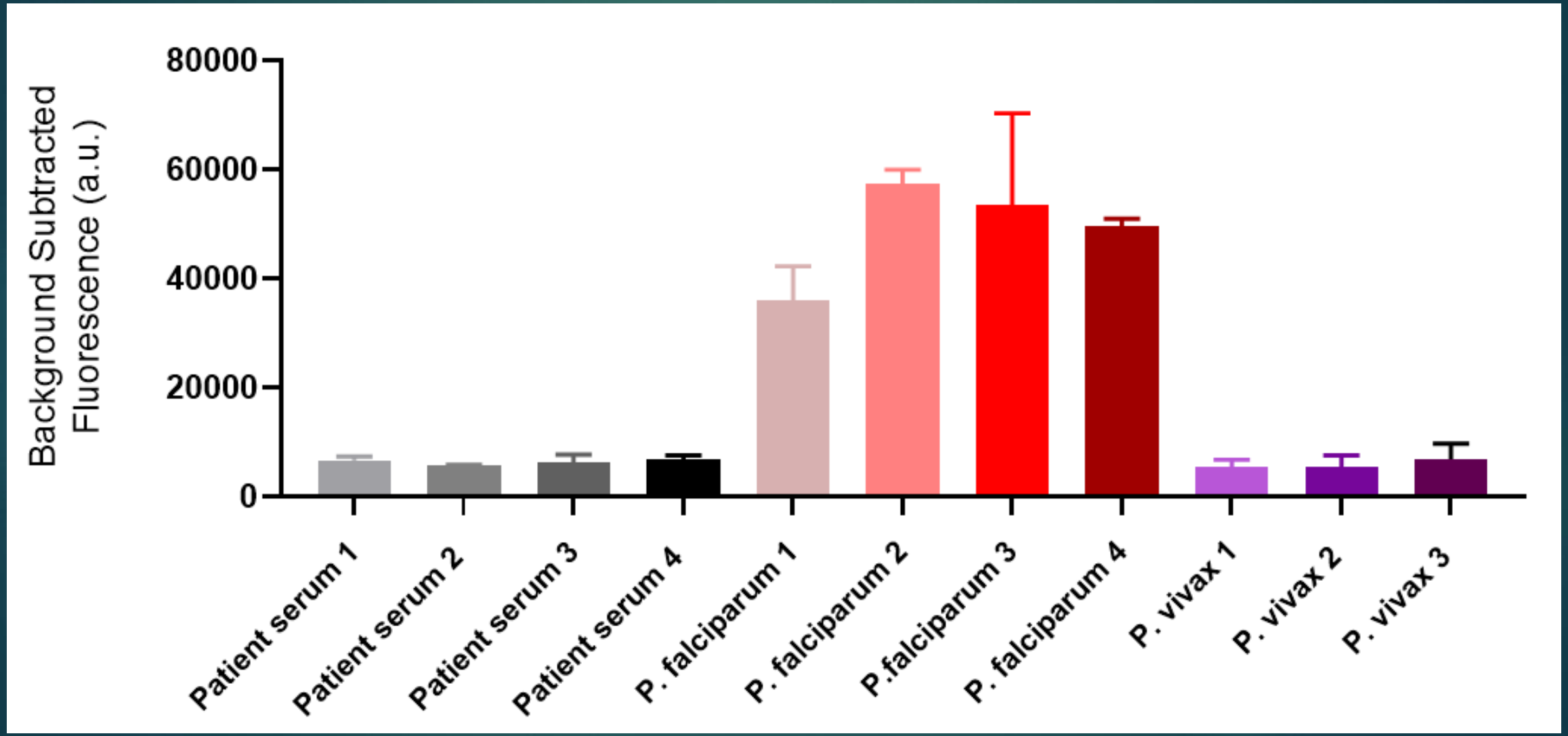
d



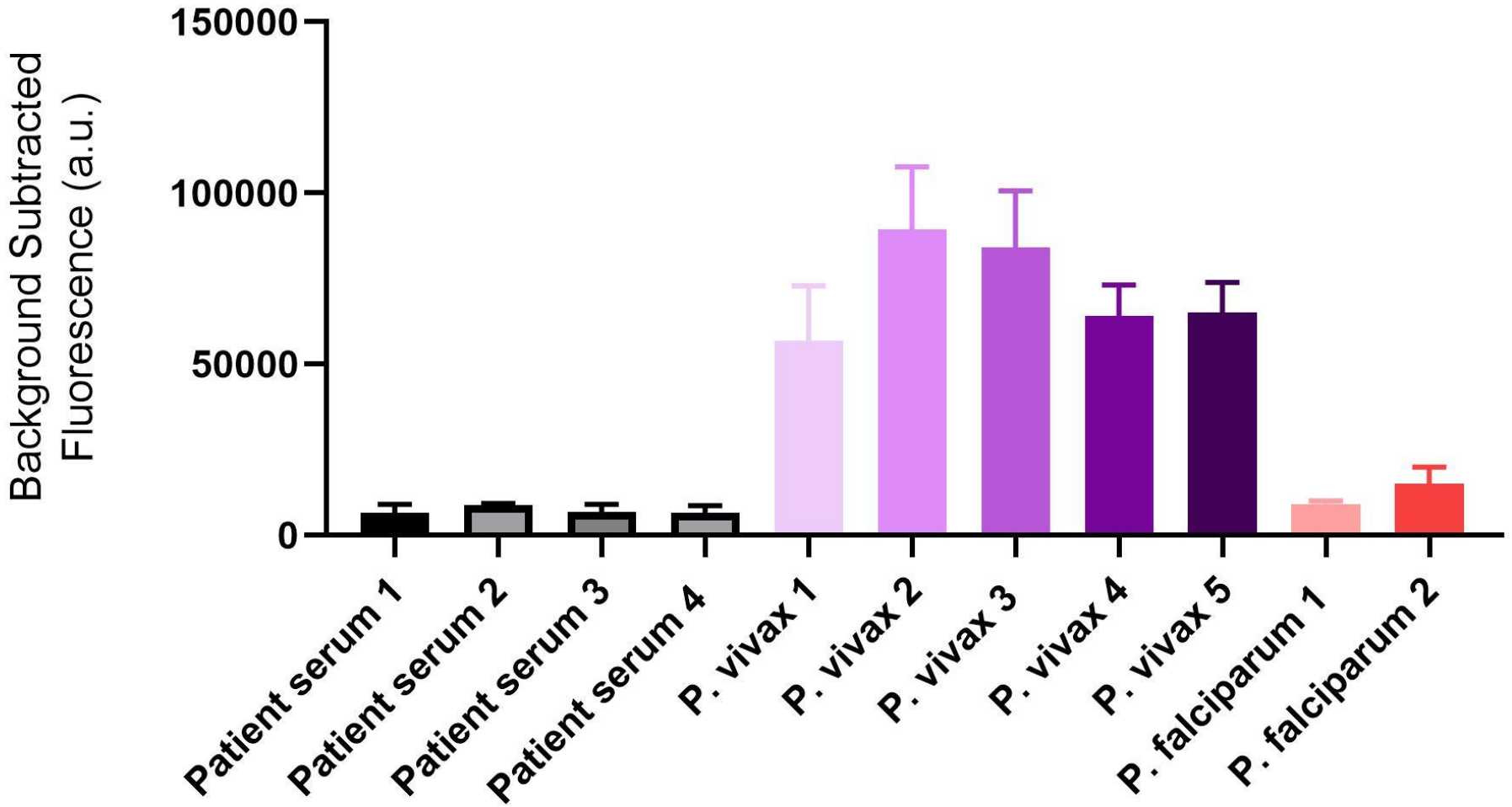
Pan-plasmodium target



Falciparum specific target

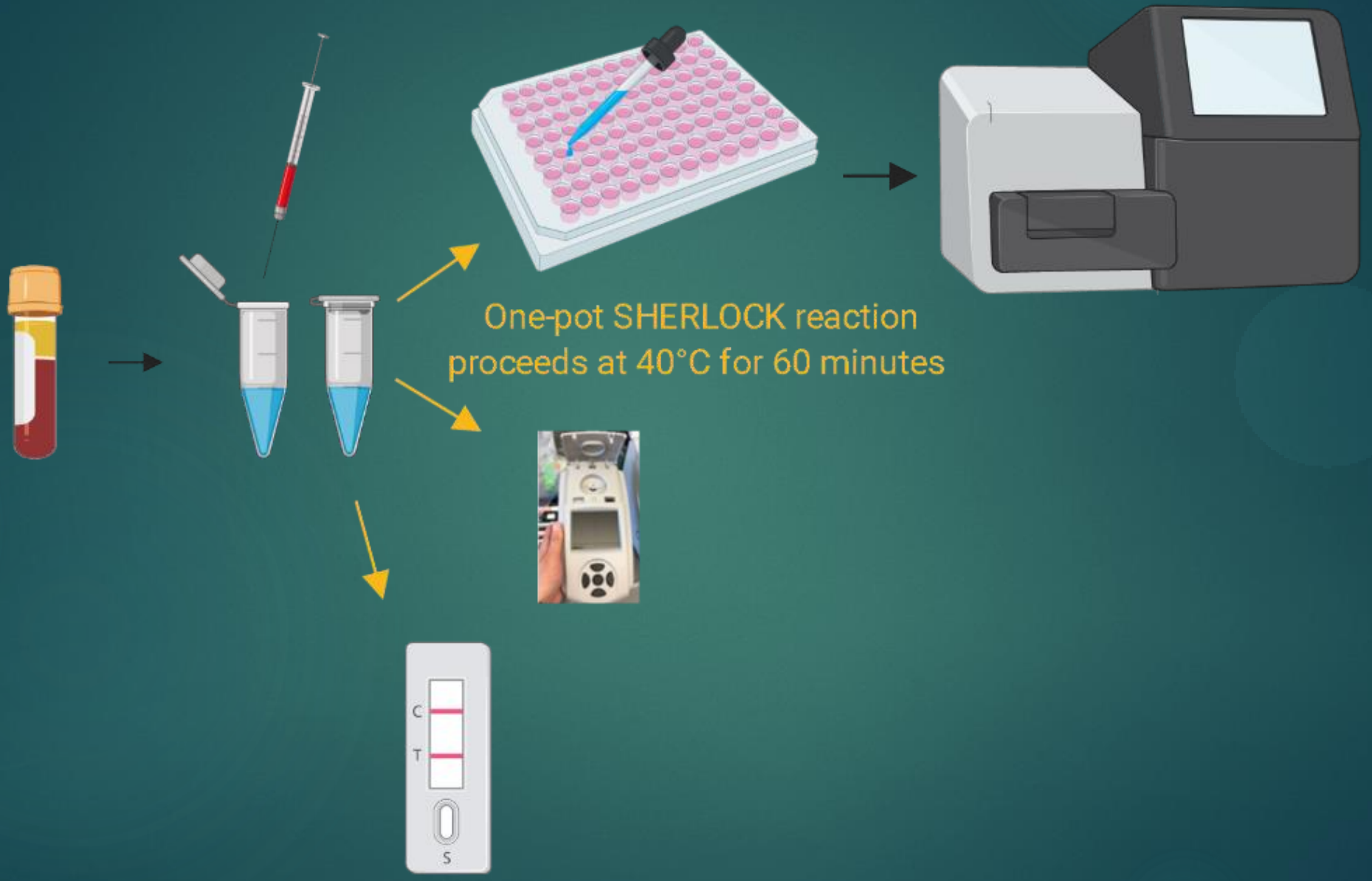


Vivax specific target



40 p/uL 2 p/uL

0 aM 1 fM 50 aM


One-pot SHERLOCK reaction
proceeds at 40°C for 60 minutes
C
T
S

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- Nira Pollock
- Jeffrey Dvorin
- Helena de Puig Guixe

Questions/Comments?