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Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia

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International conference on parasitology,2015
Philadelphia ,USA

Introduction

- Malaria is one of the deadly infectious disease worldwide especially in SSA countries like Ethiopia
- To minimize the burden of mortality and morbidity, early and accurate diagnosis of malaria is critically required

Introd..

- Current routine diagnostic methods (Microscopy and Rapid diagnostic tests)
  - used to reduce the morbidity and mortality with malaria
  - less sensitive for detection of low level infections and asymptomatic cases

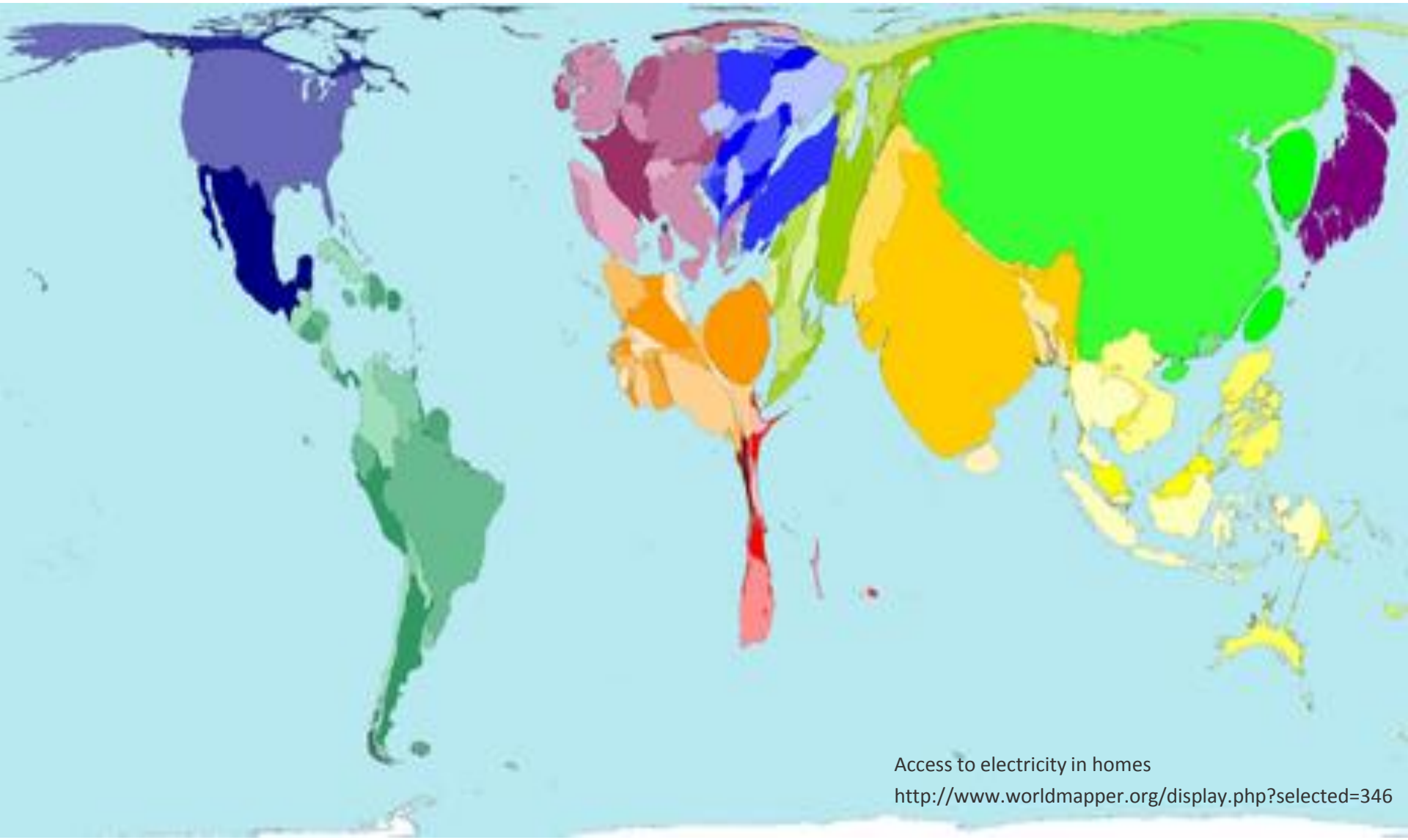
Bell D, WHO, 2010. Golassa L etal, 2013. Alemu etal , 2014.

Introd...

- PCR assays are highly sensitive and specific method that can detect low level parastemia
- PCR is not feasible in low resource settings (LRS)

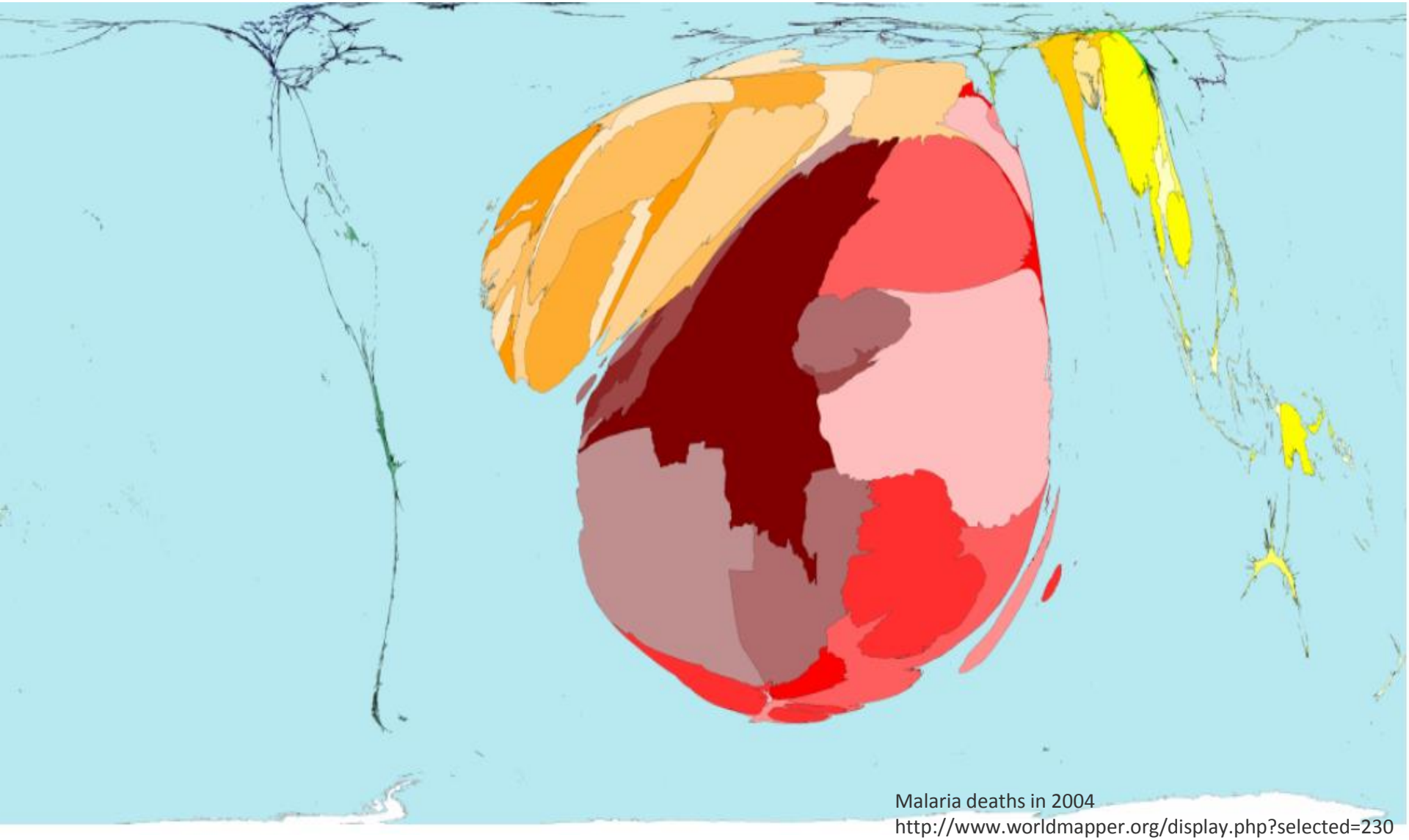
(WHO, 2014, Cordray et al,2012 ; Noemi et al, 2000; Polley et all, 2010)

Electricity Access



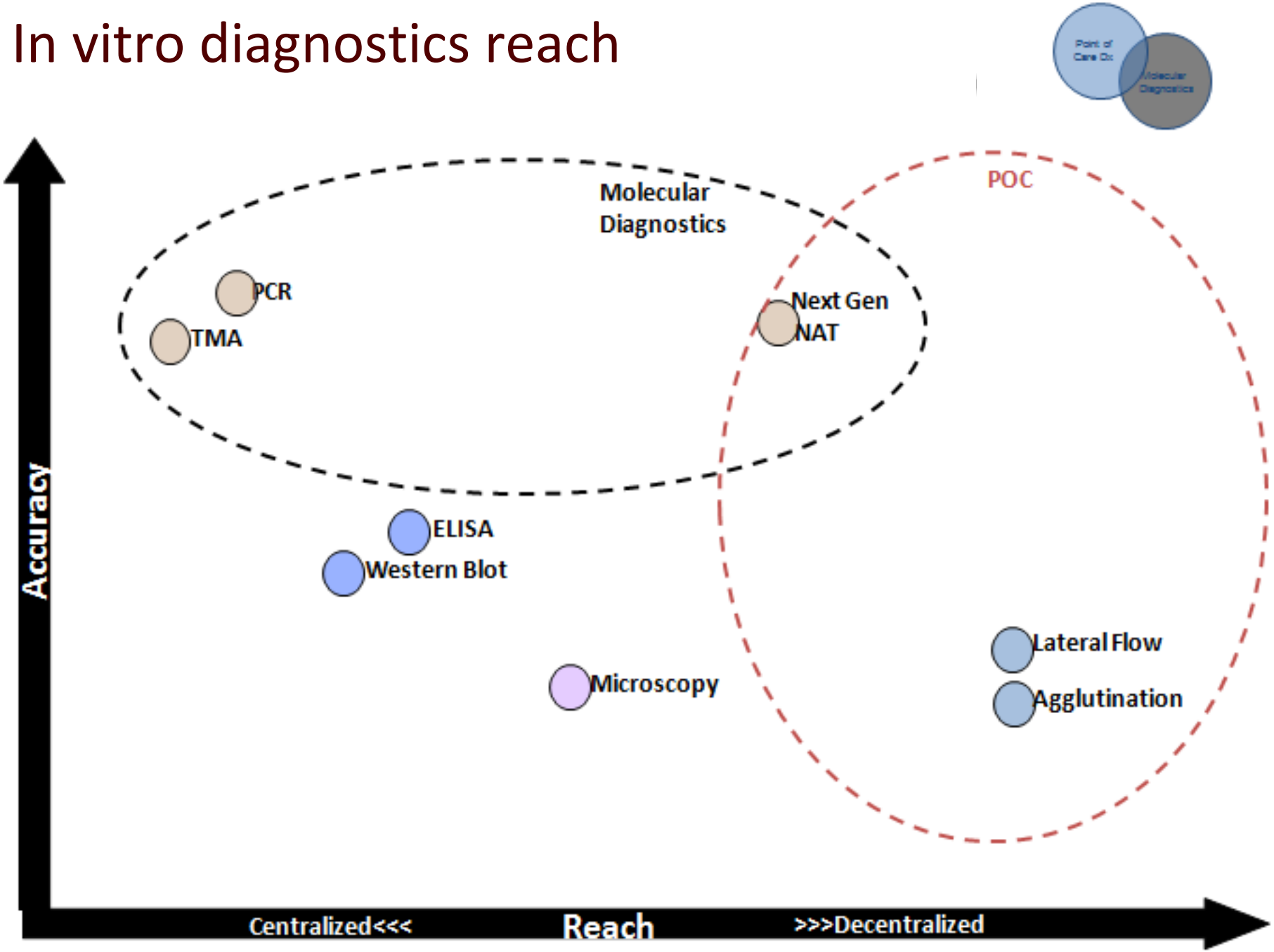
Access to electricity in homes
http://www.worldmapper.org/display.php?selected=346

Malaria Deaths

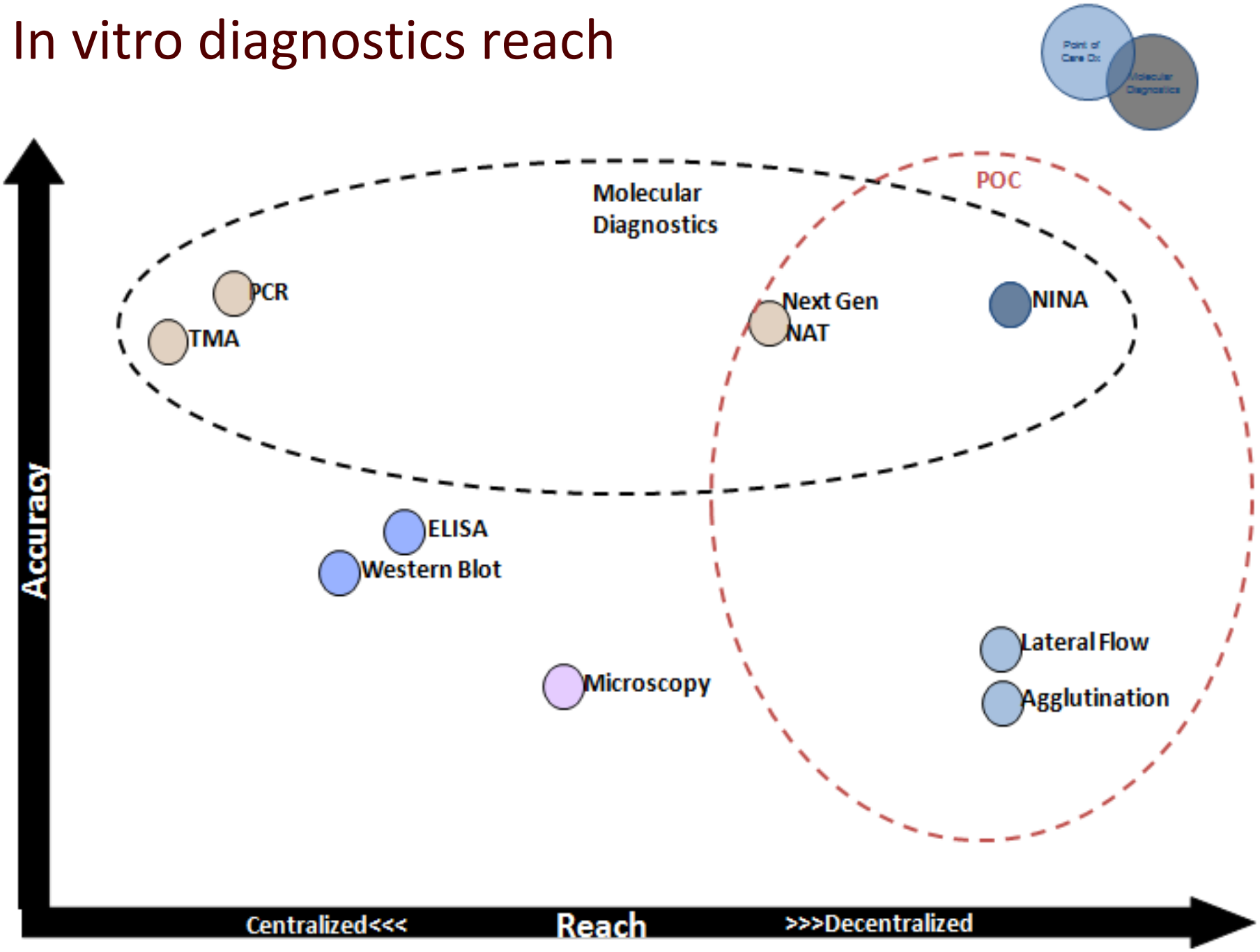


Malaria deaths in 2004
http://www.worldmapper.org/display.php?selected=230

In vitro diagnostics reach



In vitro diagnostics reach



Introd...

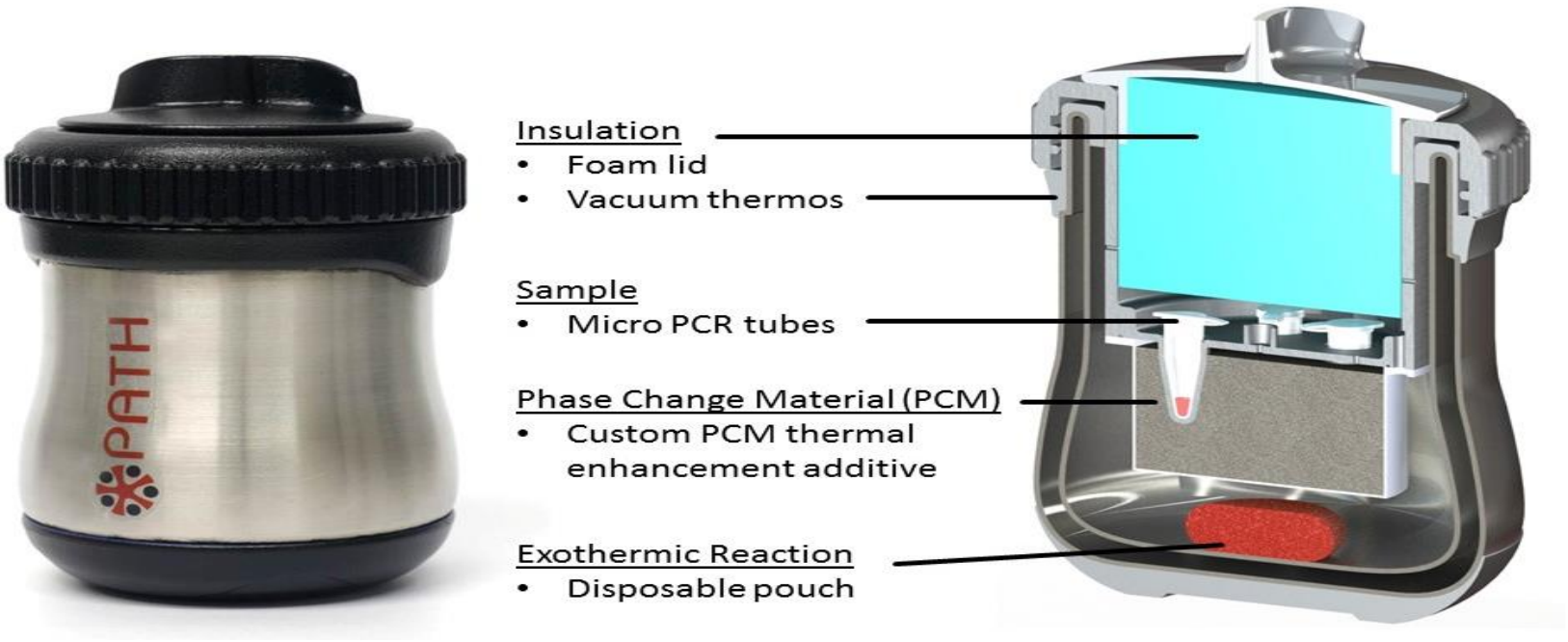
- In developing countries like Ethiopia, more sensitive, specific, cost-effective, rapid and easy method of diagnosis is crucial to the success of the NSP to eliminate and eradicate malaria

- LAMP was developed as promising simplified molecular diagnostic method appropriate in LRS

(WHO, 2014, Cordray et al,2012 ; Noemi et al, 2000; Polley et all, 2010)

Introd…

- PATH developed a variety of NINA heaters to facilitate pathogen detection via LAMP.



(Weigl et al,2008; LaBarre etal ,2010,2011; Singleton etal, 2014)

What is NINA?

(12) **United States Patent**
LaBarre et al.

(10) **Patent No.: US 8,431,387 B2**
(45) **Date of Patent: Apr. 30, 2013**

(54) **CHEMICAL TEMPERATURE CONTROL**

(75) Inventors: **Paul Donald LaBarre**, Suquamish, WA (US); **Jay Lewis Gerlach**, Kenmore, WA (US); **Bernhard Hans Weigl**, Seattle, WA (US); **Gonzalo Jose Domingo-Villegas**, Seattle, WA (US)

(56) **References Cited**

U.S. PATENT DOCUMENTS

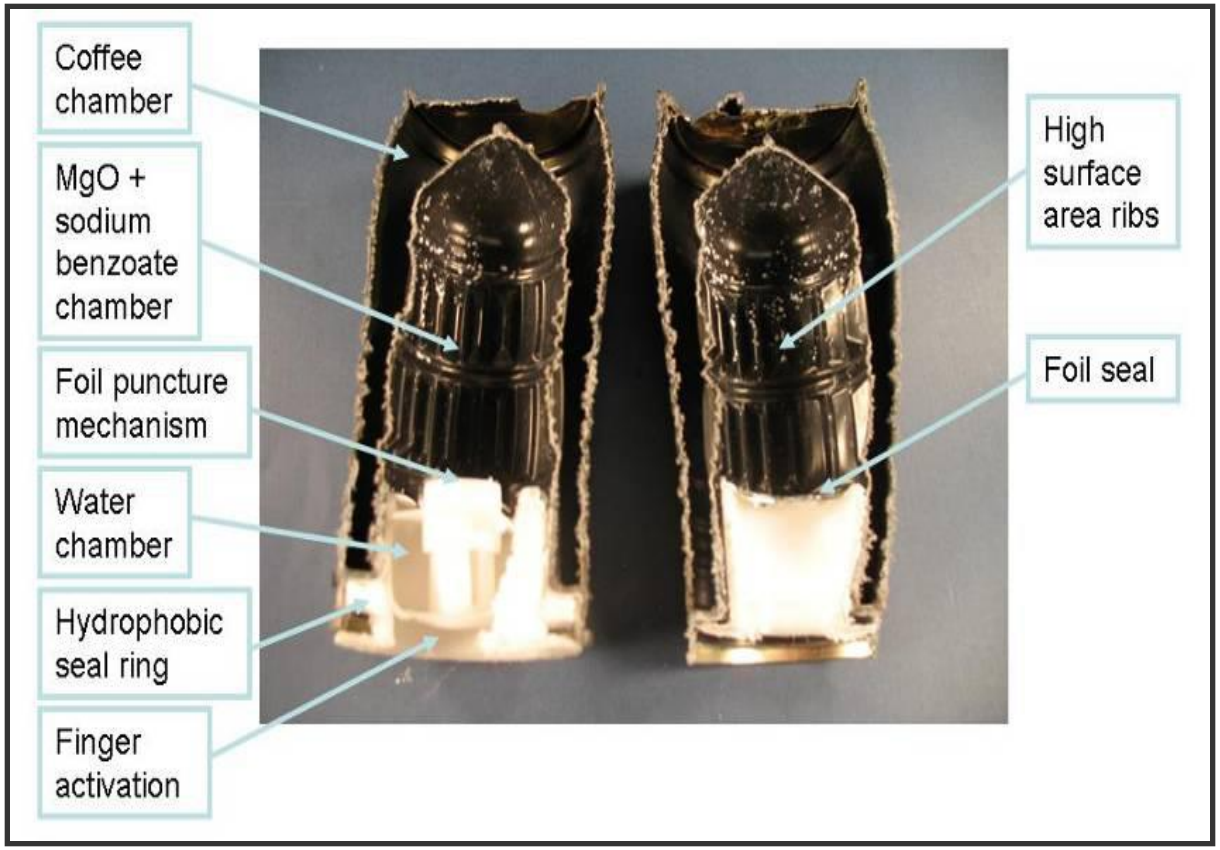
3,903,011 A 9/1975 Donnelly
3,976,049 A 8/1976 Yamashita et al.

(Continued)

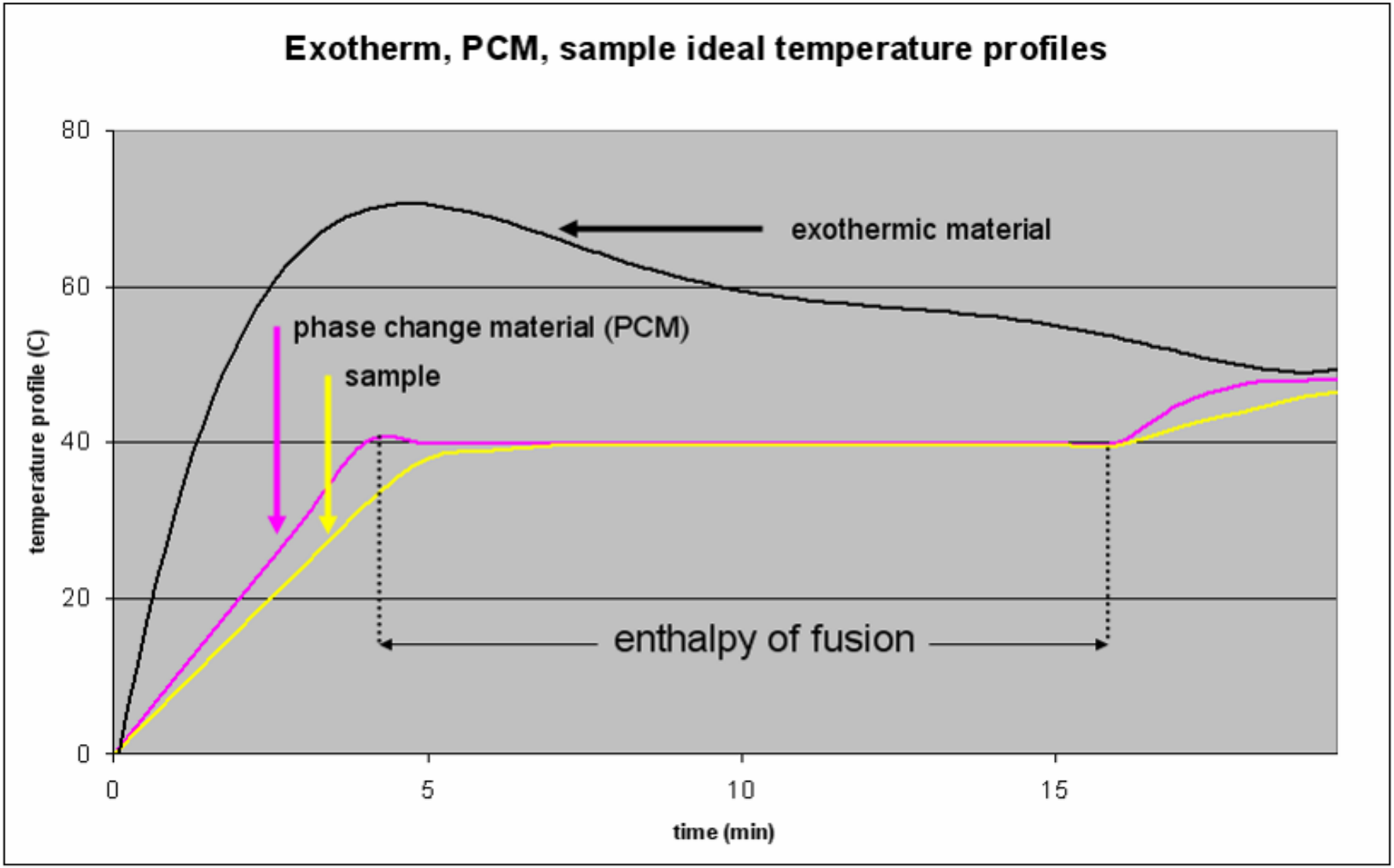
- **NINA:** stands for “non-instrumented nucleic acid amplification”
- **The NINA mission:** expanding access to accurate diagnostics wherever they are needed
- **The NINA vision:** 100% electricity free, infrastructure-free, sample to results, molecular diagnostics
- **Strategy**

NINA: Inspiration from a consumer product

Inspiration from a consumer product



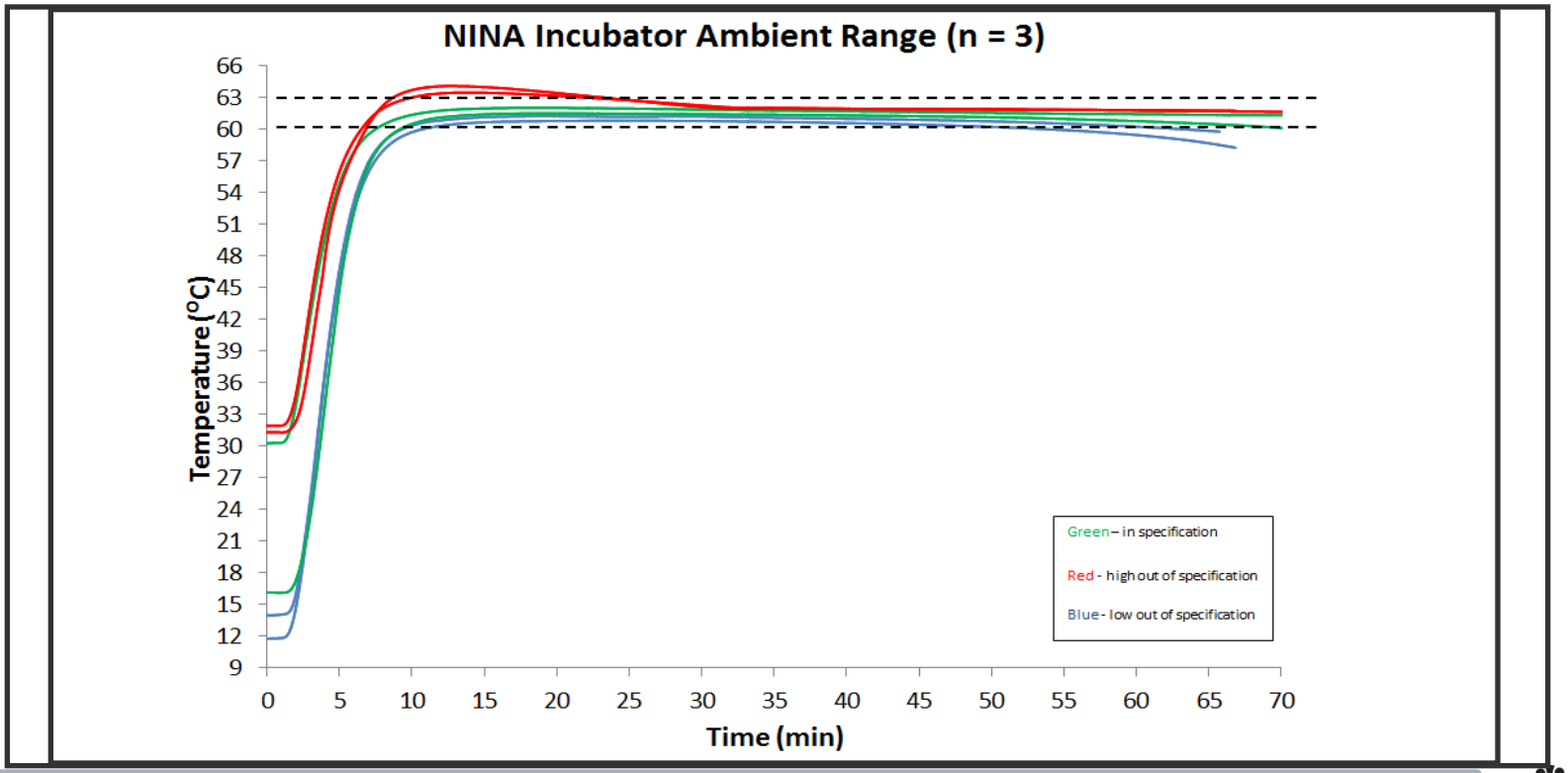
NINA: Theory of operation



RESEARCH ARTICLE

Electricity-Free Amplification and Detection for Molecular Point-of-Care Diagnosis of HIV-1

Jered Singleton[1], Jennifer L. Osborn[1], Lorraine Lillis[1], Kenneth Hawkins[1], Dylan Guelig[1], Will Price[2], Rachel Johns[1¤], Kelly Ebels[1], David Boyle[1], Bernhard Weigl[1], Paul LaBarre[1*]



Objectives

General objective

- To examine the diagnostic performance of NINA-LAMP compared to microscopy and nested PCR for the diagnosis of malaria

Specific objectives

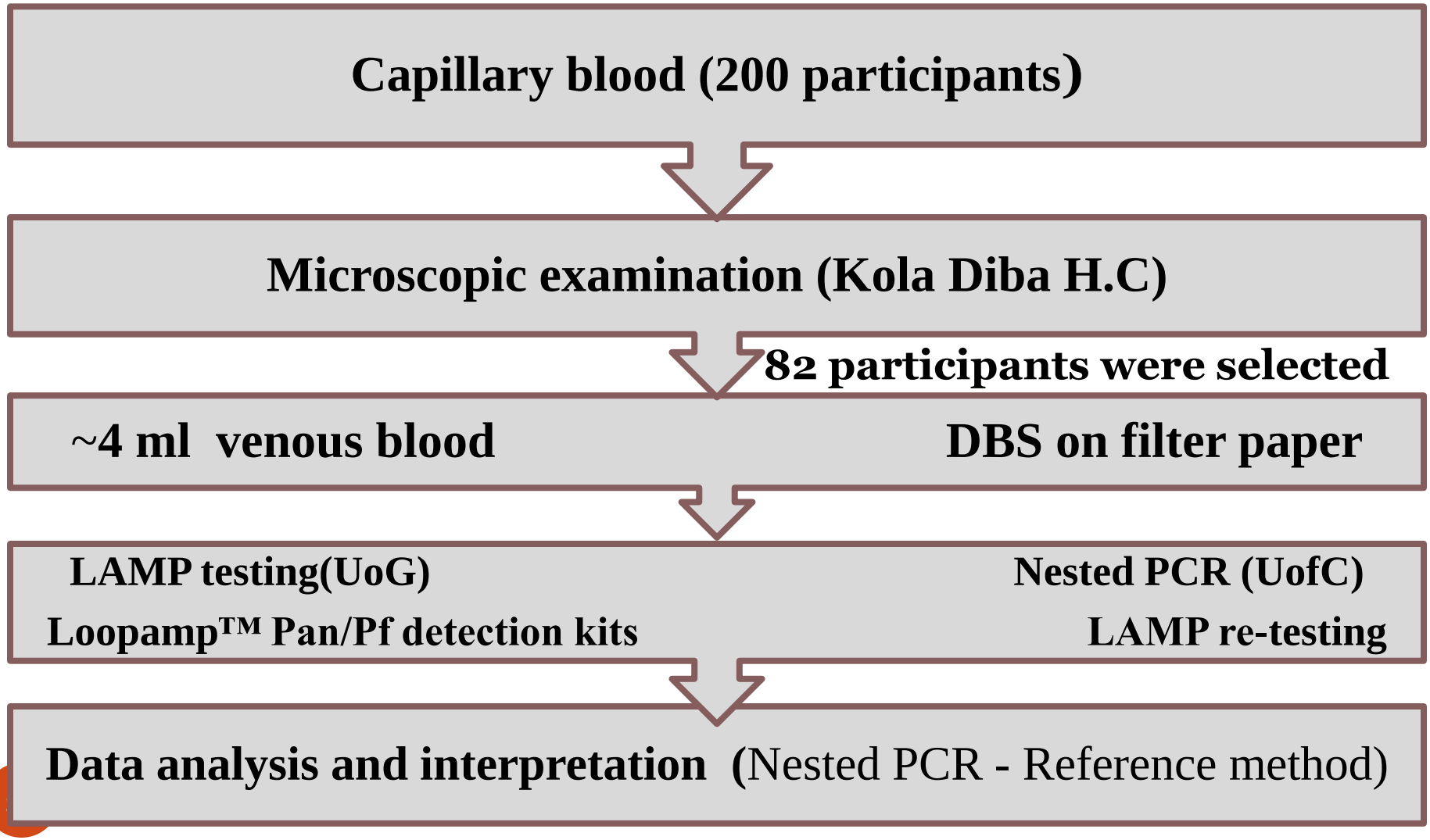
- To compare parasite positivity by Giemsa microscopy and NINA-LAMP and nested PCR
- To determine the diagnostic accuracy of NINA-LAMP and Giemsa microscopy as compared to nested PCR

Materials and methods

Study characteristic	description
Study area	Kola Diba Health center
Study design and period	Cross-sectional study, March to July 2014
Study population	All febrile malaria suspected outpatients in HC during the study period
Sample size	200 malaria suspected febrile patients
Sampling technique	Convenient sampling

Methods…

Sample collection and laboratory methods



Methods…

NINA-LAMP principle and procedure

DNA Extraction → mixing of sample and reagent → Amplification → Detection

Boil and spin method

NINA H.V6 prototype heater device

Visual detection of turbidity

R. Time

10-15 minutes

45-60 minutes

Results and discussion

Parasite positivity by microscopy, NINA-LAMP and nested PCR

Giemsa microscopy (*n*)	Primary NINA- LAMP result (n)	Nested PCR (n)
Positives (30),	Positives (38),	Positives (31),
P. falciparum (13)	*P. falciparum* (26)	*P. falciparum (13)*
P.vivax (17)	Non- *P. falciparum* (12)	*P. vivax (11), P. ovale* (7)
Negatives (52)	Negatives (44)	Negatives (51)

Result…

Diagnostic accuracy of NINA-LAMP and microscopy using nested PCR as reference method

Method		Senstivity(%)	Specificity(%)	%agreement
NINA -LAMP	Pan (Gondar)	**96.8**	**84.3**	**89.0**
	Pf (Gondar)	**100**	**81.2**	**84.1**
	Pan (Calgary)	**96.8**	**98.0**	**97.6**
	Pf (Calgary)	**100**	**100**	**100**
Microscopy	Pan	**93.6**	**98.0**	**96.3**
	Pf	**92.3**	**100**	**98.8**

Result…

- **NINA- LAMP**
  - Satisfies the WHO recommendation of diagnostic kits sensitivity of > 95%
  - Comparable performance with nested-PCR and superior to Microscopy
  - Comparable accuracy to other LAMP evaluation studies conducted using nested PCR as the reference method.

Result…

- **Good operational characteristics**

- Previous experiences was not required to perform
- Reading of test result and interpretation was easy
- The whole test procedure takes about 60-80 minutes
- Special equipments were not required
- The reagents were thermostable

Limitations of the study

- Use of relatively small sample size
- The use of microscopy for case detection
- Failure to obtain “asymptomatic” or low parasitemia cases.
- sample preparation still requires a centrifugation step

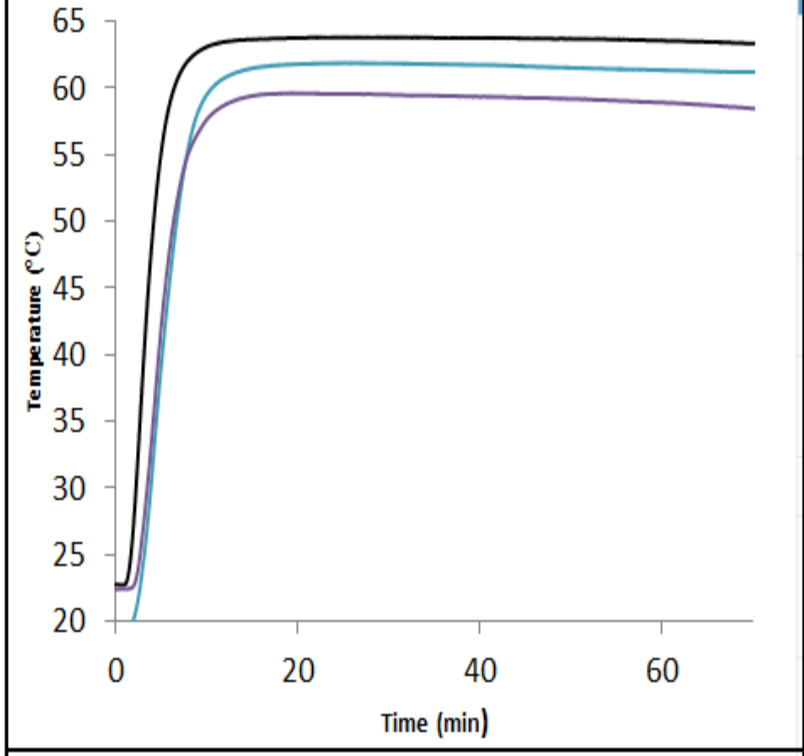
NINA Reusable Housing Platform (RHP)

- $34.37/prototype
- $0.22/test
- 5x 0.2mL PCR tubes
- Maintains 62-65°C for 60min
- Vacuum thermos insulation
- Simple activation
- 240cc device volume

NINA Single-Use Disposable (SUD)

- $1.83/prototype
- 1x 0.2mL PCR tube
- Maintains 58-60°C for 45min
- Closed-cell foam insulation
- 100cc device volume

Current collaborations & operating temps



Collaborator	Assay	Temperature Requirement	Phase Change Material (PCM)
Centers for Disease Control, USA	LAMP, HIV-1	58 – 61C	PureTemp 60
Center Pasteur, Cameroon	RT-LAMP, *Pf* gametocytes	62-67C	PureTemp 65
New England Biolabs	LAMP, Filariasis	62-64C	Palmitic Acid
University of Calgary, Canada	LAMP, *Plasmodium* spp.	62—65C	Puretemp 65
PATH, USA	LAMP, HIV-1	60—63cC	Palmitic Acid
Noguchi Research Institute, Ghana	LAMP, Buruli Ulcer	62—65C	PureTemp 65
***Proposed, Lucigen**	*OmniAmp (LAMP), Pf and EBOV*	*69-72C*	*Na2SiO3-5H2O (proposed)*

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Gebeyaw Getnet
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Sema et al. Malaria Journal (2015) 14:44
http://www.malariajournal.com/content/14/1/44

- Kola Diba health center laboratory staffs and study participants

Thank you!