Different Laboratory Tools for Case Management, Surveillance, Malaria Elimination Settings and Outbreak Investigations

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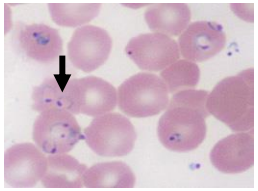
Lab Methods for Malaria Parasite Detection and Exposure

Malaria diagnosis and exposure detection

Microscopy	Rapid Diagnostic Tests (RDTs) (antigens)	Molecular Methods	Serology (parasite exposure)
			
Visualize Parasite morphology	Detect antigens	Detect nucleic acids	Measure antibodies

Case Management and Therapeutic Efficacy Studies

Microscopy

- Sensitivity range 100-200p/uL (expert LOD=10-20 p/ul)
- Most appropriate tool in Americas (Highly important to provide training and quality management)

RDTs

- Limited to settings where microscopy is not available
- Sensitivity ~100-200p/uL
- *hrp*-2 gene deletion (false negative test results)

Molecular tools

- Expensive and technically challenging (for reference lab)
- Sensitivity varies and WHO recommends 1 p/ul (**rarely met**)

Surveillance

Microscopy: Time consuming and results may not be obtained in a timely manner

RDTs: Commonly used due to ease of use

Challenge for use in Amazon countries due to pfhrp2 deletion

Molecular: Recommended for submicroscopic detection of malaria parasites

Surveillance/Elimination

Serology:

- When parasite prevalence reaches low level (<1%) it is difficult to map transmission zones

- Valuable tool for determining malaria exposure by measuring antibody levels

- Methods include ELISA and Luminex assay

Outbreak Investigations/Reintroduction of Parasites in Elimination Settings

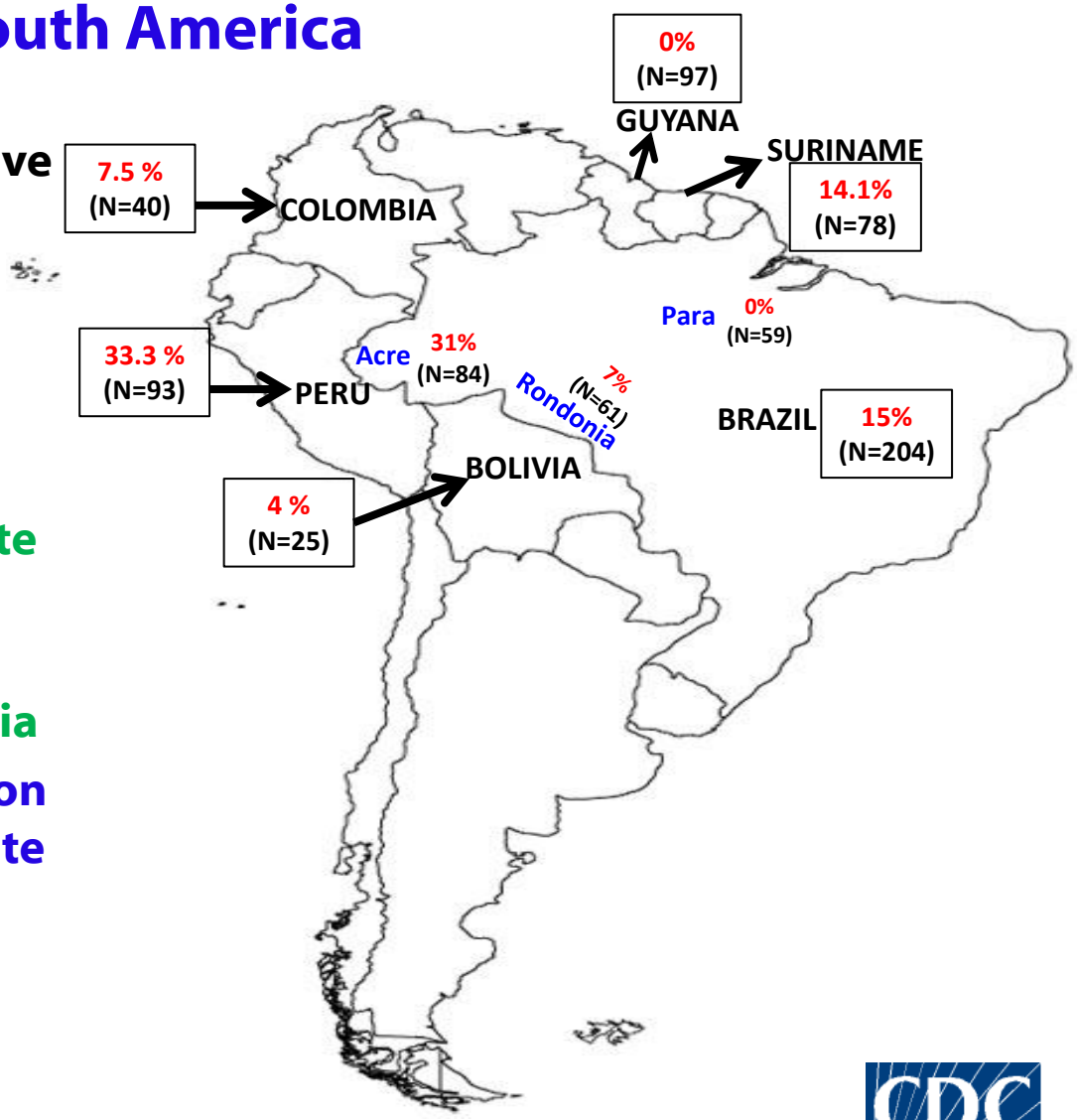
Besides microscopy and RDTs- molecular tools are increasingly used for

- Confirmation of species
- Source of parasites (geographical origin)
- Specialized tools such as microsatellites, molecular barcodes etc

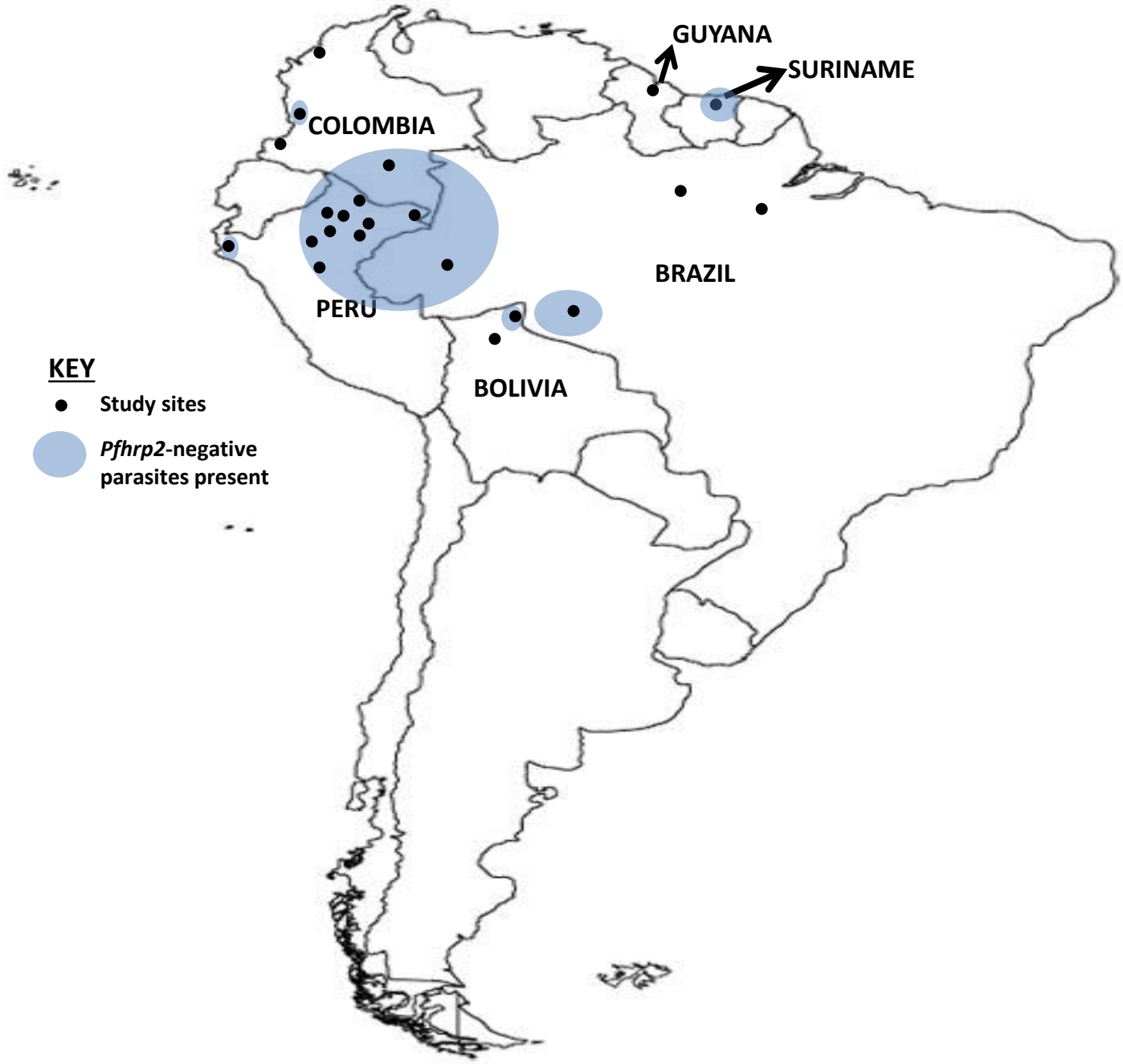
What we learned from molecular surveillance for Pfhrp2/Pfhrp3 deletions?

Distribution of *pfhrp2*-negative *P. falciparum* isolates in South America

- *pfhrp2*-negative parasites in five out of six countries
- Prevalence varied in different locations:
  - No deletion observed in Guyana and from Para state (Brazil)
  - Rare or low deletion in Coastal Peru, and Colombia
  - High rates of *pfhrp2* deletion in Peru (33%) and Acre state (Brazil; 31%)



Distribution of *pfhrp2*-negative *P. falciparum* isolates in South America



What is the Solution?

- **Amazon regions can use non-pfhrp2 based RDTs**

  Eg: LDH based tests

- **In areas where pfhrp2 based tests are used periodic molecular surveillance for pfhrp2 deletion is needed (3 year interval)**

  - **Significant knowledge gap about the prevalence of pfhrp2 deletion in some parts of Brazil (Amazonas State and other regions)**

  - **In Central America no evidence for pfhrp2 deletion found but data is limited and periodic surveillance may be required**

Molecular Tools Come in Different Forms and Applications varies

What Kind of Molecular tools?

Nested PCR: cumbersome, contamination prone, and labor intensive (qualitative method)

Realtime PCR: Quantitative method, less prone for contamination and requires instrumentation

eg: Taqman-PCR and PET-PCR (convenient for endemic countries)

LAMP: less dependent on sophisticated equipment, portable, not quantitative and different end use platforms available

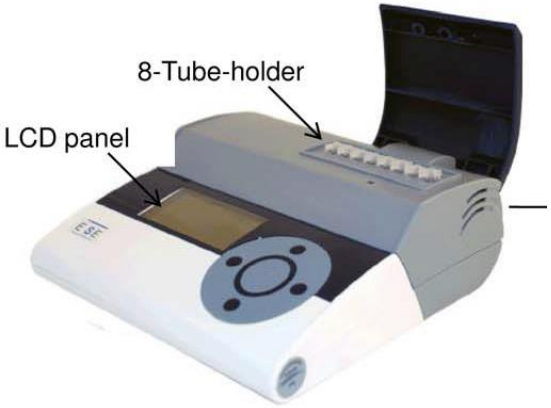
Our Experience with PET-PCR

- **Convenient method for large scale use (Peru, Brazil, Colombia, Suriname, Ecuador and Haiti)**

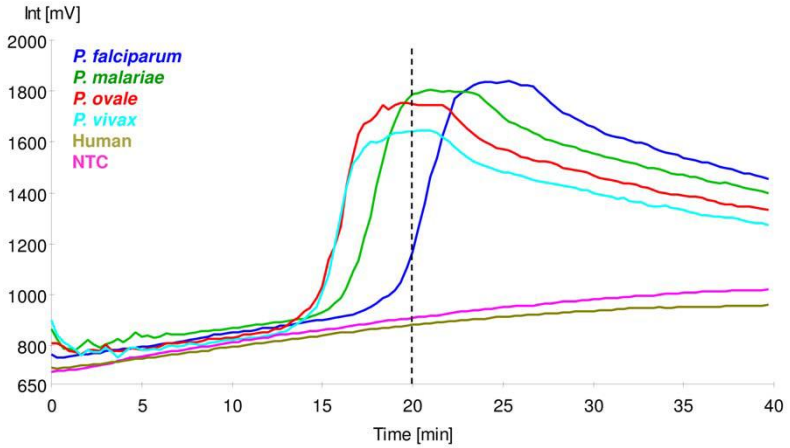
- **Used for Haiti national surveillance study of 2011 and trained Haiti National Public Health Lab staff for implementation in the country**
  - **Haiti 2011 survey (~ 3,000 samples)**
    - **three molecular tests compared; PET-PCR and Taqman Real-time PCR yielded identical results (detection limit ~ 3.2 p/μL)**
    - **Observed malaria prevalence= 0.45%**
  - **Haiti successfully used it for 2012 national surveillance study to test over 5,000 samples and CDC provided QC support**

Real-Time Loop-Mediated Isothermal Amplification (RealAmp)

PCR target region (18S ssRNA genes for all species except *P. vivax*)



SYTO-9 or SYBR Green



Field tested in Thailand and India

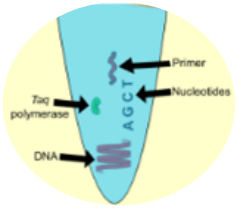
Current prospective evaluation in Para state and Acre, Brazil and Peru

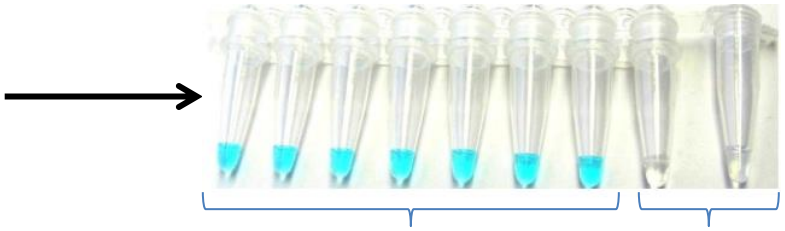
Lucchi NW, et al. (2010) PLoS ONE 5(10): e13733.
Patel JC, et al. (2013) PLoS ONE 8(1): e54986. doi:10.1371/journal.pone.0054986
Patel et al., (2014) JID

Malachite-Green LAMP for Large Scale Surveillance

- **Use heat blocks for amplification and read visually for large scale use**
- **Heat blocks with 40 well (<$300) and one can employ as many as they need**
- **End point → color change: green for positive samples; colorless for negative samples**

Mini heat block





Positive

Negative

Unpublished data

P. falciparum outbreak investigations

Outbreak Investigations: Molecular Tools Identified Parasite Strain and Geographic Origin information

- **Tumbes outbreak 2012: source population identified as a multi-drug resistant and pfhrp2 deleted P. falciparum strain Bv1 clonal lineage (Baldeviano et al., 2015, EID in press)**

- **Cusco outbreak 2013: source population identified as Bv1 clonal lineage-introduced by construction workers from Iquitos**

http://geografia.laguia2000.com/geografia-regional/america/geografia-de-peru-generalidades

[1]Ministerio de Salud del Peru, Direccion General de Epidemiologia. "Casos confirmados de malaria por *Plasmodium falciparum* en el distrito de Echarate, provincia de la Convencion y departamento del Cusco, ano 2013. 2013".

Okoth S *et al.* Manuscript in preparation.

Serology

Haiti Experience

where parasite prevalence is <1% in national surveillance study

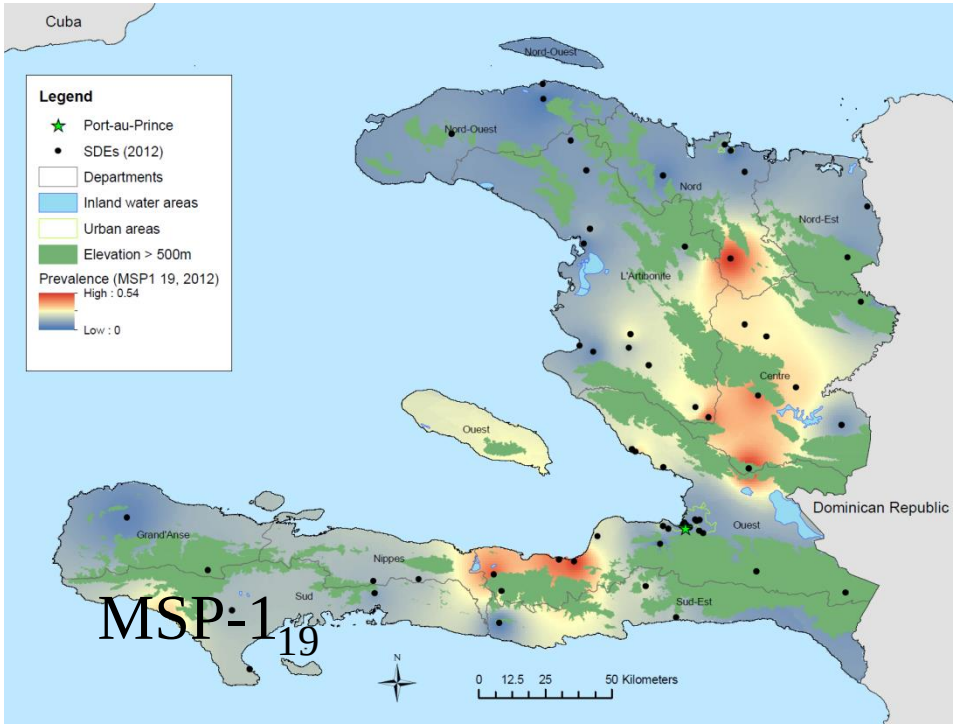
Serology as a Marker for Malaria in Low-Transmission Setting

- Serum IgG against *Plasmodium* antigens has been shown to be very specific, largely without cross-reactivity
- High confidence that serum anti-*Plasmodium* IgG indicates exposure at some point(s) in the past
- Serum IgG has much longer circulating half-life than parasite DNA or blood stage proteins, useful for active surveillance
- IgG protein much more stable for long-term sample storage
- Can stratify by age groups; if certain percentage in young age groups has IgG, it can be a marker for transmission
- Data is continuous, allowing qualitative and quantitative analyses

ELISA vs Multiplex Immunoassays

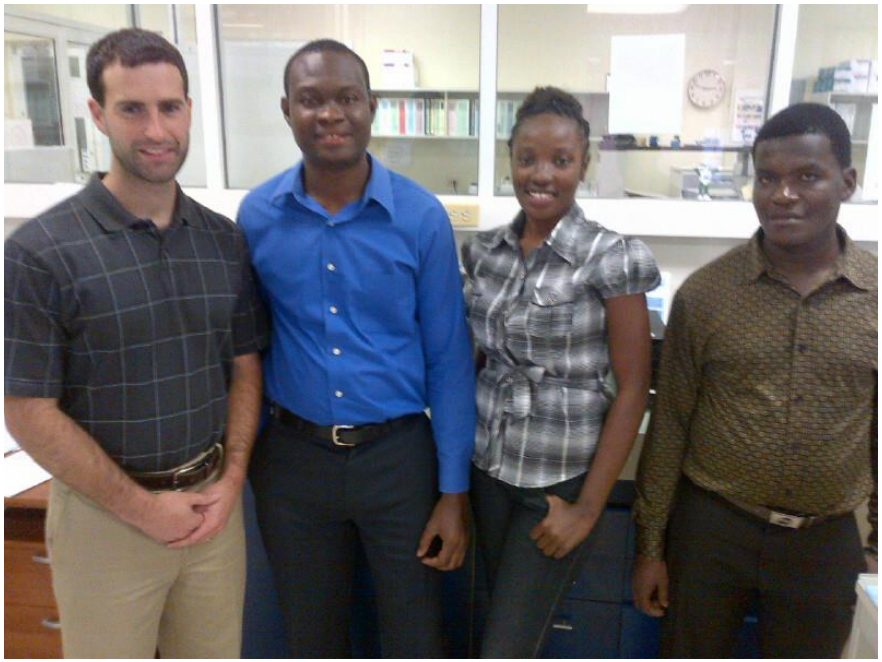
- Both have very similar protocols and sequential steps
- Currently, neither are appropriate for a field setting and must be performed in a wet lab
- ELISA assays have slightly fewer technical limitations, and may assist with building of initial laboratory capacity
- ELISA allows assaying for IgG against one antigen at a time, whereas current Multiplex technology allows for assaying of up to 100 antigens simultaneously (multiple pathogens)
- Multiplex assay has wider dynamic range and lower backgrounds allowing for greater sensitivity of IgG detection

Haiti Study 2012-Serological Response Measures can be Used to Develop Potential Transmission Risk Area Maps (work in progress Rogier E. et al unpublished)



Haiti study 2012

- Serum eluted from >5,000 blood spots
- Antibody measured using ELISA and Luminex
- Luminex had low background and yielded better data
- Serology data converted to seroprevalence curves and data plotted on this map



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