

# **Revised Protocol and Follow-up Actions to Monitor the HRP2 Gene deletion in the Region**

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## Malaria rapid diagnostic tests (RDTs)

- Malaria RDTs are parasite antigen-capture based tests that detect
  - *Plasmodium falciparum* histidine-rich protein-2 (PfHRP2)
  - lactate dehydrogenase (pLDH), or aldolase enzyme
- Majority of commercial RDTs designed to detect PfHRP2 antigen
- Deletion of the gene encoding PfHRP2 (*pfhrp2*) in natural *P. falciparum* populations led to false negative test results. This was discovered by chance in Peru during parasite collection for WHO evaluation of RDTs (Gamboa D. *et al.* 2010. *PLoS One*).
- Molecular surveillance was conducted in 6 countries between 2009-2012 to monitor the deletion of *pfhrp2* and *pfhrp3* genes in South America

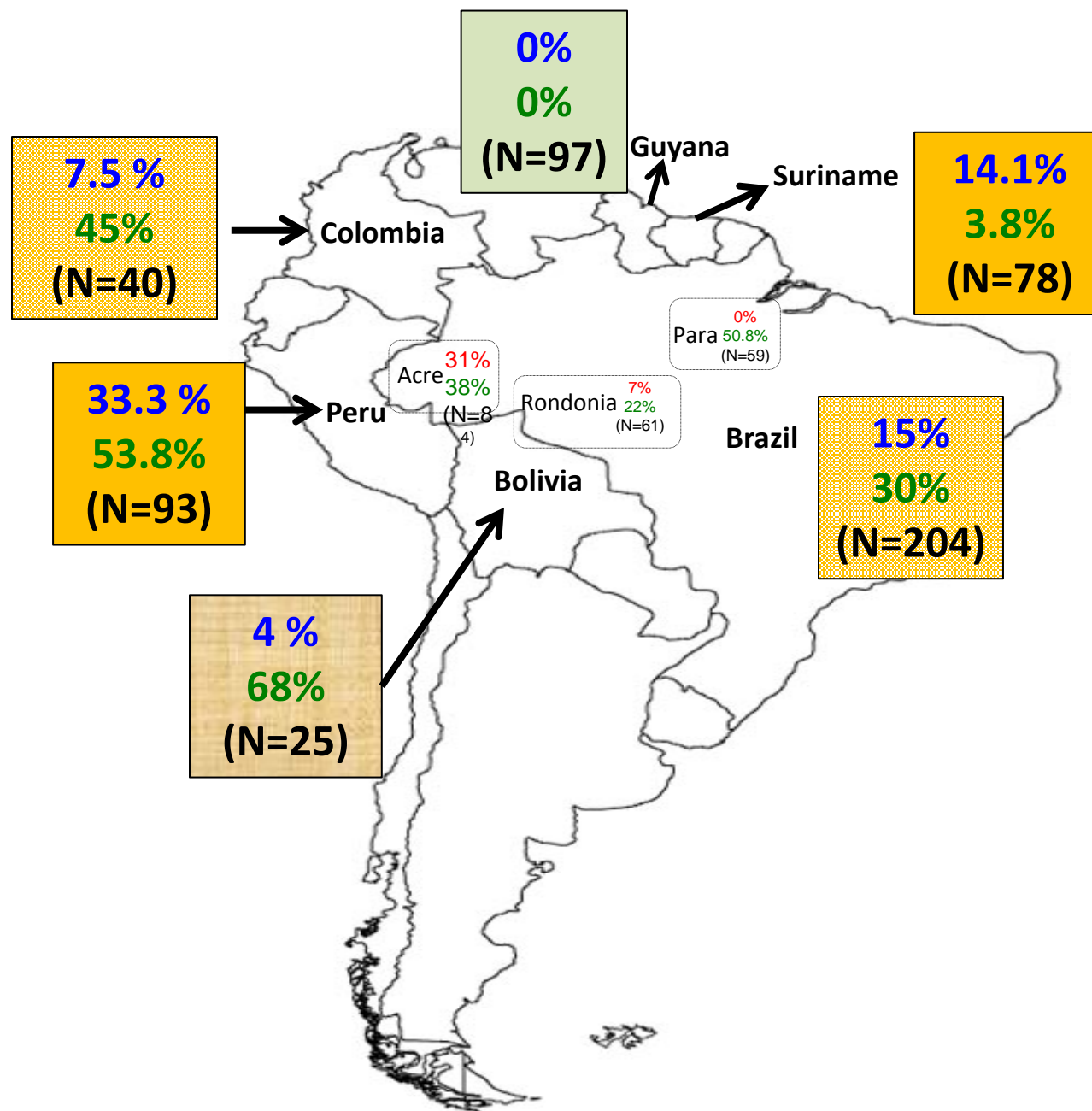


## **Major findings from the pfhrp2 and pfhrp3 deletion surveillance (2009-2012)**

- **Deletion of pfhrp2 and pfhrp3 was found in 5/6 countries**
- **Deletion was found in Peru, Brazil, Bolivia, Colombia and Suriname**
- **Guyana was the only country in which no deletion of these two genes was found**
- **Deletion was widely distributed in various parts of Peru**
- **In Brazil and Colombia deletion was found in high proportions only in some regions**
- **Pfhrp2 deleted parasites were found to spread through human migrations to regions where pfhrp2 deleted parasites were not reported previously**



## Distribution of *pfhrp2*- and *pfhrp3*-negative *P. falciparum* isolates in South America



# What do we know about *pfhrp2* deletion in Ecuador?

It did not participate in prospective surveillance



## Esmeraldas outbreak

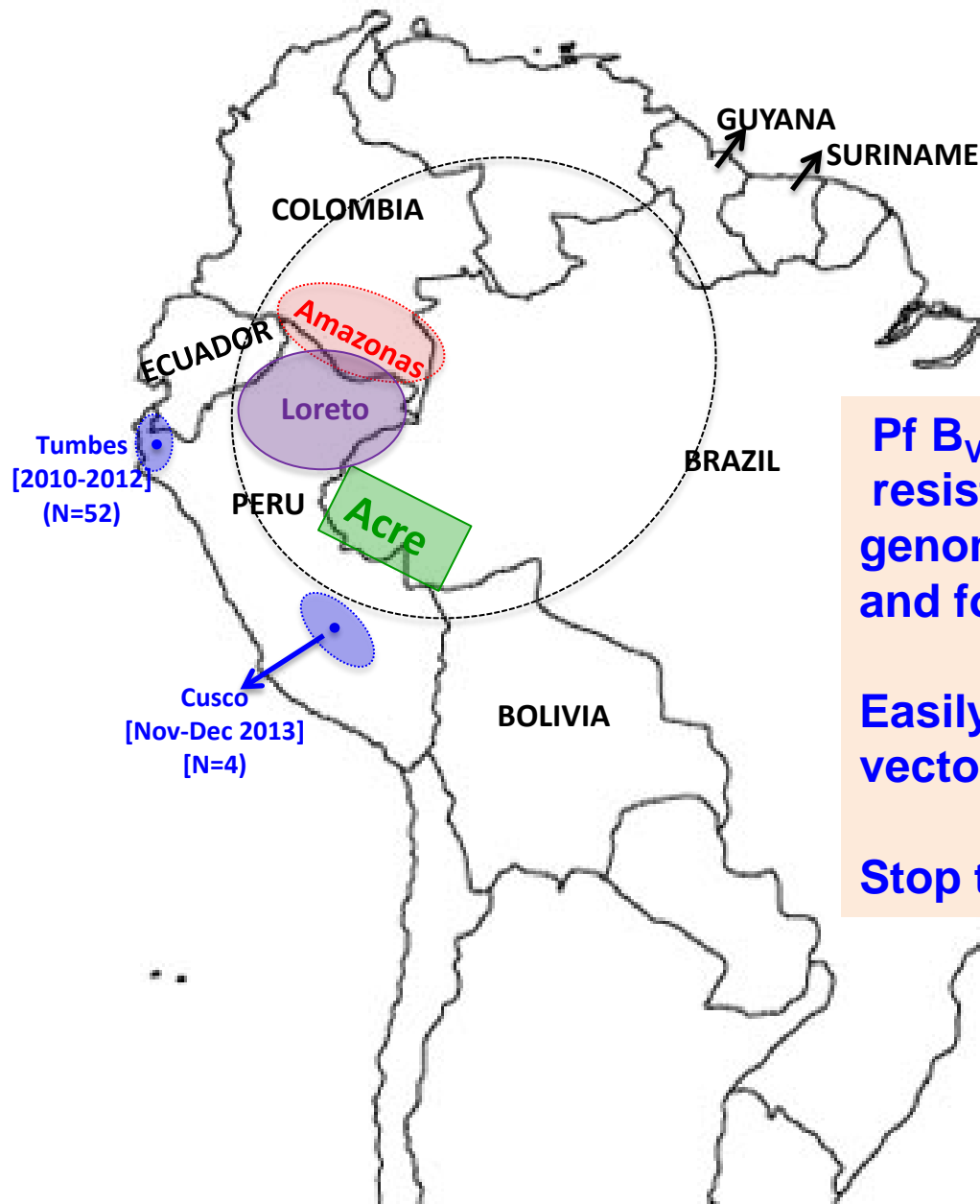
Feb 2013 to Nov 2013

32 samples analyzed

and one sample was lacking *pfhrp2*  
*but serological confirmation not done*

Dr. Fabian from Quito

# Distribution of B<sub>V1</sub> parasites in the Amazon Basin



**Pf B<sub>V1</sub> strain with multidrug resistant and HRP2 deleted genome is spreading rapidly and found in 3 countries**

**Easily adapted to new vectors and ecological zones**

**Stop the spread of this strain**

# Summary

- **An interim progress report was submitted in October 2012 to AMI/PAHO and country partners.**
- **Summary of molecular surveillance test results were presented to AMI in April 2013 (Peru meeting).**
- **Manuscripts are being prepared and planned to be submitted in 2014.**



# Lessons Learned

- **Care must be exercised when HRP-2 based RDTs are selected for use in South American countries.**
- **Non-HRP-2 based RDTs will be appropriate when more than 10% deletion of HRP2 occurs in a region.**
- **With limited surveillance data so far there is no evidence for presence of HRP-2 deleted parasites in central American region but further surveillance is necessary to confirm this.**
- **If countries continue to use HRP-2 based RDTs they need to plan a periodic molecular surveillance to determine the suitability of using such tests.**





# Training Activities

- **Molecular Training Workshop for the detection of HRP2/3 genetic deletions, Instituto Evandro Chagas, Belem, Brazil (Aug 30<sup>th</sup>-Sep 10<sup>th</sup>, 2010)**
- **Trainees from 5 sites in Brazil (IEC, Belem is national reference lab)**
- **Guyana (Javin Chandrabose)**
- **Suriname (Mergior Y Bracho Garrido)**
- **Dr. Fabian from Ecuador was trained in July 2013**
- **Capacity for the detection of HRP2 deletion detection established in Peru, Colombia, Brazil and Ecuador**



# **Molecular Surveillance for HRP-2 and HRP-3 Genetic Deletions in South America**

## **2<sup>nd</sup> Round of Monitoring**

- **Purpose:**
  - Determine the extent of HRP-2 and HRP-3 genetic deletions in *P. falciparum* populations of South America.
  - Brazil, Colombia, Guyana and Suriname
  - When to start? 2015

## Basic Protocol

- Febrile patient, >5 yr., Microscopic diagnosis or pLDH RDT
- Single Pf infection, inform & consent
- 3 ml venous blood draw or FTA filter paper, information form, thick & thin film
- 3 aliquots of plasma and cells each (local and national reference material)
- Molecularly analyze cell samples:
  - 1. test for species-specific 18S rRNA gene and MSP2
  - 2. Pf HRP2/3 and
  -
- Total 4 different PCR with many repetitions
- Quantitatively assay plasma for HRP2



# Data Analysis

Species confirmation using *18S rRNA* gene amplification (only *P. falciparum* positive will be further taken for analysis)



Amplify for *msp2* gene and take samples that are positive for this gene and *18S rRNA* to determine *pfhrp2* and *pfhrp3* expression/gene status



Test for *pfhrp2* and *pfhrp3* gene presence using PCR amplification. Repeat tests if *pfhrp2* or *pfhrp3* amplification reactions are negative



Serology to test for the expression of PfHRP2 as needed to confirm the molecular test results



*Pfhrp2* absence confirmed when *pfhrp2*-specific PCR shows no amplification in two consecutive tests



*Pfhrp3* absence confirmed when *pfhrp3*-specific PCR shows no amplification in two consecutive tests

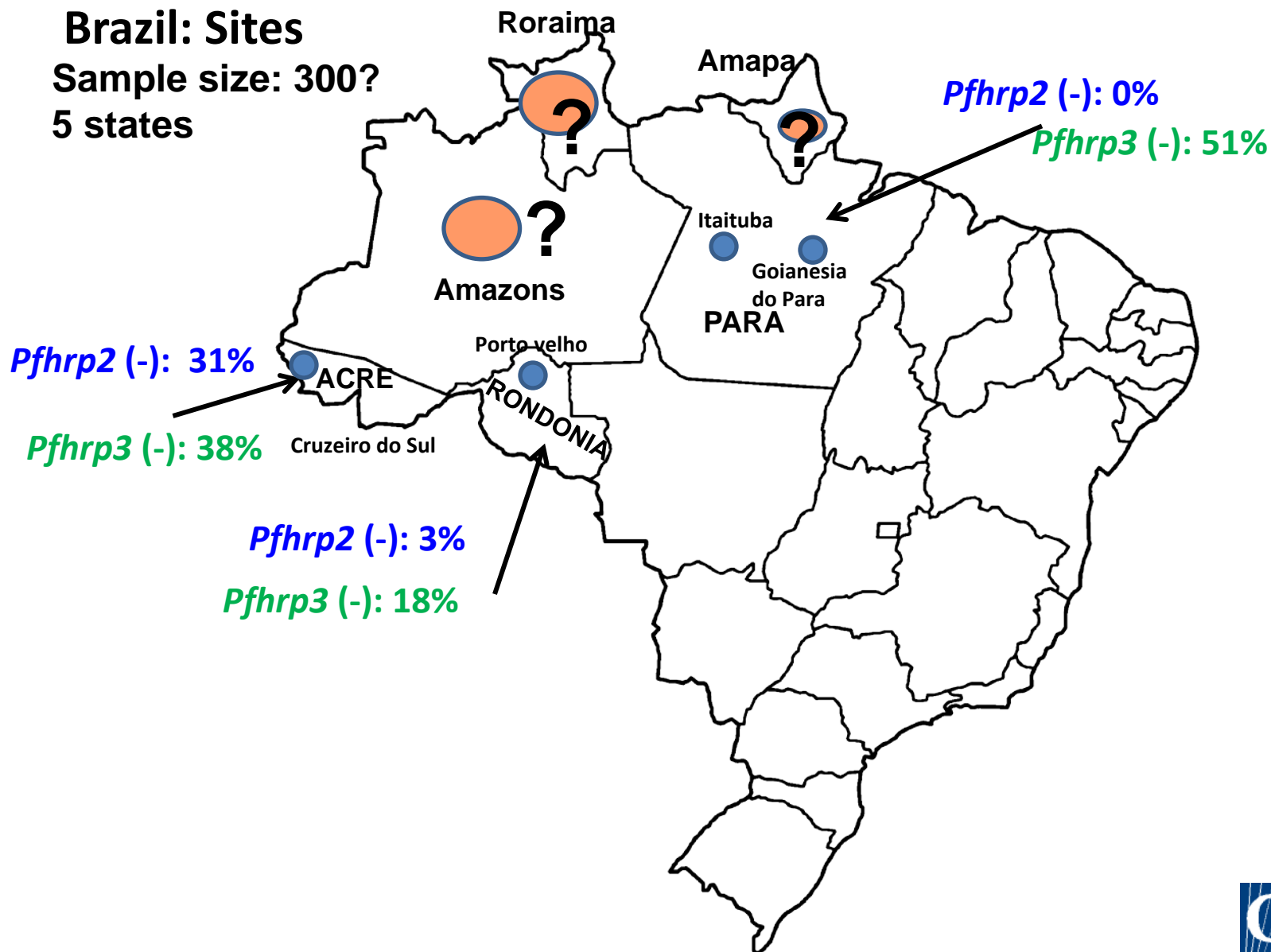
# Study Sites



## Brazil: Sites

Sample size: 300?

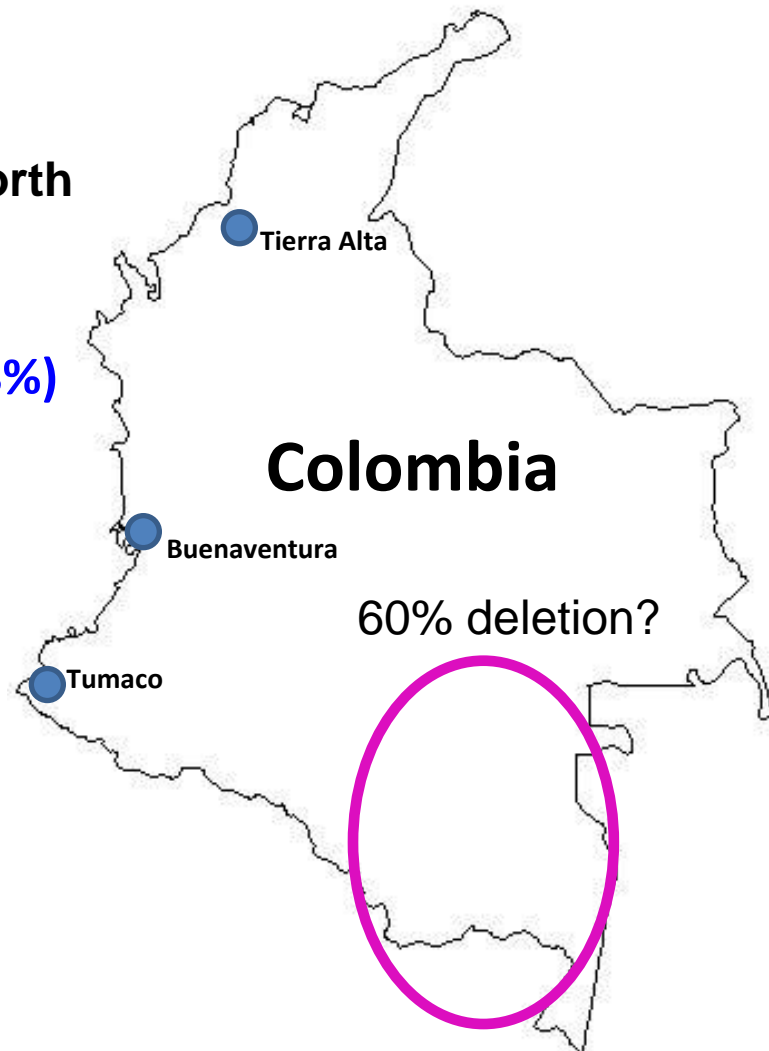
5 states



## Colombia

Coastal regions in South and North  
Sample size: 200 total?

*Pfhrp2* (-): 3 (8%)



Claribel Murillo-Solano

Samples collected 2009-2010

Total number of samples collected: 53

**Number of qualified samples: 40**

# Guyana

## Sample size: 100

### Georgetown

*No pfhrp2 or pfhrp3  
deletion in 2010*

Dr. Nicolas Ceron

Mr. Krishnalal

Samples collected in 2010

Total number of samples collected: 100

**Number of qualified samples: 97**





# Suriname

***Pf* very low**

**Can we collect  
sufficient sample  
size?**

***Pfhrp2* (-): 11/78 (14%)**

***Pfhrp3* (-): 3/78 (4%)**



**Dr. Malti Adhin**

**Samples collected in 2009, 2010, 2011**

**Total number of samples collected: 103**

**Number of qualified samples: 78**



# Who will do the lab work?

- **Lab capacity established in Peru, Colombia, Ecuador and Brazil**
- **Encourage countries to do it**
- **CDC can assist with QA/QC (confirm results using a subset of samples) and support additional training and performance as needed**
- **CDC can support data analysis and report writing**



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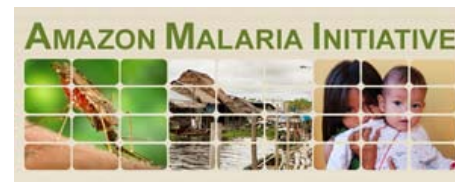
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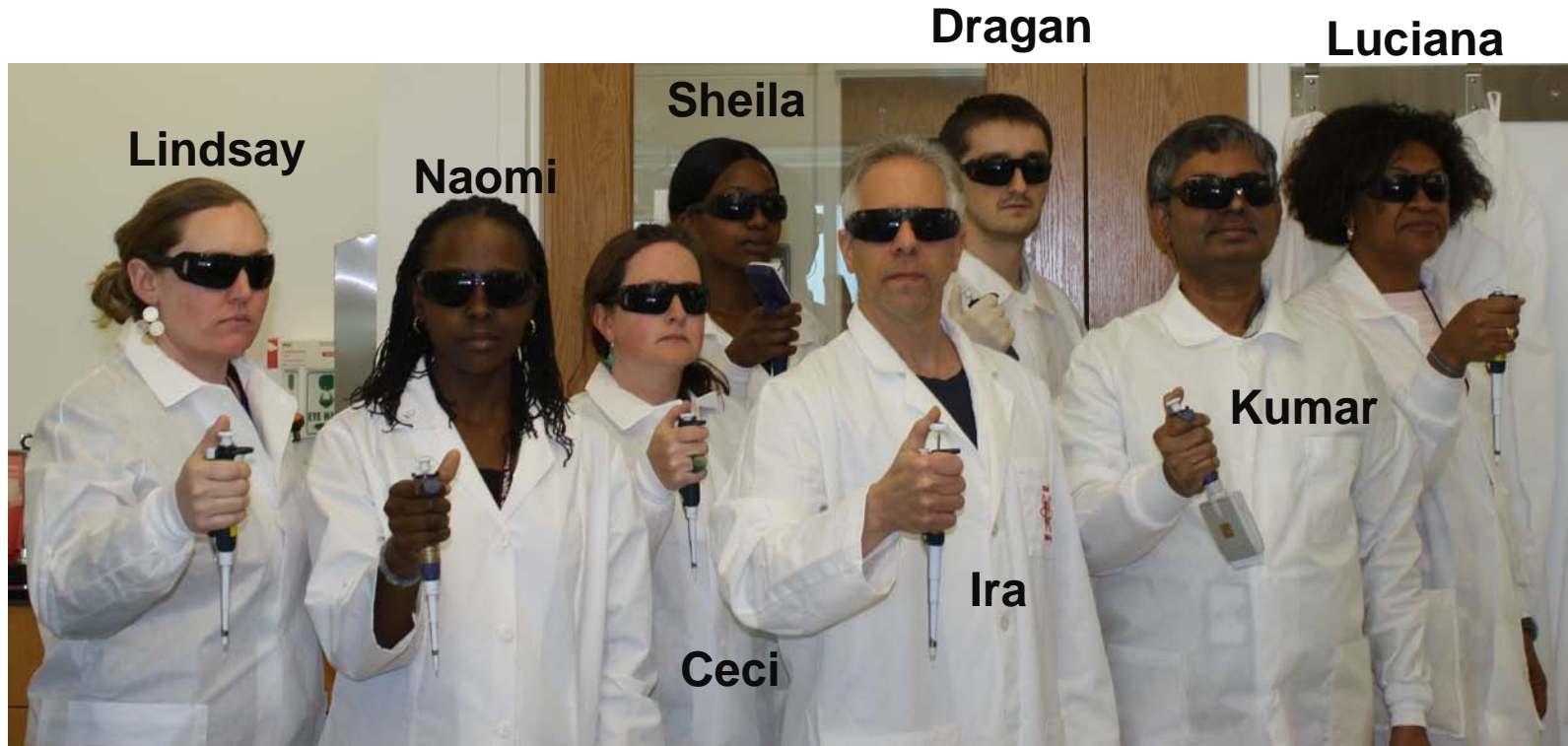
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# Hasta La Vista, Malaria



THE  
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