Molecular Profile and Survey of HRP2 and HRP3 Genetic Deletions in South America: An Update

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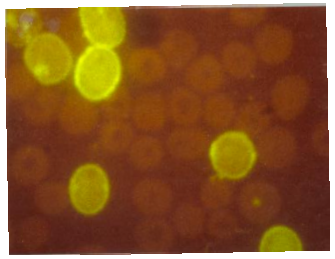
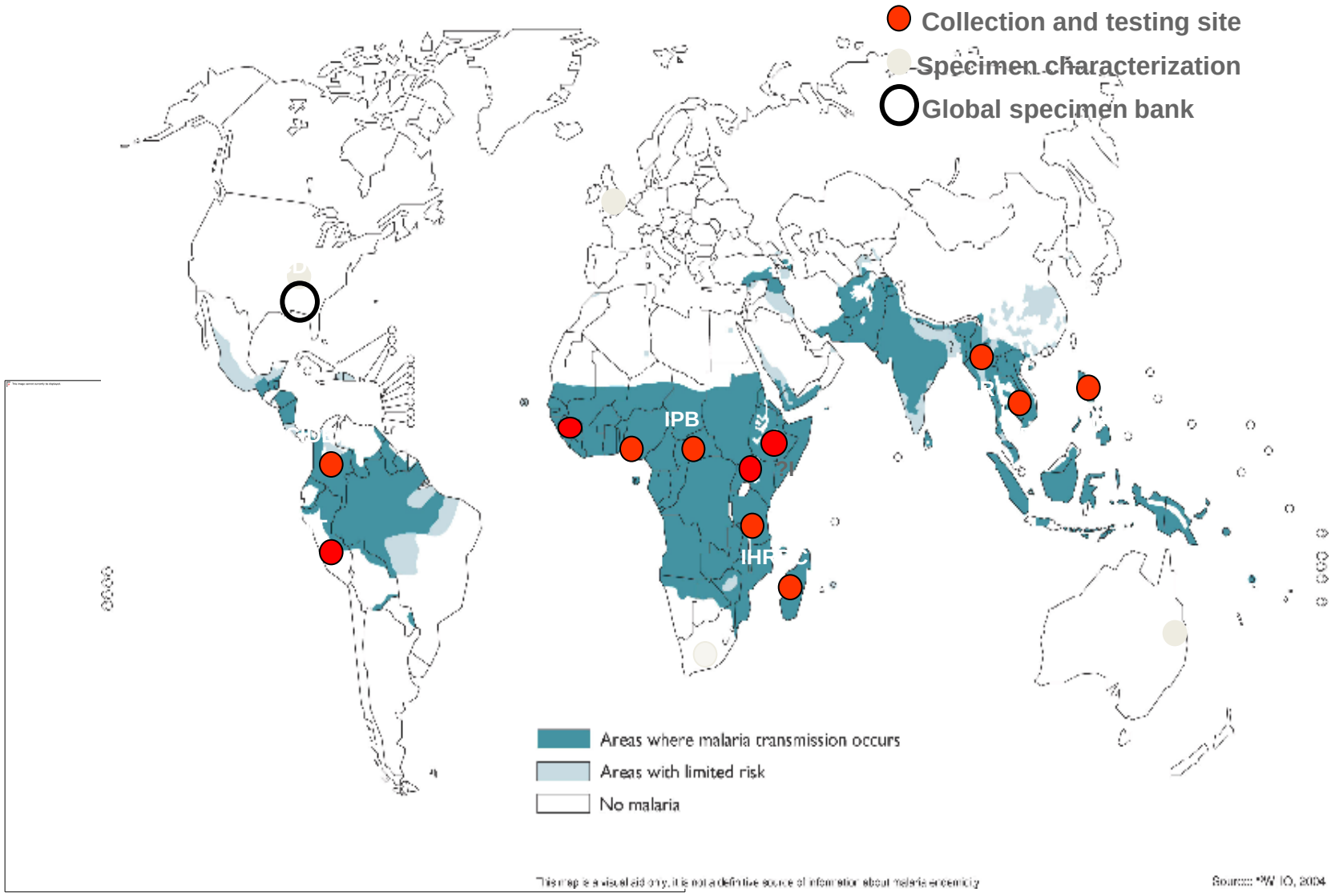
Background - Rapid Diagnostic Tests (RDTs) and malaria diagnosis



- RDTs are antigen-capture based assays detecting in whole blood:
  1. ***P. falciparum*** **hisitidine-rich protein-2 (HRP-2)**
  2. *Plasmodium* lactate dehydrogenase (pLDH)
  3. *Plasmodium* aldolase enzyme
- HRP-2 is a stable soluble exported protein composed of largely histidine, alaline and aspartic acid amino acids arranged in a variety of tandem repeats.

WHO/TDR Laboratory network for RDT Specimen Bank & Performance Evaluation Testing



Deletion of hrp-2 and hrp-3 genes in *P. falciparum* populations in the Peruvian Amazon

OPEN ACCESS Freely available online

PLoS one

A Large Proportion of *P. falciparum* Isolates in the Amazon Region of Peru Lack *pfhrp2* and *pfhrp3*: Implications for Malaria Rapid Diagnostic Tests

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- Detected during establishment of a specimen bank for RDT QA/QC and Performance Evaluation Program.
- Instituto de Medicina Tropical/Universidad Peruana Cayetano Heredia, AAMI/QIMR, FIND, WHO, CDC, and HTD (London)

Current and Retrospective Survey of hrp2/hrp3 deletions in the South America

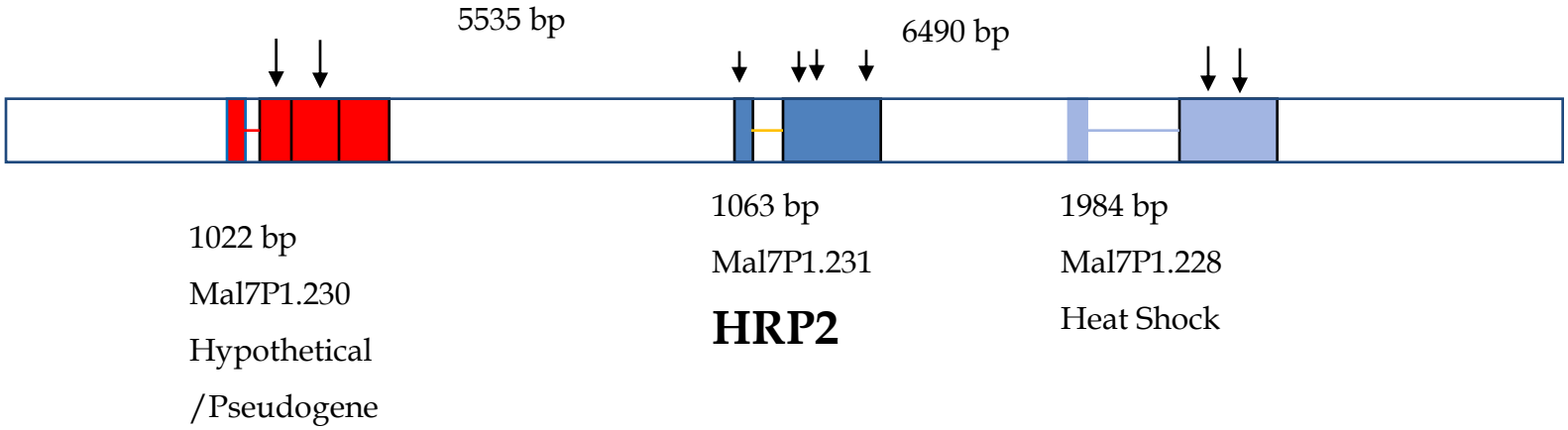
- Purpose:
  - Determine the current extent of *P. falciparum* populations with deletions of the Histidine-rich protein 2 (HRP2) and Histidine-rich protein 3 (HRP3) genes in the Amazon Basin and adjoining areas of South America.
  - Retrospectively determine the origin and population history of the HRP2 and HRP3 deletions in South America through archived samples.

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
- 3. flanking genes
- Total 8 different PCR with many repetitions
- Quantitatively assay plasma for HRP2

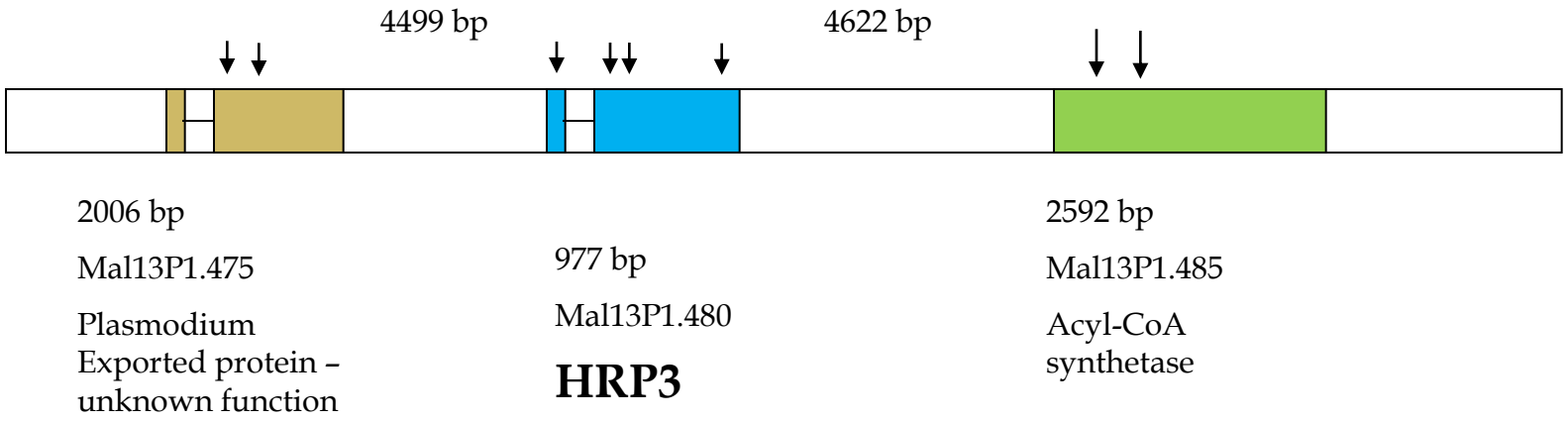
Genes of Interest in *hrp2* Locus

- **HRP2**:
  - Located on chromosome 7 (same chromosome as *Pfcrt* gene)
  - Contains numerous histidine repeats (key trait exploited by RDTs for detection)
  - Mal7P1_230 and Mal7P1_228 are the immediate upstream and downstream genes respectively.

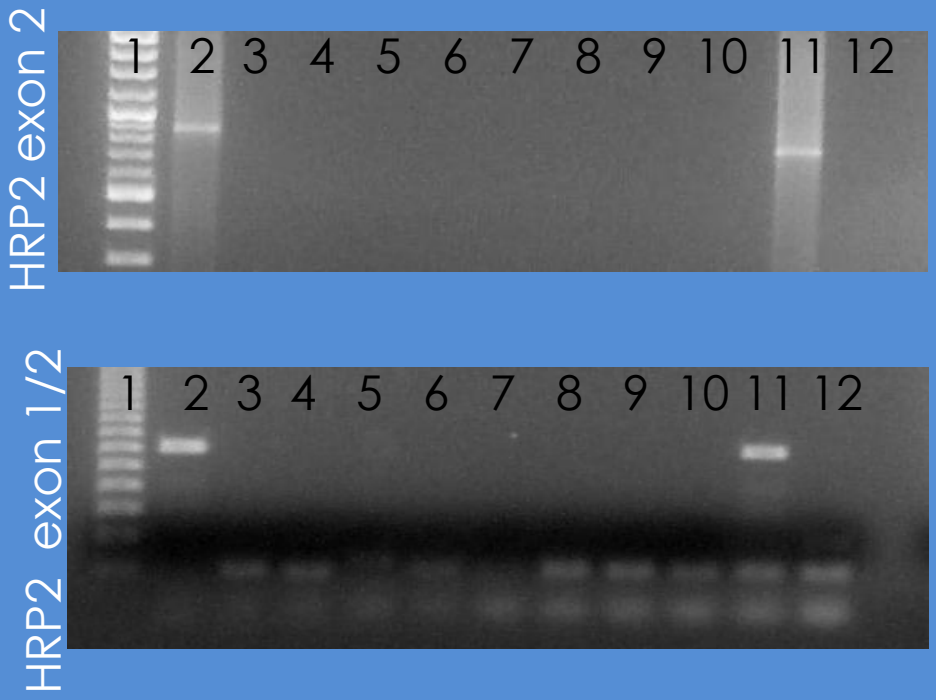


Genes of Interest in *hrp3* Locus

- **HRP3**:
  - Located on chromosome 13 near the telomeric region.
  - Also contains numerous histidine repeats.
  - Mal13P1_485 and Mal13P1_475 are the immediate upstream and downstream genes respectively.

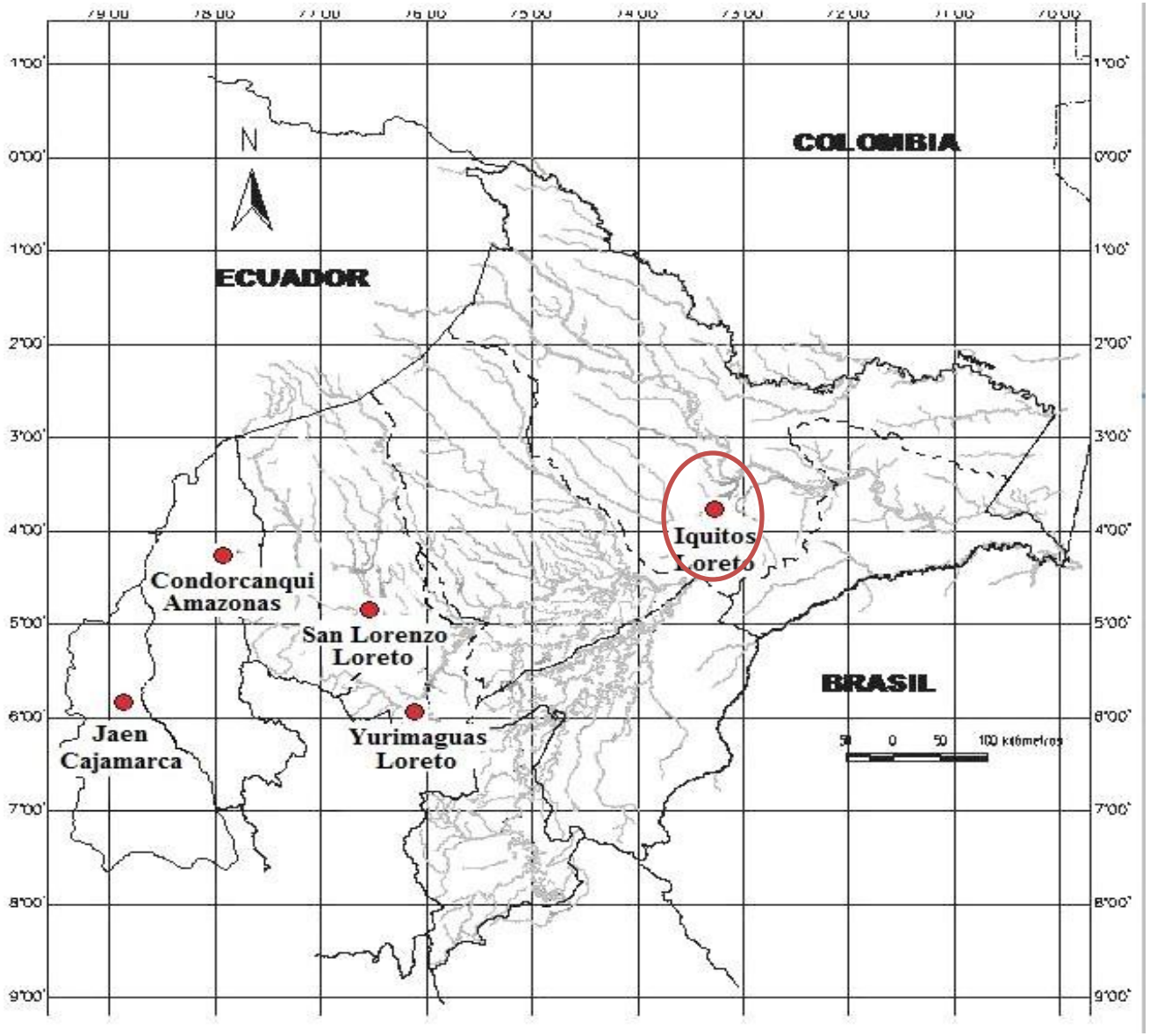


Looking for HRP2…



Lane 1: Marker
Lane 2: PE01 F04
Lane 3: PE01 F06
Lane 4: PE01 F07
Lane 5: PE01 F11
Lane 6: PE01 F15
Lane 7: PE01 F16
Lane 8: PE01 F17
Lane 9: PE01 F18
Lane 10: PE01 F19
Lane 11: 3D7
Lane 12: No DNA

Collection sites for hrp2/hrp3 genotype profiling (2003 - 2008)

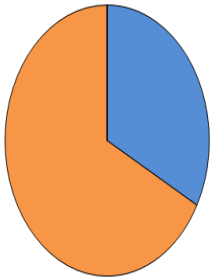


Sample Collection Sites

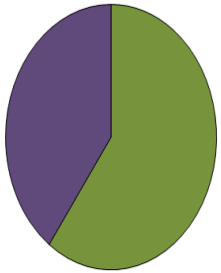
Results for hrp2/hrp3 genotype profiling (2003 - 2008)

Area	No.	*pfhrp2* – PCR		*pfhrp3* – PCR		*pfhrp2/pfhrp3*	
		Pos (%)	*Neg (%)*	Pos (%)	*Neg (%)*	*double positive (%)*	*double negative (%)*
Iquitos	114	73 (64)	41 (36)	35 (31)	79 (69)	18 (15.8)	21 (18.4)
Condorcanqui	5	0 (0)	5 (100)	4 (80)	1 (20)	0 (0)	1 (20)
San Lorenzo	15	8 (53)	7 (47)	2 (13)	13 (87)	1 (6.7)	6 (40)
Yurimaguas	12	6 (50)	6 (50)	2 (17)	10 (83)	1 (8.3)	4 (33)
Jaén	2	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)
Combined	**148**	87 (59)	**61 (41)**	45 (30)	**103 (70)**	20 (13.5)	32 (22)

HRP2/HRP3 deletion status in 2009-2010 samples-Peru



HRP2 (-): 32.9%
HRP2 (+): 67.1%



HRP3 (-): 60%
HRP3 (+): 40%

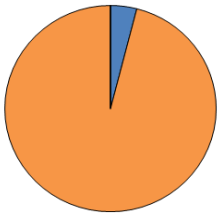
Dionicia Gamboa (University of Cayetano Peru)

Total number of samples: 94
Number of qualified samples: 88?

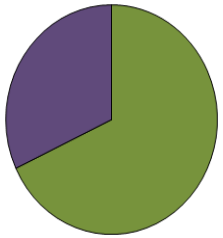
Malaria reemergence is changing the landscape of spread of HRP2 deleted parasites in Peru

Introduction of HRP2 deleted Pf in 2010 to the coast

Bolivia -HRP2/HRP3 deletion survey (2010)



HRP2 (-): 4%
HRP2 (+): 96%

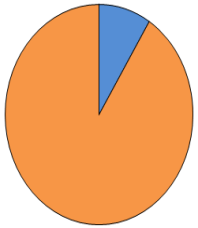


HRP3 (-): 68%
HRP3 (+): 32%

Dr. Arletta Anez
Total number of samples: 27
Number of qualified samples: 25
Additional 75 samples need to be tested when available

Submitted the report to the country

Colombia- HRP2/HRP3 deletion survey (2010)



HRP2 (-): 9%
HRP2 (+): 91%

HRP3 (-): 47.7%
HRP3 (+): 52.3%

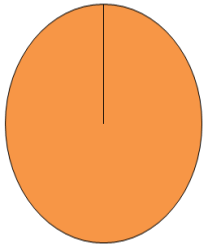
Dr. Claribel Murillo-Solano, CIDEIM, Cali,

Total number of samples: 54
Number of qualified samples: 44

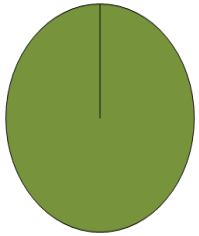
Final report will be submitted to the country this month

Guyana-HRP2/HRP3 deletion survey

HRP2 (-): 0%
HRP2 (+): 100%



HRP3 (-): 0%
HRP3 (+): 100%

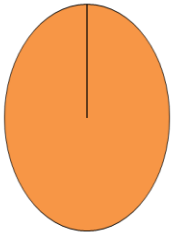


Dr. Nicolas Ceron and
Mr. Krishnalal
Total number of samples: 100
Number of qualified samples: 97

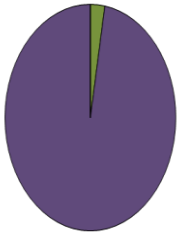
Submitted the report to the country
Pending: Serological conformation

Suriname-HRP2/HRP3 deletion surveu

HRP2 (-): 0%
HRP2 (+): 100%



HRP3 (-): 2.7%
HRP3 (+): 97.3%



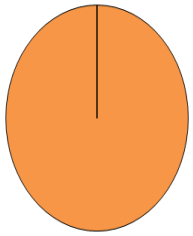
Dr. Malti Adhein
Total number of samples: 53
Number of qualified samples: 37

Additional samples provided 50
Testing ongoing and serology pending

Submitted the report to the country

Honduras-HRP2/HRP3 deletion survey

HRP2 (-): 0%
HRP2 (+): 100%



HRP3 (-): 44.1%
HRP3 (+): 55.9%

Dr. Rosa Elena Mejia and
Dr. Tamara Mancero
Total number of samples: 68

Training and Capacity Development

Katherine Jessica Torres-Fajardo, (Dioni's lab) IMTAH, Univ. Cayetano, Peru (7/20/09 to 9/18/2009)

Claribel Murillo-Solano, CIDEIM, Cali, Columbia (Feb 14, 2010 to March 27, 2010)

Lucia Ortiz-Batsche and Maria E. Castellanos-Reynosa, Univasidad de Valle of Guatemala, Guatemala (March 21 to April 17, 2010)

Gustavo A. Fontecha-Sandoval, Universidad Nac. Autonoma de (Honduras) and Meisy E. Mendoza-Montoya, Laboratorio de Malaria, Tegucigalpa, Honduras (Jan 22-Feb 23, 2011)

Training Activities (Cont.)

- **Molecular Training Workshop for the detection of HRP2/3 genetic deletions, Instituto Evandro Chagas, Belem, Brazil (Aug 30th-Sep 10th, 2010)**
- **Trainees from 5 sites in Brazil (IEC, Belem is national reference lab)**
- **Guyana (Javin Chandrabose)**
- **Suriname (Mergiory Y Bracho Garrido)**
- **On site training in Bolivia and Guyana (Kumar and Alex Macedo)- March 2010**
- **Betzabe Mara Rodriguez, Molecular Diagnosis Lab, Ministry of Health, Nicaragua (ongoing)**

Alex in the training session with Dr. Anez in Bolivia

Guyana lab team training

Workshop in Brazil

Dr. Marinete Pova lecturing

Dr. John Barnwell in discussion Brazil Workshop

Maru and Lucia (Guatemala) with our lab team

Summary

- **High level of deletion in Peru (40%)**
- **Evidence for deletion in Colombia, Bolivia-Additional testing needed**
- **No evidence for deletion in Guyana, Suriname, Honduras and other countries in Central America needs to be tested**
- **Pending investigation in Brazil and Ecuador**
- **What this means for programs?**
- **What recommendations?**

Acknowledgements

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WHO

David Bell

PAHO

Keith Carter

CDC

Alexandre Macedo de Oliveira

Tonya Mixson-Hayden

Curtis Huber

Amanda Poe

Joeseph Abdallah

Guyana/PAHO

Nicholas Ceron

Suriname

Malti Adhin

IEC/Brazil

Marinete Marins Povoa

USAID/AMI

FIND: Mark Perkins and Sandra Incardona

¡Gracias!