



# Malaria testing by PCR

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HEMORIO



**IPFA/PEI 29<sup>th</sup> International Workshop on  
Surveillance and Screening of Blood-borne Pathogens**

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I have no conflict of interest to declare

# Outline

- Why perform the NAT test for malaria on all donations across Brazil
- NAT test for malaria adopted for blood donors in Brazil
- Initial NAT results for malaria testing in Hemorio Blood Center

# Boletim Epidemiológico

Secretaria de Vigilância em Saúde | Ministério da Saúde

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## Malária | 2021

145.188 cases/year (2020)

O Brasil registrou no ano de 2020 um total de 145.188 casos de malária (Figura 1). Comparando com o ano de 2019, quando foram registrados 157.452 casos da doença, houve uma redução de 7,8%. Diferente do observado em relação ao total de casos de malária

notificados no País, o número de casos de malária por *Plasmodium falciparum* e malária mista aumentou no período. Em 2019 haviam sido registrados 17.140 casos desta espécie e em 2020 foram registrados 23.757, um aumento de 38,6%.



**Legal Amazon - 99.9% of cases**



[Home](#) > [Assuntos](#) > [Saúde de A a Z](#) > [M](#) > [Malária](#) > [Situação Epidemiológica da Malária](#) > [Boletins Epidemiológicos de Malária](#) > [Boletim Epidemiológico Vol.53 Nº30](#)  
- 2022 - Malária na região extra-amazônica do Brasil série histórica de 2010 a 2021

## Boletim Epidemiológico Vol.53 Nº30 - 2022 - Malária na região extra-amazônica do Brasil série histórica de 2010 a 2021

| Regiões/UF             | ANO DE NOTIFICAÇÃO |       |      |      |      |      |      |      |      |      |      |       | Total |
|------------------------|--------------------|-------|------|------|------|------|------|------|------|------|------|-------|-------|
|                        | 2010               | 2011  | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 | 2021* |       |
| Região extra-amazônica | 1.280              | 1.052 | 950  | 828  | 567  | 518  | 496  | 509  | 734  | 543  | 319  | 521   | 8.317 |
| Casos autóctones       | 173                | 90    | 100  | 88   | 54   | 90   | 83   | 95   | 247  | 50   | 18   | 99    | 1.187 |

Case Reports > Rev Inst Med Trop Sao Paulo. 2011 Jan-Feb;53(1):55-9.

doi: 10.1590/s0036-46652011000100010.

## Transfusion-transmitted malaria: case report of asymptomatic donor harboring *Plasmodium malariae*

Patricia Scuracchio<sup>1</sup>, Sergio Domingos Vieira, Denise Albuquerque Dourado, Luciana Moro Bueno, Rafael Colella, Eduardo Milton Ramos-Sanchez, Giselle F M Castro Lima, Juliana Inoue, Maria Carmen Arroyo Sanchez, Silvia Maria Di Santi

Affiliations + expand

PMID: 21412621 DOI: 10.1590/s0036-46652011000100010

Free article

### Abstract

Malaria in Brazil is endemic in the Amazon region, but autochthonous cases with low parasitaemia occur in the Atlantic Forest area of the country. According to Brazilian legislation no test is mandatory for blood donors from non-endemic areas. However if they have traveled to malaria transmission regions they are deferred for six months before they can donate. This report describes a transfusion-transmitted malaria case in Sao Paulo, Brazil, where one recipient received infected blood and developed the disease. He lived in Sao Paulo and had no previous transfusion or trips to endemic areas, including those of low endemicity, such as Atlantic Forest. Thick blood smears confirmed *Plasmodium malariae*. All donors lived in Sao Paulo and one of them (Donor 045-0) showed positive hemocopy and PCR. This asymptomatic donor had traveled to Juquia, in the Atlantic Forest area of Sao Paulo State, where sporadic cases of autochthonous malaria are described. DNA assay revealed *P. malariae* in the donor's (Donor 045-0) blood. Serum archives of the recipient and of all blood donors were analyzed by ELISA using both *P. vivax* and *P. falciparum* antigens, and IFAT with *P. malariae*. Donor 045-0's serum was *P. malariae* IFAT positive and the *P. vivax* ELISA was reactive. In addition, two out of 44 donors' archive sera were also *P. vivax* ELISA reactive. All sera were *P. falciparum* ELISA negative. This case suggests the need of reviewing donor selection criteria and deferral strategies to prevent possible cases of transfusion-transmitted malaria.

Maselli et al. *Malaria Journal* 2014, **13**:224  
http://www.malariajournal.com/content/13/1/224



### RESEARCH

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## Detection of *Plasmodium falciparum* and *Plasmodium vivax* subclinical infection in non-endemic region: implications for blood transfusion and malaria epidemiology

Luciana MF Maselli<sup>1,2†</sup>, Debora Levy<sup>1†</sup>, Gabriel Z Laporta<sup>4,5</sup>, Aline M Monteiro<sup>3</sup>, Linah A Fukuya<sup>3</sup>, Maria F Ferreira-da-Cruz<sup>6</sup>, Claudio T Daniel-Ribeiro<sup>6</sup>, Pedro E Dorlhiac-Llacer<sup>3</sup>, Maria Anice M Sallum<sup>4</sup> and Sérgio P Bydlowski<sup>1\*</sup>

> Rev Soc Bras Med Trop. 1998 Jan-Feb;31(1):27-34. doi: 10.1590/s0037-8682198000100004.

## [Control of blood transfusion malaria in an endemic and in a non-endemic region in Brazil]

[Article in Portuguese]

A Sáez-Alquézar<sup>1</sup>, A M Ramos, S M Di Santi, M S Branquinho, K Kirchgatter, I. Cordeiro, M Murta, J C Saraiva, S G Oliveira, M G Bochetti, J A Pirolla, D Guerzoni, D A Chamone

[Malar J.](#) 2017; 16: 78.

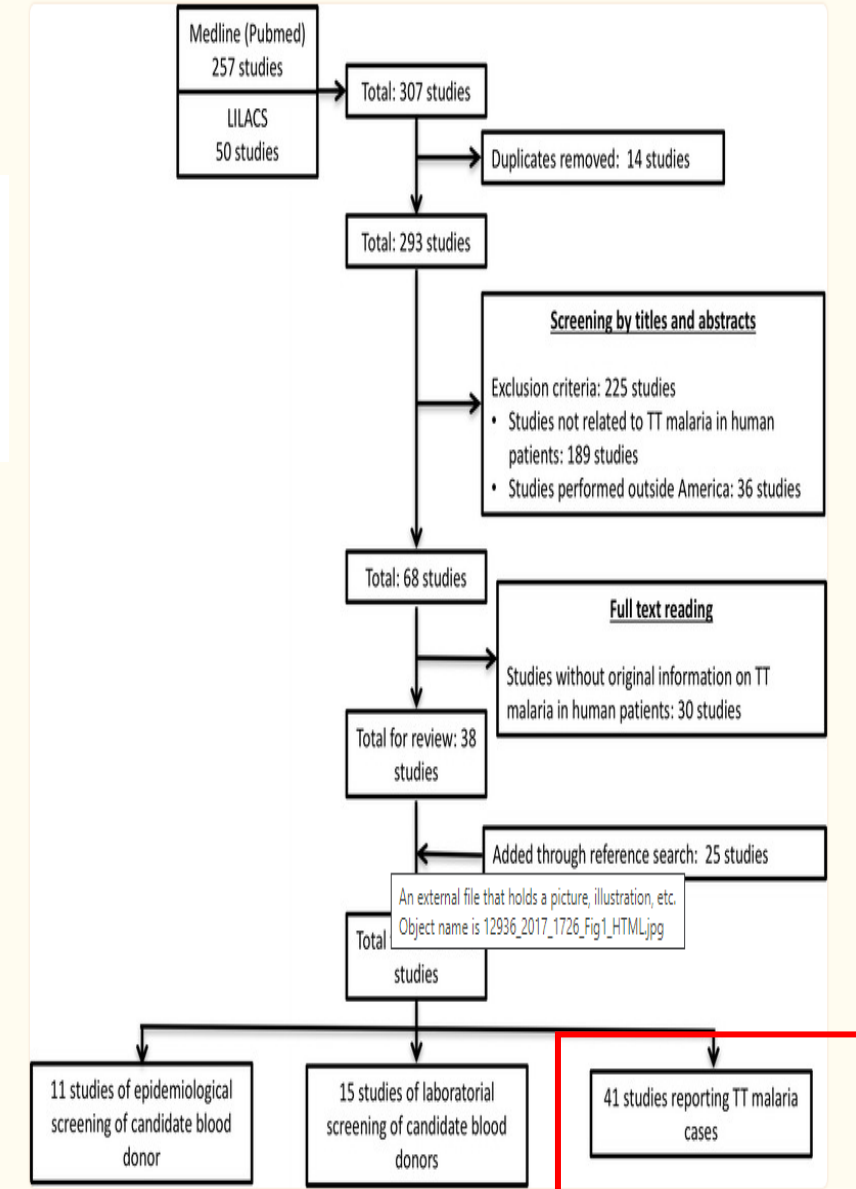
Published online 2017 Feb 15. doi: [10.1186/s12936-017-1726-y](https://doi.org/10.1186/s12936-017-1726-y)

## Alternative transmission routes in the malaria elimination era: an overview of transfusion-transmitted malaria in the Americas

[Regina M. Alho](#)<sup>1,2</sup>, [Kim Vinícius Amaral Machado](#)<sup>3</sup>, [Fernando F. A. Val](#)<sup>1,3</sup>, [Nelson A. Fraiji](#)<sup>2</sup>, [Marcia A. A. Alexandre](#)<sup>1</sup>,

[Gisely C. Melo](#)<sup>1,3</sup>, [Judith Recht](#)<sup>4</sup>, [André M. Siqueira](#)<sup>5</sup>, [Wuelton M. Monteiro](#)<sup>1,3</sup> and [Marcus V. G. Lacerda](#)<sup>1,3,6</sup>

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Epidemiological characteristics of 422 transfusion-transmitted malaria cases reported in the American continent

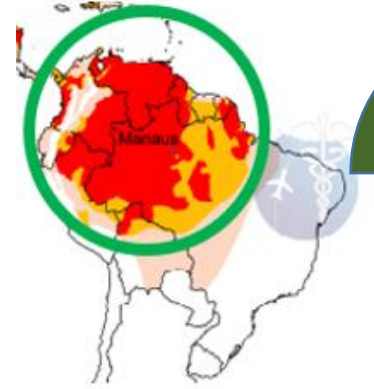
| Characteristics                          | Number of cases | Proportion (%) |
|------------------------------------------|-----------------|----------------|
| Country (n = 422; completeness = 100.0%) |                 |                |
| Mexico                                   | 214             | 50.7           |
| USA                                      | 170             | 40.3           |
| Brazil                                   | 28              | 6.6            |
| Canada                                   | 4               | 0.9            |
| Venezuela                                | 3               | 0.7            |
| Peru                                     | 2               | 0.5            |
| Colombia                                 | 1               | 0.3            |

# Donor deferrals related to malaria exposure in Americas

Deferral rates related to malaria exposure in the American continent

| Country  | Location and period                                                                                                                  | Period               | Subjects                                                   | Sample size | Deferral cause                                                                                                  | Findings                  | Reference                 |
|----------|--------------------------------------------------------------------------------------------------------------------------------------|----------------------|------------------------------------------------------------|-------------|-----------------------------------------------------------------------------------------------------------------|---------------------------|---------------------------|
| Canada   | Nationwide data, except Québec                                                                                                       | 2007–2012            | Candidate blood donors                                     | 1,625,432   | Travel to endemic countries                                                                                     | 76,312 deferred subjects  | O'Brien et al. [26]       |
| USA      | Six blood centres located in San Francisco (CA), Milwaukee (WI), Cincinnati (OH), Pittsburgh (PA), Dedham (MA) and Douglasville (GA) | 2006                 | Overall deferred donors due to travel to endemic countries | 2108        | Travel to Mexico                                                                                                | 885 deferred donors       | Spencer et al. [31]       |
| USA      | Six blood centres located in San Francisco (CA), Milwaukee (WI), Cincinnati (OH), Pittsburgh (PA), Dedham (MA) and Douglasville (GA) | 2006                 | Overall non-eligible donors at moment                      | 13,007      | Travel to endemic countries                                                                                     | 2108 deferred subjects    | Spencer et al. [30]       |
| Canada   | Nationwide data                                                                                                                      | Jul to Dec 2004      | Candidate blood donors                                     | 37,165      | Travel to endemic countries                                                                                     | 1105 deferred subjects    | O'Brien et al. [25]       |
| USA      | Nationwide data                                                                                                                      | 2000–2006            | Candidate blood donors                                     | 28,933,936  | Travel to endemic countries                                                                                     | 316,495 deferred subjects | Leiby et al. [24]         |
| USA      | Mississippi Valley Regional Blood Center                                                                                             | Apr 2004 to Mar 2005 | Candidate blood donors                                     | NA          | Travel or residence in endemic countries                                                                        | 156 deferred subjects     | Katz et al. [23]          |
| USA      | Six blood centres located in San Francisco (CA), Milwaukee (WI), Cincinnati (OH), Pittsburgh (PA), Dedham (MA) and Douglasville (GA) | 2003                 | Candidate blood donors                                     | NA          | Travel to endemic countries                                                                                     | 12,310 deferred subjects  | Spencer et al. [29]       |
| Brazil   | Manaus                                                                                                                               | NA                   | Candidate blood donors                                     | 324         | Previous history of malaria                                                                                     | 38 deferrals              | Torres et al. [32]        |
| Colombia | Cali                                                                                                                                 | 2002                 | Candidate blood donors                                     | 286         | Travel or living in endemic areas in the last year                                                              | 115 deferrals             | Castillo et al. [22]      |
| Canada   | Nationwide data                                                                                                                      | 2002                 | Candidate blood donors                                     | NA          | Travel to endemic countries                                                                                     | 7216 deferred subjects    | Shehata et al. [28]       |
| Brazil   | São Paulo (SP)                                                                                                                       | NA                   | Candidate blood donors                                     | 1200        | Travel to endemic areas without prophylaxis in the last 6 months, living in malaria endemic areas (deferral for | 36 deferred subjects      | Sáez-Alquézar et al. [27] |

# Criteria for donor deferral in Brazil based on malaria exposure risk



## Donor deferral

### ENDEMIC AREA / AMAZON



DEFERRED

I - Who had Malaria in the **12 months** prior to the donation

II - With **fever** or **suspected Malaria** in the last **30 days**

III - Who **moved** to or **came** from a high-risk area (Annual Parasite Index/A.P.I. greater than 49.9) less than **30 days** before donation

IV – Areas with active transmission, regardless the A.P.I.: a malaria test must be carried out to detect Plasmodium



### NON-ENDEMIC AREA / EXTRA-AMAZON REGION

I - Who traveled to or came from municipalities located in endemic areas:

***31 days to 12 months after displacement, provided they tested negative for Plasmodium or Plasmodium antigens***

# Detection of *Plasmodium sp* in blood donors

## MICROSCOPY

Thick blood smears

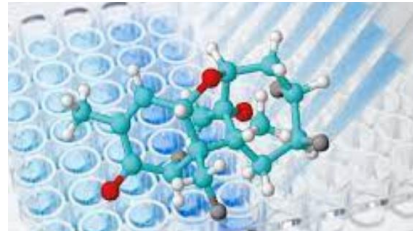


Detection of the parasite  
(*Plasmodium sp.*)



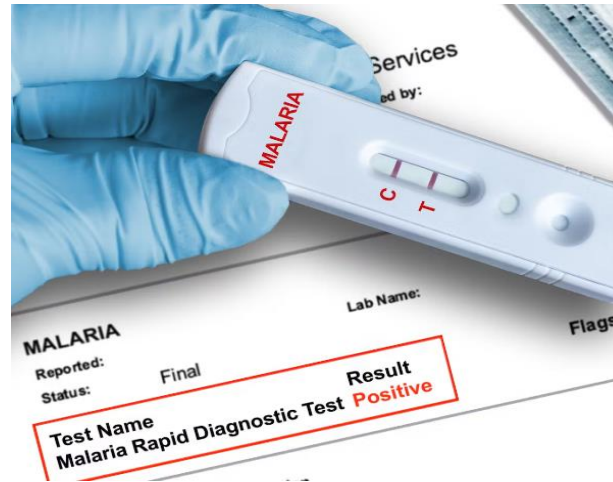
## SEROLOGY

Antibody detection: IFA / ELISA



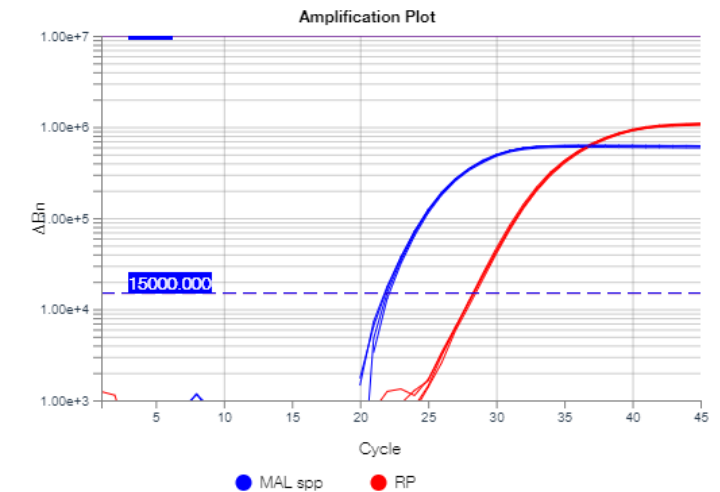
## RAPID TEST

Detection of parasite antigens



## MOLECULAR DETECTION

DNA of *Plasmodium sp*



| Window cases  |            |           |            | Positive samples |
|---------------|------------|-----------|------------|------------------|
| Ano           | HIV        | HCV       | HBV        | Malaria          |
| 2008 (EP)     | 0          | 0         | 0          |                  |
| 2010(EM)      | 2          | 0         | 0          |                  |
| 2011          | 1          | 1         | 0          |                  |
| 2012          | 4          | 1         | 0          |                  |
| 2013          | 14         | 6         | 0          |                  |
| 2014          | 12         | 5         | 0          |                  |
| 2015          | 15         | 6         | 18         |                  |
| 2016          | 19         | 11        | 25         |                  |
| 2017          | 36         | 7         | 25         |                  |
| 2018          | 30         | 9         | 20         |                  |
| 2019          | 21         | 8         | 16         |                  |
| 2020          | 8          | 3         | 5          |                  |
| 2021          | 1          | 0         | 4          |                  |
| 2022          | 8          | 1         | 3          |                  |
| 2023          | 0          | 0         | 0          | 5                |
| <b>TOTAL:</b> | <b>171</b> | <b>58</b> | <b>116</b> | <b>5</b>         |

Rocha et al. *Malar J* (2020) 19:275  
<https://doi.org/10.1186/s12936-020-03345-y>

Malaria Journal

RESEARCH

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# Use of a NAT-based assay to improve the surveillance system and prevent transfusion-transmitted malaria in blood banks

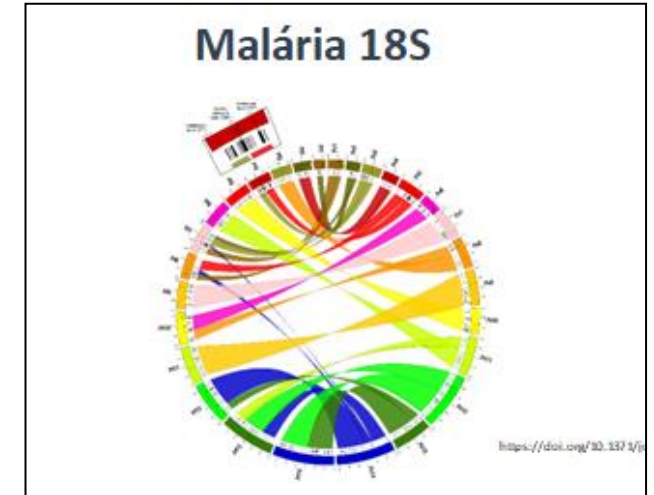
Daniele Rocha<sup>3,5</sup>, Gisely Cardoso de Melo<sup>1,2</sup>, José Marcelo Hipólito Carneiro<sup>6</sup>, Marisa Ribeiro<sup>3,5</sup>, Sthefanie Ribeiro<sup>3</sup>, Daniela Tupy de Godoy<sup>3</sup>, Anne Cristine Gomes de Almeida<sup>1</sup>, Elisabete Ferreira de Andrade<sup>3,5</sup>, Cláudia Maria de Moura Abrahim<sup>6</sup>, Nelson Abrahim Fraiji<sup>6</sup>, Antonio Gomes Pinto Ferreira<sup>3</sup>, Wuelton Marcelo Monteiro<sup>1,2</sup>, Rodrigo Brindeiro<sup>5</sup>, Amilcar Tanuri<sup>5</sup>, Marcus Vinicius Guimarães de Lacerda<sup>1,2,4</sup> and Patrícia Alvarez<sup>3\*</sup> 

**Table 2 Cycle threshold (Ct) results for FMT–HVD samples using the BioRobot MDx (silica extraction) and NAT Plus System (bead extraction) workstations, and in-house 18S RNA gene quantification**

| Patient | Sample      | MDx (silica)      |                        | NAT Plus (beads)  |                        | 18S RNA quantification<br>Copies/μL |
|---------|-------------|-------------------|------------------------|-------------------|------------------------|-------------------------------------|
|         |             | Single (Ct value) | Pool of six (Ct value) | Single (Ct value) | Pool of six (Ct value) |                                     |
| 1       | Whole blood | 12.45             | 14.36                  |                   |                        | 550,345.9                           |
|         | Plasma      | 16.1              | 17.53                  | 15.64             | 18.51                  | 22,732.9                            |
| 2       | Whole blood | 18.66             | 20.22                  |                   |                        | 38,766.9                            |
|         | Plasma      | 17.53             | 20.33                  | 21.41             | 19.91                  | 89.5                                |
| 3       | Whole blood | 20.47             | 23.61                  |                   |                        | 13,727.3                            |
|         | Plasma      | 25.99             | 28.83                  | 20.01             | 24.96                  | 150.8                               |
| 4       | Whole blood | 13.17             | 15.17                  |                   |                        | 53,443.5                            |
|         | Plasma      | 23.42             | 27.4                   | 19.58             | 22.95                  | 161.7                               |
| 5       | Whole blood | 17.19             | 21.87                  |                   |                        | 194,645.8                           |
|         | Plasma      | 20.95             | 24.23                  | 17.45             | 21.08                  | 399.9                               |
| 6       | Whole blood | 15.7              | 21.29                  |                   |                        | 103,623.2                           |
|         | Plasma      | 20.16             | 26.7                   | 15.39             | 19.74                  | 192.9                               |
| 7       | Whole blood | 18.2              | 20.08                  |                   |                        | 368,428.4                           |
|         | Plasma      | 22.32             | 25.61                  | 19.75             | 23.38                  | 24,047.6                            |
| 8       | Whole blood | 25.22             | 29.92                  |                   |                        | 161.5                               |
|         | Plasma      | Non-reagent       | Non-reagent            | 28.12             | 31.84                  | 7.1                                 |
| 9       | Whole blood | 18.91             | 21.97                  |                   |                        | 14,431.3                            |
|         | Plasma      | 23.61             | 26.75                  | 20.51             | 24.09                  | 1155.3                              |
| 10      | Whole blood | 24.43             | 26.93                  |                   |                        | 1358.9                              |
|         | Plasma      | 28.76             | Non-reagent            | 26.07             | 28.49                  | 2.9                                 |
| 11      | Whole blood | 12.35             | 14.71                  |                   |                        | 186,777.7                           |
|         | Plasma      | 26.53             | 29.1                   | 22.52             | 25.74                  | 471.7                               |
| 12      | Whole blood | 11.89             | 14.0                   |                   |                        | 239,674.9                           |
|         | Plasma      | 26.7              | 29.36                  | 22.67             | 25.9                   | 109.2                               |
| 13      | Whole blood | 13.37             | 15.71                  |                   |                        | 372,567.5                           |
|         | Plasma      | 22.57             | 25.73                  | 18.3              | 21.44                  | 1156.3                              |
| 14      | Whole blood | 16.86             | 19.5                   |                   |                        | 19,880.9                            |
|         | Plasma      | 27.23             | 29.8                   | 21.84             | 25.84                  | 113.2                               |

# Molecular test for malaria (NAT PLUS™)

|                          |                                                           |
|--------------------------|-----------------------------------------------------------|
| Test features            | Multiplex Real-time PCR (HBV, HCV, HIV and Plasmodium sp) |
| Manufacturer             | Biomanguinhos/Fiocruz                                     |
| Type of sample           | Plasma                                                    |
| Required volume          | 1.5 mL of plasma from each donation                       |
| Minipool size            | 6                                                         |
| Target                   | Conserved region of the 18S rRNA gene                     |
| Sensibility              | 200 copies/mL                                             |
| Limit of detection (LoD) | 50.97 copies/mL (95% CI)                                  |



Test able to detect *Plasmodium vivax*, *falciparum*, *malariae*, *ovale* and *knowlesi*  
Pan-Plasmodium - but not discriminatory

## NAT PLUS HIV/HBV/HCV/MALARIA Bio-Manguinhos KIT

Detection of  
*Plasmodium spp* in  
human **PLASMA**

**CHALLENGE**



Nucleic Acid  
Extraction  
**Magnetic Beads**



Confirmatory  
Analysis in **red**  
**blood cells**;  
**plasma** /  
Malaria Typing  
qPCR

Automated  
Platform



# NAT for malaria in Hemorio: first results

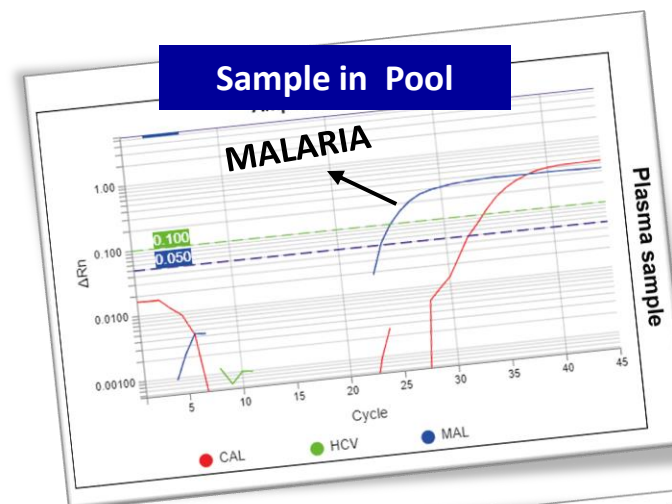
|                                                                     |                                                                                           |
|---------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| <b>Implementation date</b>                                          | <b>Oct 1<sup>st</sup>, 2022</b>                                                           |
| <b>Donor samples tested up to May 9<sup>th</sup><br/>(7 months)</b> | <b>194,813</b>                                                                            |
| <b>Origin of samples</b>                                            | <b>Rio de Janeiro and Espírito Santo states</b>                                           |
| <b>Positive cases</b>                                               | <div> 2 <div> - <i>P. vivax</i> – 1 case<br/> - <i>P. malariae</i> – 1 case </div> </div> |
| <b>Prevalence</b>                                                   | <b>1.02/100,000 donations</b>                                                             |

# CASE 1

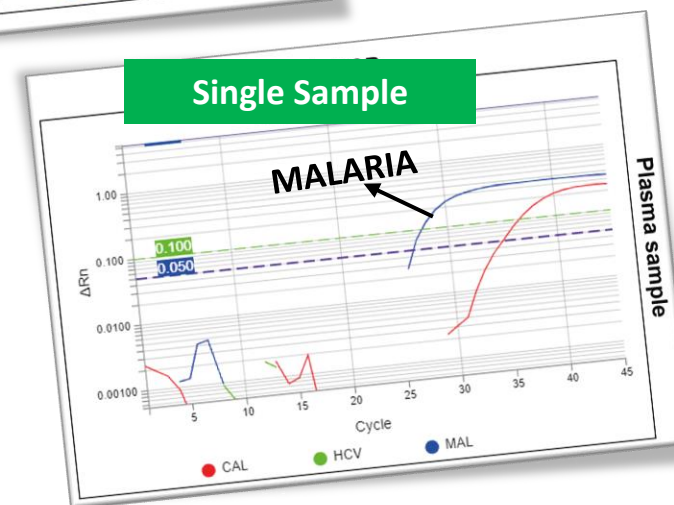


## Detection of molecular target **MALARIA** (*Plasmodium sp.*) in human plasma samples

- 🔴 37 years old Brazilian male
- 🔴 Lives in RJ since 2018
- 🔴 No malaria-related symptoms
- 🔴 From Maranhão state, part of Legal Amazon
- 🔴 Frequent visits to his hometown – but last visit 15 months before; he had traveled to Guyana (former English Guyana) in 2021



**Ct: 27**  
**Upper limit: 38**





ternal use

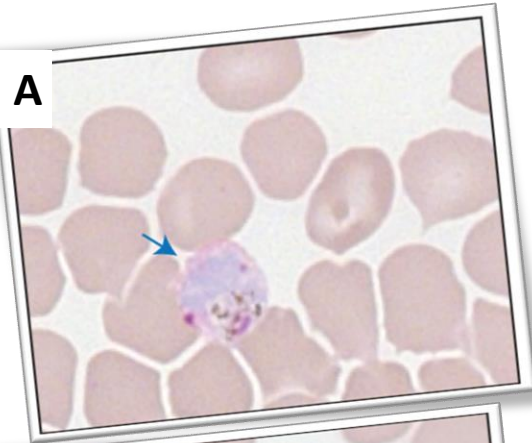
## CASE 1

### CONFIRMATORY ANALYSIS

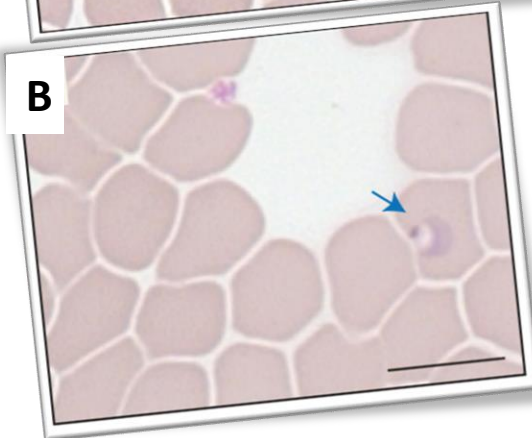
#### THIN BLOOD SMEARS



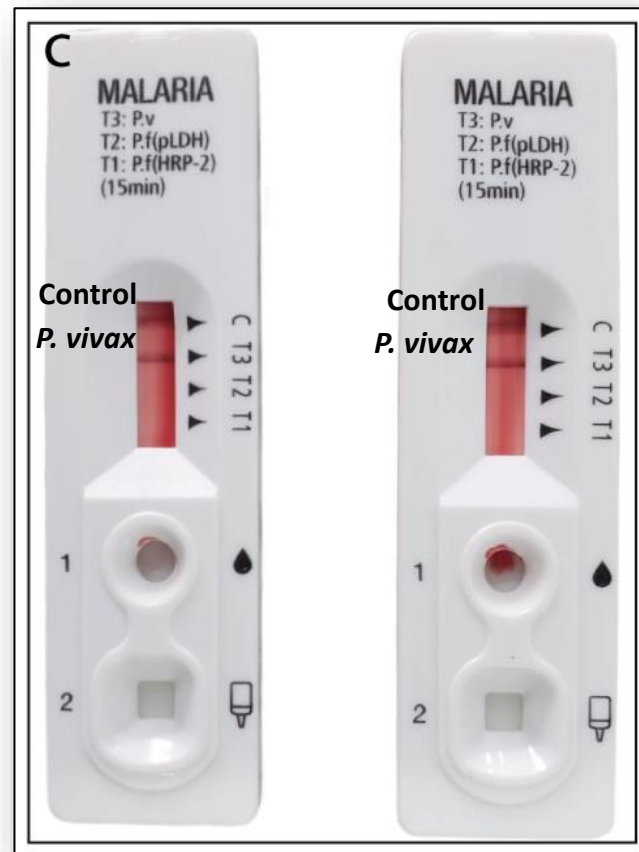
A



B



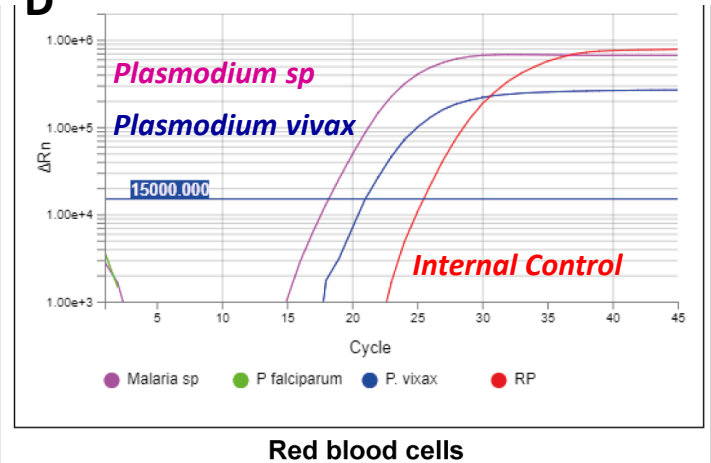
#### RAPID TEST



#### MALARIA TIPYNG qPCR

Bio-manguinhos 

D



**Plasmodium vivax**

NUCLEIC ACID  
SEQUENCING

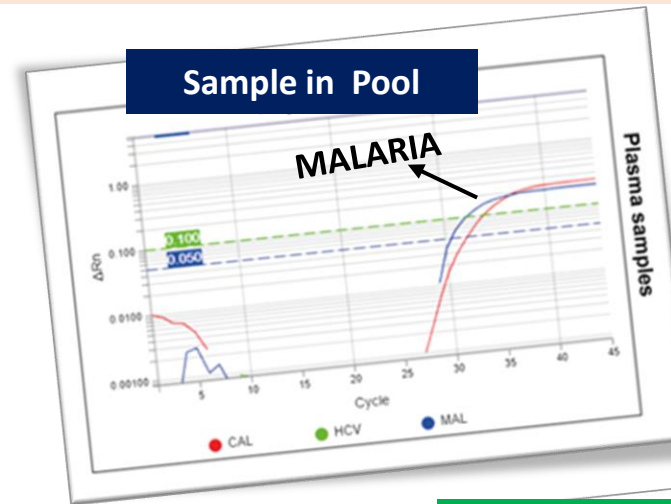




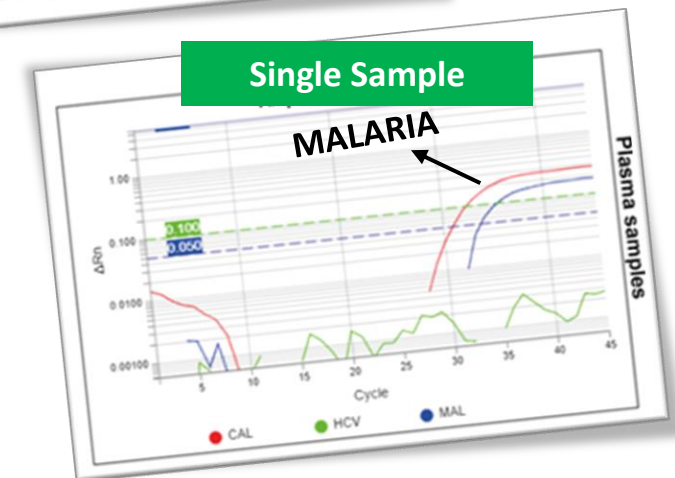
## CASE 2

### Detection of molecular target MALARIA (*Plasmodium sp.*) in human plasma samples

- 🔴 A 51 years old Brazilian male
- 🔴 No malaria-related symptoms
- 🔴 Reports that had traveled to the Atlantic Forest regions in Rio de Janeiro state 3 months before blood donation
- 🔴 Sporadic cases of autochthonous Malaria have already been described in those areas



Ct: 30.5



# CASE 2



## CONFIRMATORY ANALYSIS



## MALARIA TIPYNG q-PCR Bio-Manguinhos



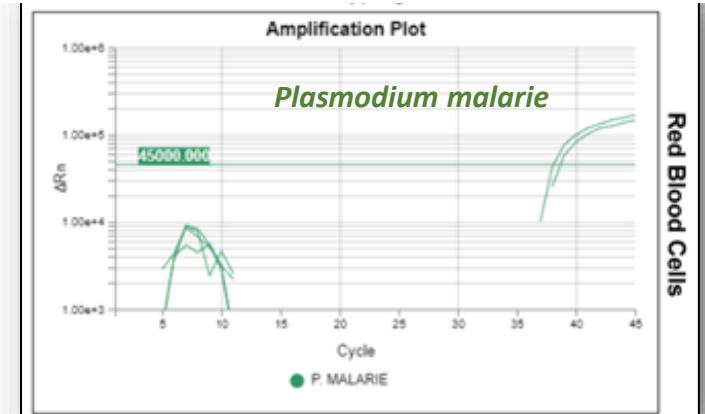
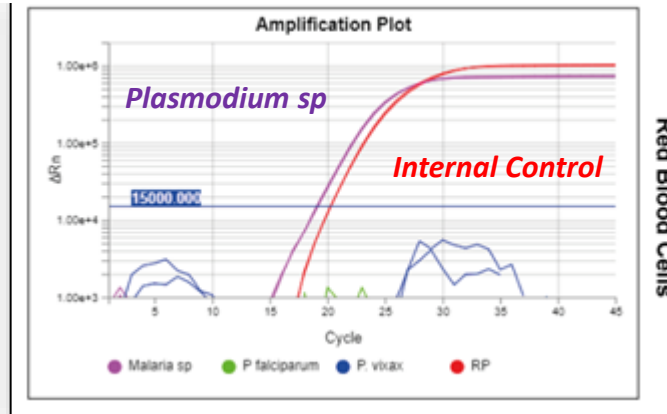
**THICK BLOOD SMEARS:**  
**NEGATIVE**



**RAPID TEST:**  
**NEGATIVE**



**THICK BLOOD SMEARS:**  
**NEGATIVE**



NUCLEIC ACID  
SEQUENCING

***Plasmodium malariae***



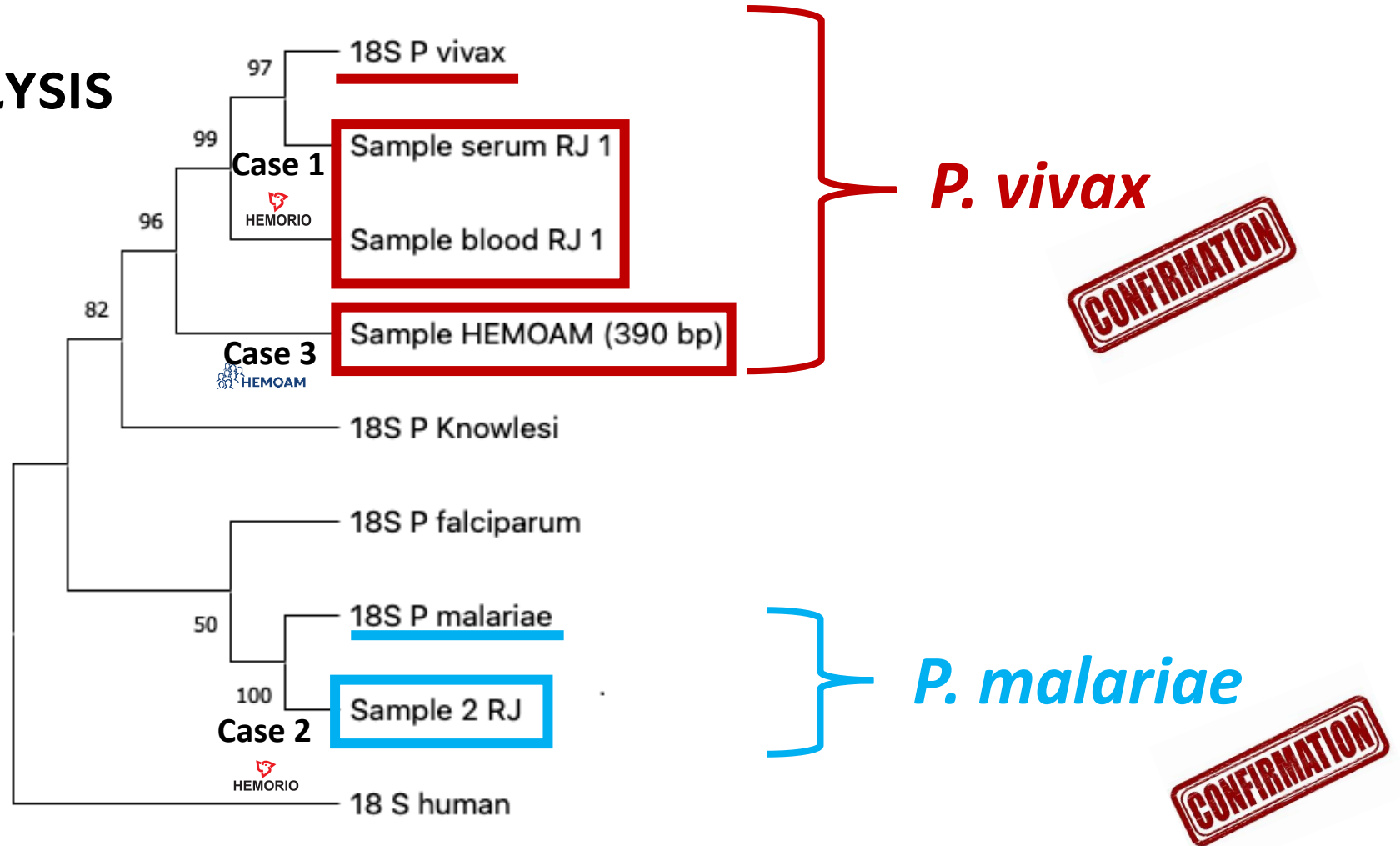
## CASE 2



### LOOK BACK

- RBC from the previous donation transfused to a child who underwent Bone Marrow Transplantation
- Current sample (whole blood) from the child tested for malaria with NAT Plus (Hemorio) and Pan-malaria-Bio (Fiocruz): negative results

## SEQUENCING DATA PHYLOGENETIC ANALYSIS



# CASE 3



Detection of molecular target MALARIA  
(*Plasmodium sp.*) in human plasma samples

1

NAT PLUS: Pool and Single:  
**Malaria Detected**

Thick blood smears:  
**Positives**



***Plasmodium vivax***



NUCLEIC ACID  
SEQUENCING

🔴 No symptoms

🔴 The person lives in an endemic area: Amazon

# Detection of molecular target **MALARIA** (*Plasmodium sp.*) in human plasma samples



## NEW CASES



🔴 No symptoms.

🔴 The two blood donors live in endemic area: Roraima state (Amazon region)

4

NAT PLUS: Pool and  
Single: **Malaria  
Detected**

Thick blood smears:  
**Positive**

*Plasmodium vivax*

5

NAT PLUS: Pool and  
Single: **Malaria  
Detected**

Thick blood smears:  
**Positive**

*Plasmodium falciparum*

## Donor deferral due to malaria exposure risk

| Period                                | Deferred donors | %     | p < 0,01 |
|---------------------------------------|-----------------|-------|----------|
| Oct 2021 – Sep 2022                   | 139             | 0,15% |          |
| Oct 2022 – May, 10 <sup>th</sup> 2023 | 23              | 0,04% |          |

# Preliminary conclusions

- Malaria NAT test implementation seems totally justified
- The use of plasma as a test material favors the logistics and speed of testing
- The potential risk reduction and decrease in donor deferral related to malaria exposure looks promising