Original article

Improving blood safety: NAT-based detection of *Plasmodium* spp. in blood donors in endemic areas of Brazil

Anne C. Gomes de Almeida[1,2*], Ana C. Shuan Laco[2*], Maewia Gomes Rodrigues[3], Vitória G. Godinho de Siqueira[1], Fransuellem Batista de Moura Brito[4], Cynara Melo Gato[3], Jady S. Mota Cordeiro[2], José M. Hipólito Carneiro[3], Sergio R. Lopes Albuquerque[1,3], Yury Oliveira Chaves[3,4], Maria de Fátima Ferreira da Cruz[5], Patrícia Alvarez Baptista[6], Marcelo Addas-Carvalho[7], Andrea Monteiro Tarragô[1,3,8], Gisely Cardoso de Melo[1,2,9]

*These Authors have contributed equally

[1]*Programa de Pós-Graduação em Ciências Aplicadas à Hematologia (PPGH-UEA), Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil;*
[2]*Programa de Pós-Graduação em Medicina Tropical (PPGMT-UEA), Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil;*
[3]*Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas (HEMOAM), Manaus, Amazonas, Brazil;*
[4]*Instituto Leônidas & Maria Deane (ILMD- Fiocruz Amazônia), Manaus, Amazonas, Brasil.*
[5]*Instituto Oswaldo Cruz (IOC/Fiocruz), Rio de Janeiro, Brazil;*
[6]*Institute of Technology in Immunobiology Bio-Manguinhos, Fundação Oswaldo Cruz/Fiocruz, Rio de Janeiro, Brazil;*
[7]*Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil;*
[8]*Programa de Pós-graduação em Imunologia Básica e Aplicada (PPGIBA), Universidade Federal do Amazonas,Manaus, Amazonas, Brazil;*
[9]*Fundação de Medicina Tropical Doutor Heitor Vieira Dourado. Manaus, Amazonas, Brazil*

Arrived: 30 July 2025
Revision accepted: 28 October 2025
Correspondence: Gisely Cardoso de Melo
e-mail: cardosogisely@gmail.com

Background - Transfusion-transmitted malaria remains a significant challenge in endemic areas and is a leading cause of blood donation deferral. Rigorous clinical and molecular screening is essential to ensure the safety of blood components. The presence of low-density *Plasmodium* spp. parasitemia in asymptomatic individuals can compromise transfusion safety and perpetuate disease transmission. This study aimed to describe malaria positivity detected by the NAT PLUS HIV/HBV/HCV/Malaria kit (Bio-Manguinhos), as well as to characterize the epidemiological profile of blood donors and the main deferral causes at a reference blood center in the Brazilian Amazon.

Materials and methods - This was a retrospective observational study using secondary data from the HemoSys database, which included sociodemographic variables, clinical screening, and self-reported malaria history from blood donors. Cases of clinical malaria were investigated among NAT PLUS-positive donors within six months before and after the reactive donation.

Results - A total of 67,114 blood donors tested using the NAT PLUS assay were included. The median age was 35 years (IQR 25-43), with a predominance of male donors (65.1%). Most self-identified as mixed race (80.5%) and had completed high school (53.3%). Malaria-related factors accounted for 2.6% of deferrals (No.=27,434). Malaria positivity detected by NAT PLUS assay was 0.01% (8/67,114), with all reactive donors being asymptomatic at screening. Most positive cases (6/8) occurred during the low-transmission period (rainy season). One donor (12.5%) developed symptomatic malaria (*P. falciparum*), confirmed by thick blood smear and notified in the SIVEP-Malaria system shortly after donation.

Discussion - Individuals testing positive for *Plasmodium* spp. by NAT PLUS assay may progress to clinical malaria post-donation, posing risks to both donor health and transfusion safety. The frequent absence of information on malaria exposure during clinical screening highlights the need for standardized protocols and molecular testing to improve early identification of at-risk donors in endemic regions.

Keywords: *malaria, NAT Plus assay, hemovigilance, screening, blood screening.*

INTRODUCTION

Malaria remains a public health challenge in the Brazilian Amazon, where persistent transmission hinders elimination efforts. In 2022, Brazil reported 131,224 cases, mostly caused by *Plasmodium vivax* (*P. vivax*) (84.2%), followed by *Plasmodium falciparum* (*P. falciparum*) (13.9%), the latter associated with severe disease. Although typically less severe, *P. vivax* can also cause severe malaria[1,2]. Additionally, mixed infections accounted for 1.8% of cases, and *Plasmodium malariae* (*P. malariae*) infections were reported in less than 0.1% of cases[1]. Transfusion-transmitted malaria (TTM) poses additional risks, especially for immunocompromised recipients, due to the difficulty of detecting low parasitemia in asymptomatic donors[3-5]. In the Americas, *P. falciparum* is most often linked to TTM (43.1%), followed by *P. malariae* (27.7%) and *P. vivax* (23.1%)[4].

Molecular screening, based on nucleic acid amplification tests (NAT), offers a highly sensitive method for detecting malarial infections in blood donors, even in instances of low-density or asymptomatic infections that may go undetected by conventional methods. The implementation of NAT assay is essential to ensure transfusion safety and minimize the risk of TTM. The NAT PLUS HIV/HBV/HCV/Malaria Bio-Manguinhos kit (Instituto de Tecnologia em Imunobiológicos de Bio-Manguinhos, Rio de Janeiro, Brazil), which has been routinely used, has demonstrated excellent analytical performance, with sensitivity ranging from 92.45 to 100% and specificity from 99.8 to 100% (95% CI). It is designed to detect the *Plasmodium 18S rRNA* target gene using a plasma sample collected from the blood donor[6]. With molecular screening in place, it becomes possible to identify submicroscopic parasitemia in asymptomatic donors. Previous studies underscore the presence and significance of asymptomatic infections with low parasitemia and gametocytemia contributing to the persistence of malaria and disease-related morbidity in endemic areas[7-9].

Before the implementation of molecular screening, donor eligibility in Brazil for individuals potentially exposed to malaria was determined based on clinical and epidemiological history, including the Annual Parasite Incidence (API) of the donor's municipality of residence. This information, obtained during the clinical screening interview, was critical for assessing deferral. In the absence of more sensitive diagnostic tools, self-reported exposure remained the only available source of epidemiological data. According to Consolidation Ordinance Nº 5/2017, individuals residing in municipalities with an API greater than 49.9 were temporarily deferred for 30 days. Those with a previous *Plasmodium* spp. infection were subject to a 12-month deferral following confirmed treatment and cure, regardless of the species, except for *P. malariae* infections, which required permanent deferral[10].

The implementation of the NAT PLUS assay for blood donor screening led to updates in Brazilian regulations. In non-endemic regions, the deferral period was reduced from 12 months to 30 days, provided donors test negative with the NAT PLUS HIV/HBV/HCV/Malaria assay. In endemic areas with active transmission, current regulations require *Plasmodium* spp. testing regardless of the API, although molecular methods such as NAT PLUS are not mandatory[11]. Additionally, species-specific deferral periods were established, 12 months for *P. falciparum*, three years for *P. vivax* or *P. ovale* due to relapse risk, and permanent deferral for *P. malariae*, owing to its potential for prolonged parasitemia and recrudescence[11].

Globally, sustained surveillance among blood donors in endemic areas is critical. A systematic review across 21 countries (Africa, the Americas, Asia, Europe, and Oceania), involved approximately 985,000 blood donors, and reported a global parasitemia prevalence of 10.54% by microscopy, 5.36% by molecular testing, and 0.38% by rapid diagnostic tests (RDTs). The African continent presented the highest burden, with a prevalence of 36% detected through molecular assays, predominantly attributed to *P. falciparum*, especially in sub-Saharan Africa[12]. In Ghana, 2.5% of asymptomatic and clinically eligible donors tested positive for malaria, underscoring the silent risk of TTM[13]. In the Arabian Peninsula, *Plasmodium* spp. was found in 0.7% of more than 64,000 blood donations analyzed[14].

Recent studies conducted in Brazilian blood centers have found that donors who tested positive for malaria by molecular assay reported no symptoms of the disease during clinical screening[15,16]. Despite the importance of NAT PLUS HIV/HBV/HCV/Malaria screening for blood supply safety, local data on malaria prevalence among blood donors in the Brazilian Amazon and the impact of NAT PLUS assay screening on the detection of asymptomatic cases remain limited. This study aims to fill this gap by providing valuable information to guide TTM

screening and prevention policies in the Amazon region. This study aimed to investigate the frequency of positivity and the epidemiological characteristics of donors tested by NAT PLUS assay for malaria, as well as the occurrence of symptomatic malaria, in a blood center located in the Brazilian Amazon.

MATERIALS AND METHODS

Study site

This study was conducted at the Amazonas State Foundation for Hematology and Hemotherapy (*Fundação Hospitalar de Hematologia e Hemoterapia do Amazonas [HEMOAM])*, located in Manaus, Brazil. HEMOAM is a regional reference center for the diagnosis and treatment of onco-hematological diseases and coordinates blood collection, distribution, and routine serological screening. Through the NAT PLUS platform, it also performs molecular screening for blood donors from Acre, Rondônia, and Roraima, thus expanding its molecular diagnostic coverage across Northern Brazil.

The city of Manaus is the capital of Amazonas, the state responsible for over 99% of malaria cases in Brazil, positioning it as the main endemic area for the disease in the country[1,17]. Transmission is unstable and perennial, with seasonal peaks influenced by rainfall patterns that determine the availability of breeding sites for the primary vector, *Anopheles darlingi*[18]. Regarding the etiological agents, *P. vivax* is the predominant species, but *P. falciparum* remains a significant cause of malaria, particularly in transmission hotspots[19]. A key epidemiological feature of the Amazon region is the high prevalence of asymptomatic and submicroscopic infections, which serve as a silent parasite reservoir[19,20]. Data on the Entomological Inoculation Rate (EIR) are scarce for the Brazilian Amazon. To provide regional context, estimates from the southern Venezuelan Amazon indicate an EIR of approximately four infective bites per person per year, suggesting that even low vector density can sustain malaria transmission[21]. This underscores the importance of molecular screening to ensure transfusion safety in malaria-endemic settings.

NAT Plus platform for molecular screening

HEMOAM implemented the NAT PLUS HIV/HBV/HCV/Malaria assay (Bio-Manguinhos) in March 2022 for simultaneous molecular screening of HIV, HBV, HCV, and *Plasmodium* spp. in blood donor samples. Plasma samples were tested in six-sample pools using a 96-well plate format. Sample pipetting was automated via the Janus system (Revvity, Waltham, MA, USA), and nucleic acid extraction was performed using the Chemagic Prime platform (Revvity). The eluted DNA was analyzed by multiplex real-time PCR on a QuantStudio Dx instrument (Thermo Fisher, Waltham, MA, USA), targeting the *18S rRNA* gene for malaria detection, as described by Rocha *et al.*[16] and according to the manufacturer's instructions[22]. The NAT software automatically interprets amplification curves and internal control parameters to classify samples as reactive, non-reactive, or invalid.

Clinical and epidemiological data collection and donor eligibility criteria

The donor data analyzed in this study were obtained from the HemoSys online platform (HEMOAM, Manaus, Brazil), which contains the clinical screening data collected from each donor candidate. Data were gathered on the profile of all donations and donors between January 2023 and June 2024, including sociodemographic variables, clinical screening information, malaria exposure, and NAT PLUS HIV/HBV/HCV/Malaria results. Malaria-related factors were defined based on the candidates' malaria history, travel, and residence in malaria-endemic areas. In addition, the overall reasons for blood donation deferral and the malaria-related deferral causes were collected for the same period.

Malaria-related deferrals were defined according to the information provided by donor candidates during the clinical screening questionnaire, which relied on self-reported data. Candidates were considered deferred if they reported having stayed in areas classified as endemic for malaria transmission regardless of the duration of residence or visit or if they reported a malaria episode within the 12 months preceding the donation attempt.

All donor candidates who completed both clinical and laboratory screening steps were considered eligible for inclusion in the study, regardless of donation eligibility outcome, thus comprising both eligible and deferred donors. No exclusion criteria were applied, ensuring that all records available in the database during the study period were included, thereby preserving the completeness and integrity of the dataset.

Investigation of clinical cases of malaria in blood donors

To confirm potential episodes of symptomatic malaria following positive NAT PLUS HIV/HCV/HBV/Malaria blood donation, a search was conducted in the National Malaria Case Notification System (SIVEP-MALARIA). Positive thick blood smear results were examined for the six months prior to and the six months following the date of the malaria-reactive donation.

Ethical aspects

This study was approved by the HEMOAM Research Ethics Committee (CAAE: 74332523.8.0000.0009; Approval No. 6.483.195). Donor data were accessed following approval of the Data Use Agreement and waiver of informed consent.

Data analysis

To ensure the quality and consistency of the data, two independent searches were conducted in the SIVEP-Malaria system at different time points using identifiers of NAT PLUS-positive donors. A similar duplicate extraction procedure was applied to the HemoSys database to retrieve clinical and laboratory information. These parallel searches aimed to enhance reproducibility and reduce potential data entry errors. Epidemiological and clinical-laboratory data were compiled in Microsoft Excel 2023 and analyzed using Stata version 15 (StataCorp LP, College Station, TX, USA). The Shapiro-Wilk test was used to assess the distribution of continuous variables. Normally distributed variables were presented as mean and standard deviation (SD), while non-normal variables were expressed as median and interquartile range (IQR). Categorical data were summarized as absolute and relative frequencies (%).

RESULTS

From January 2023 to June 2024, a total of 98,362 blood donations were recorded at the HEMOAM. These donations were made by 67,114 unique donors, acknowledging that some individuals may have made multiple donations during this period. Of the total blood donations, 96,962 (98.6%) underwent molecular screening using the NAT PLUS HIV/HBV/HCV/Malaria kit. A total of 1,400 donations were not tested using the NAT PLUS platform due to sample-related issues, such as hemolysis and lipemia (**Figure 1**).

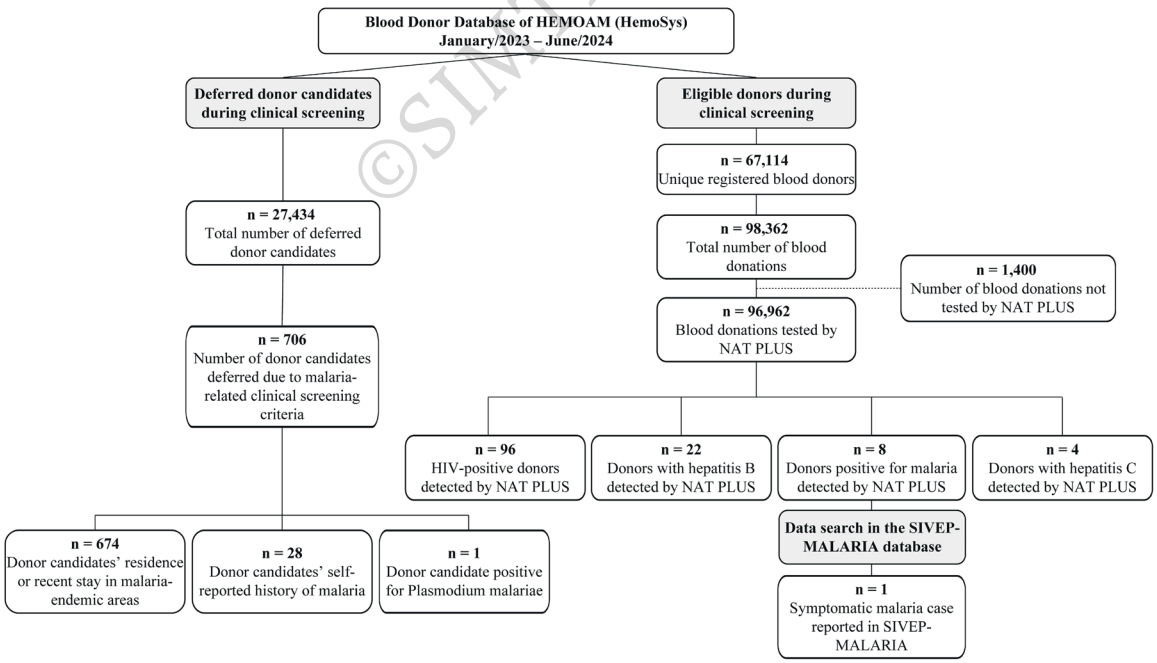

Figure 1 - Flowchart of blood donations, NAT PLUS assay, and malaria detection among unique donors at HEMOAM (January 2023 - June 2024)
Data were obtained from the HemoSys blood donor database, including both eligible and deferred donor candidates according to clinical screening outcomes. In addition, a data search was performed in the SIVEP-Malaria database for donors who tested positive in the NAT PLUS assay.

The median age of blood donors was 34 years (IQR=25-43 years; *Online supplementary* **Table SI**). The majority were male (65.1%) and self-identified as mixed race (80.5%). Approximately 93% resided in Manaus, and 83.2% were born in the state of Amazonas, while 16.8% were from other states. Regarding educational background, 56.25% completed high school. O+ was the most prevalent blood group (59.3%) (**Table I**).

Regarding malaria exposure, 85.0% (No.=57,059) of blood donors reported no history of the disease, while 0.30% (No.=204) reported a previous malaria diagnosis (**Table I**). In terms of travel history, 85.3% (No.=57,246) of donors had traveled or resided in another country. An assessment of recent exposure to endemic areas revealed that 85.3% (No.=57,221) had not traveled to malaria-endemic regions in the past 30 days. However, 0.04% (No.=23) reported staying in such areas during this period (**Table I**).

Among the reactive donations screened using the NAT PLUS HIV/HCV/HBV/Malaria assay, positivity rates were 0.02% for HIV (No.=22), 0.10% for HBV (No.=96), 0.004% for HCV (No.=4), and 0.01% for malaria (No.=8), among 96,962 evaluated blood units. Considering unique donors (No.=67,114), the overall frequency of malaria was also 0.01% (8/67,114). Notably, malaria-positive donors did not present co-infection with any of the other screened pathogens in the NAT PLUS assay.

Table I - *Epidemiological characteristics of donors evaluated at the HEMOAM from January 2023 to June 2024*

Characteristics	Donors No. 67,114
Age, median (IQR)	34 (25-43)
Gender (%)	
Male	43,687 (65.1)
Female	23,427 (34.9)
Self-reported race/ethnicity of donors (%)	
Brown/mixed-race	52,041 (80.5)
White	6,328 (9.8)
Black	2,231 (3.4)
Yellow	391 (0.6)
Indigenous	146 (0.2)
Not declared	3,505 (5.4)
City of residence, No. (%)	
Manaus	62,666 (93.4)
Other	4,448 (6.6)
Origin, No. (%)	
Amazonas	55,833 (83.2)
Other	11,281 (16.8)
Education level, No. (%)	
High school	35,537 (53.2)
Complete higher education	15,505 (23.2)
Incomplete higher education	9,471 (14.2)
Elementary education	2,930 (4.4)
Postgraduate	1,386 (2.1)
Professional education	809 (1.2)
Up to 4th grade	232 (0.3)
Master's degree	139 (0.2)
Illiterate	31 (0.05)

Characteristics	Donors No. 67,114
Education level, No. (%) - *follows*	
Doctorate	25 (0.04)
Adult literacy	4 (0.01)
Not reported	669 (1.0)
ABO phenotype, No. (%)	
O+	39,792 (59.3)
A+	16,355 (24.4)
B+	5,215 (7.8)
O-	2,802 (4.2)
A-	1,143 (1.7)
AB+	1,131 (1.7)
B-	375 (0.6)
AB-	78 (0.1)
A1+	1 (0.00)
Not reported	222 (0.3)
Malaria-endemic area exposure	
Malaria, No. (%)	
No	57,060 (85.0)
Yes	204 (0.3)
Not reported	9,850 (14.7)
Traveled or lived in another country, No. (%)	
Yes	57,246 (85.3)
No	9,868 (14.7)
Traveled to areas of malaria in the last 30 days, No. (%)	
No	57,221 (85.3)
Yes	23 (0.04)
Not reported	9,870 (14.7)

Among the eight donors who tested positive by the NAT PLUS assay, the mean age was 31.4 years (SD ±12.0; *Online supplementary* **Table SII**), with a predominance of males (75%) and self-identified mixed-race individuals (87.5%). Most resided in Manaus (75%) and were born in Amazonas (87.5%). High school education was reported by 75%, and occupations included self-employment, security, farming, military, and administrative roles; two did not report. Blood group O+ was the most frequent (50%), followed by A+ (25%) (**Table II**).

All donors were asymptomatic, meaning that they did not report fever, chills, headache, sweating, or any malaria-related symptoms during the clinical screening interview, and all denied recent travel to endemic areas. Regarding clinical screening, 75% reported no recent travel or previous history of malaria, although some had missing exposure data. Three had prior non-reactive donations, and one donor subsequently developed symptomatic *P. falciparum* malaria, characterized by the onset of typical clinical manifestations and laboratory confirmation by thick blood smear, which was notified in the SIVEP-MALARIA system (**Figure 2**).

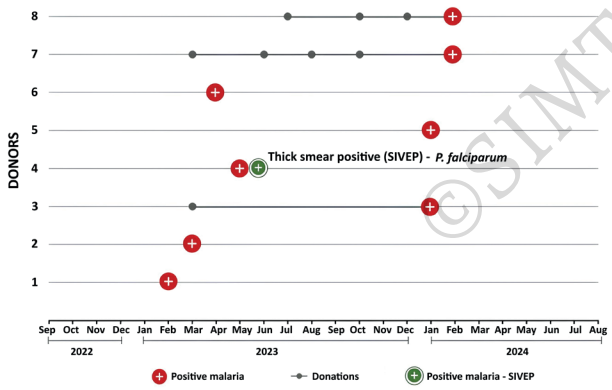

Figure 2 -Temporal distribution of donations by donor and malaria positivity in the NAT PLUS HIV/HCV/HBV/Malaria kit, related to the occurrence of clinical malaria (SIVEP-MALARIA) within six months before and after donation

The vertical axis lists donors (1 to 8), while the horizontal axis shows the timeline from September 2022 to August 2024. Gray dots represent donations with no malaria detection, red circles indicate donations that tested positive by the NAT PLUS assay, and the green circle marks a clinically confirmed case of malaria (*P. falciparum*) in the SIVEP-MALARIA system. Donors 1 and 2 tested positive for malaria in their first recorded donation. Donor 3 made an initial donation in March 2023 and tested positive in a second donation in January 2024. Donor 4 tested positive in May 2023 and developed symptomatic malaria within one month, confirmed by thick blood smear in SIVEP-MALARIA. Donors 5 and 6 also tested positive in their first donations, in January and April 2024, respectively. Donor 7 made four donations throughout 2023 and tested positive in the fifth donation in February 2024. Donor 8 had three donations in 2023 and tested positive in February 2024.

Table II - *Characteristics of donors testing positive for malaria by NAT PLUS assay information on malaria-endemic area exposure*

Malaria positive donors	**8**
Age mean (SD)	31.38 (12.01)
Gender, No. (%)	
Male	6 (75.0)
Female	2 (25.0)
Self-reported race/ethnicity of donors, No. (%)	
Brown	7 (87.5)
Not declared	1 (12.5)
City of Residence, No. (%)	
Manaus	6 (75.0)
Tefé	2 (25.0)
Residence Zone, No. (%)	
East Zone, Manaus	3 (37.5)
North Zone, Manaus	2 (25.0)
West Zone, Manaus	1 (12.5)
Urban Zone, Tefé	1 (12.5)
Rural Zone, Tefé	1 (12.5)
Origin, No. (%)	
Amazonas	7 (87.5)
Maranhão	1 (12.5)
Education level, No. (%)	
High school	6 (75.0)
Complete higher education	1 (12.5)
Incomplete higher education	1 (12.5)
Profession, No. (%)	
Other	2 (25.0)
Self-employed	2 (25.0)
Security guard	1 (12.5)
Farmer	1 (12.5)
Army soldier	1 (12.5)
Administrative assistant	1 (12.5)
ABO phenotype, No. (%)	
O+	4 (50.0)
A+	2 (25.0)
O-	1 (12.5)
B+	1 (12.5)
Malaria, No. (%)	
No	6 (75.0)
Not reported	2 (25.0)
Traveled or lived in another country, No. (%)	
No	6 (75.0)
Not reported	2 (25.0)
Traveled to areas of malaria in the last 30 days, No. (%)	
No	6 (75.0)
Not reported	2 (25.0)

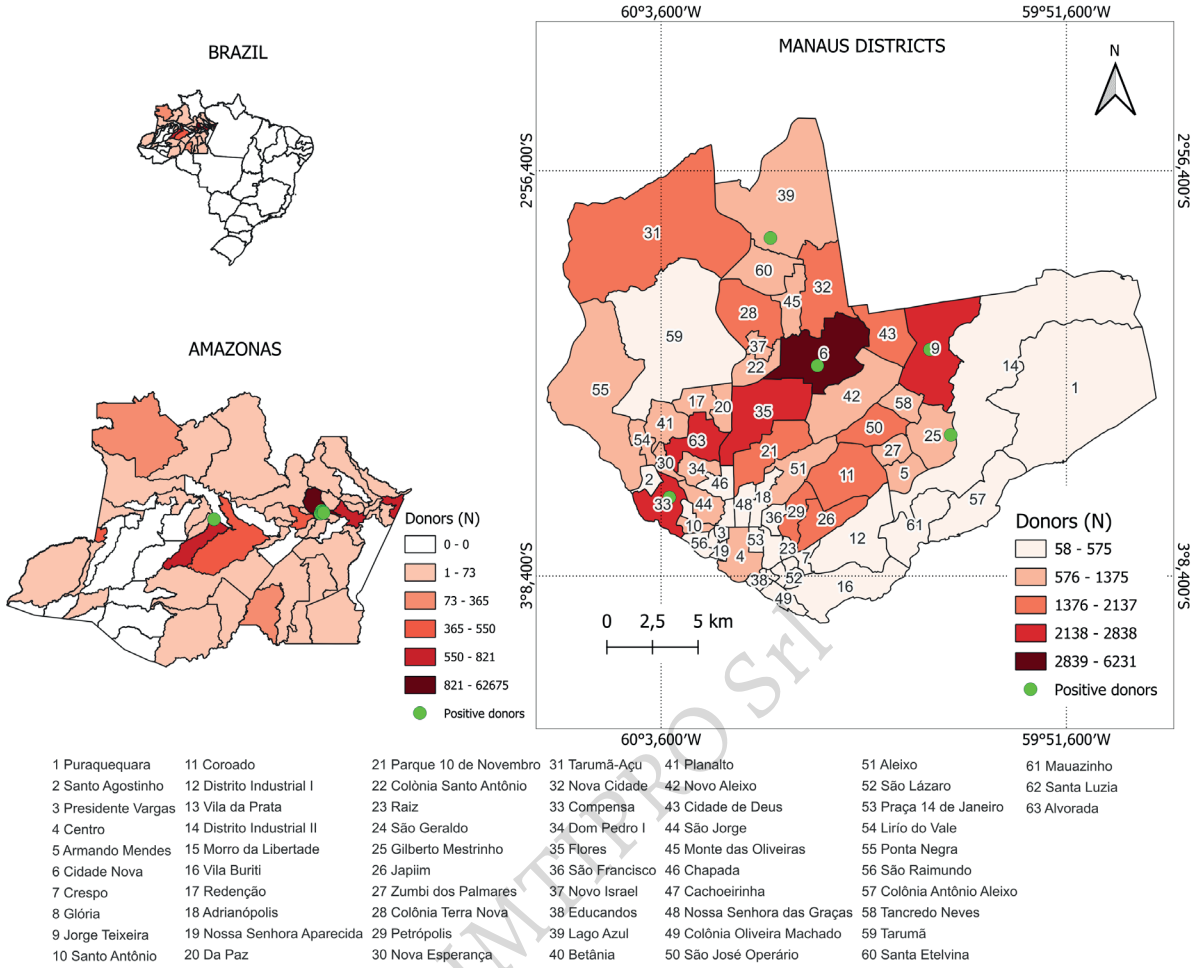

Figure 3 - Spatial distribution of blood donors included in the study in the state of Amazonas, Brazil (2023-2024)
Georeferenced map showing the residential locations of blood donors from 2023 to 2024, including 47 municipalities across the state of Amazonas and 63 neighborhoods within the city of Manaus. Donors who tested positive for *Plasmodium spp.* by the NAT PLUS HIV/HCV/HBV/Malaria assay are highlighted in green. Of the eight malaria-positive donors, six resided in Manaus and two in the municipality of Tefé. The 47 municipalities recorded were: Alvarães, Anamã, Anori, Apuí, Atalaia do Norte, Barcelos, Barreirinha, Benjamin Constant, Boa Vista do Ramos, Borba, Caapiranga, Canutama, Careiro, Careiro da Várzea, Careiro Castanho, Coari, Eirunepé, Fonte Boa, Humaitá, Iranduba, Itacoatiara, Itapiranga, Japurá, Lábrea, Manacapuru, Manaquiri, Manaus, Manicoré, Nhamundá, Nova Olinda do Norte, Novo Airão, Novo Aripuanã, Parintins, Presidente Figueiredo, Rio Preto da Eva, Santa Isabel do Rio Negro, Santo Antônio do Içá, São Gabriel da Cachoeira, Silves, Tabatinga, Tapauá, Tefé, Uarini, Urucará, and Urucurituba.

The georeferencing of blood donors' residential addresses enabled the construction of a spatial distribution map covering municipalities in the state of Amazonas and neighborhoods of Manaus. This analysis highlighted the geographic distribution of donors, including those testing positive for malaria. A total of 47 municipalities and 63 neighborhoods were represented in the sample (**Figure 3**).

During the study period, a total of 27,434 blood donation deferrals were recorded, corresponding to 27.9% of the 98,361 donations performed. The primary reasons for deferrals included low hematocrit, which accounted for 44.3% (No.=12,164) of cases, donor withdrawal (4.1%, No.=1,111), and arterial hypertension, leading to 3.0% (No.=821) of deferrals.

Blood donation deferrals were categorized into eleven groups, following criteria established by Ordinance No. 158. The most frequent cause was clinical or physical conditions identified during pre-donation screening (61.3%), followed by medication use (7.0%), infections or temporary conditions (6.6%), and invasive procedures (6.5%). Chronic diseases and reproductive health each accounted for 5.3%, while risk behavior (4.4%), recent

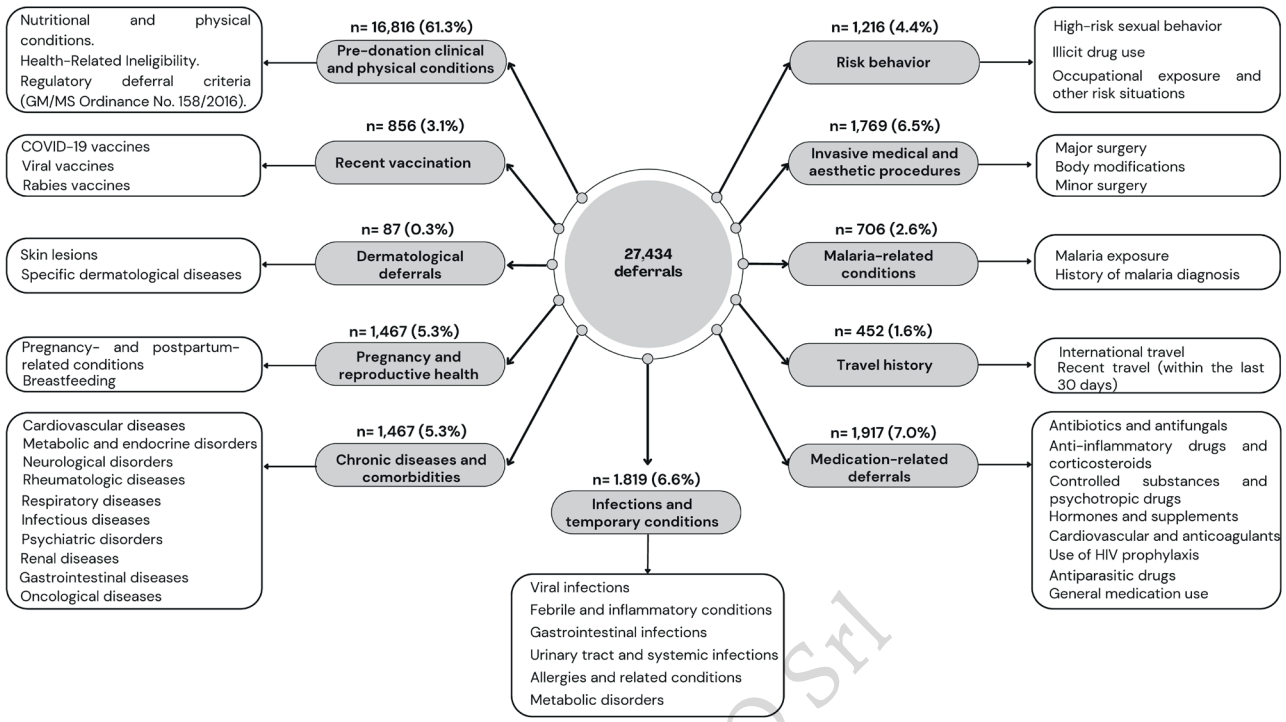

Figure 4 - Reasons for blood donation deferral recorded from January 2023 to June 2024 at HEMOAM
Donor deferrals (No.=27,434) were categorized according to clinical and epidemiological screening criteria established by national regulations.

vaccination (3.1%), malaria-related criteria (2.6%), travel history (1.6%), and dermatological conditions (0.3%) were less frequent. These findings highlight the role of standardized clinical-epidemiological screening in transfusion safety (**Figure 4**).

Among the 706 malaria-related deferrals, the majority were associated with residence or recent stay in malaria-endemic areas (No.=674; 95.5%). Additionally, 28 cases (4.0%) were due to a self-reported history of malaria, and one case (0.1%) involved an infection caused by *P. malariae*.

DISCUSSION

Transfusion-transmitted malaria poses a significant challenge for disease control in both endemic and non-endemic areas[23]. Individuals receiving blood components are more vulnerable, and if laboratory screening fails, transmission can occur, leading to complications in the health status of recipients. In the case of *P. falciparum*, it can result in severe malaria, particularly in individuals who are primarily infected, and for *P. vivax*, there is a risk of relapses due to the latent forms of this species[4,24].

The inclusion of molecular malaria screening in public blood banks in Brazil represents an innovative and strategic advance toward interrupting TTM. This pioneering effort aligns with the "Global Technical Strategy for Malaria 2016-2030" aiming to prevent malaria resurgence in endemic and previously malaria-free areas[25]. This is the first study to report malaria-positive blood donors detected by the NAT PLUS HIV/HCV/HBV/Malaria kit (Bio-Manguinhos) in Amazonas, since its implementation at HEMOAM in 2022. An additional strength was the integration of SIVEP-MALARIA data to assess notifications before and after donation, enabling the evaluation of clinical screening failures and subsequent diagnoses.

Regarding donor characteristics, the profile observed was consistent with previous Brazilian studies. Approximately 65% were male, with a median age of 34 years; 80% self-identified as brown, and most had completed high school (53.2%) or had higher education (23%). A study in Southeast Brazil with 246 participants identified the predominant donor age group as 31-40 years (25%), with higher education attainment in

Blood Transfus. 2026; 24(1): 65-76 doi: 10.2450/BloodTransfus.1168

35% of cases, contrasting with the non-donor profile (younger, female, lower socioeconomic status) [26]. Another analysis of 410,423 donors across three Brazilian states showed that most identified as brown in Minas Gerais and Pernambuco (47.1% and 60.8%, respectively), while in São Paulo, 54.3% identified as white[27].

Out of 96,962 blood donations tested using the NAT PLUS HIV/HCV/HBV/Malaria assay, eight were positive, resulting in a frequency of 0.01%. All cases were later confirmed by thick blood smear and microscopy performed at the blood center. These donors were asymptomatic at the time of clinical screening yet may still contribute to malaria transmission in endemic regions. Studies conducted in the Brazilian Amazon have demonstrated that asymptomatic individuals often exhibit low parasitemia, presence of gametocytes, and the potential to infect Anopheles vectors, reinforcing the importance of detecting and controlling these cases[9,28-30]. For *P. vivax*, hypnozoites can remain latent for varying periods, depending on the location of the infection[31]. Furthermore, low parasitemia in asymptomatic individuals has been associated with an increased risk of clinical recurrence within six months of the initial episode[32]. These data reinforce the importance of molecular assays with high sensitivity, such as the NAT PLUS assay, for hemovigilance in endemic regions. All positive donors in Manaus (75% from total) lived in peri-urban or rural areas, closer to forest and river, ideal locals for vector proliferation[33]. Also, it is worth noting that the population goes to these areas on weekends and holidays for recreation, in addition to being areas designated for agricultural and fish culture activities[9,34].

Previous studies have investigated the frequency of malaria-positive donors in the Amazon region. A multicenter survey across blood services in Rondônia, Amapá, Pará, and Acre found an average positivity rate of 2.3% using nested-PCR[35]. In Amazonas, a PCR standardization study reported a 0.3% positivity among 286 donors[36]. Using rapid diagnostic tests and microscopy, no malaria cases were detected among 407 eligible and deferred donors screened at HEMOAM[37]. In Acre, real-time PCR analysis of 230 donations revealed a 1.7% positivity rate, while all samples were negative by microscopy[15]. Following the implementation of NAT PLUS in Rio de Janeiro, two positive cases were detected (0.001%): one *P. vivax* infection acquired in the Amazon and one autochthonous case of *P. malariae*[16].

In a systematic review, a variable frequency of TTM detected by molecular method was reported depending on the continent evaluated, with Africa having the highest prevalence (36%), followed by Asia (4%), America (2%) and Europe (1%)[12]. Regarding the Americas, the frequencies of TTM detected by PCR ranged from 7.5 to 0%, with *P. falciparum* infection being most frequently found in donors, followed by mixed infections by *P. falciparum* and *P. vivax*, and infections by *P. vivax*[4]. Another systematic review that verified the occurrence of TTM in non-endemic areas reported that few studies use the molecular method to detect the parasite in donors, especially studies conducted in the United States and Canada[38]. In Brazil, the frequencies of TTM detected by PCR ranged from 3 to 0% in studies conducted in the Brazilian Amazon[4].

In this study, most malaria-positive donors were male, self-identified as brown, with a mean age of 31.3 years and a high school education. These findings are consistent with previous data from HEMOAM, where 77% of donors were male and 76% were aged 18-39 years[37]. Similar profiles were observed in Acre, where 58.7% of donors were male and 38.3% had completed high school[15]. A systematic review on TTM in the Americas also reported predominance of males (80%) and donors aged 21-30 years (52.9%)[4]. As shown in **Table I**, 65% of the blood donors evaluated in the study were male, and this is the most common gender among blood donors, as reported in other studies conducted at the HEMOAM[39]. These individuals also tend to have greater exposure to or contact with endemic rural or peri-urban areas due to professional activities such as farming or military service.

Eight individuals deemed eligible during clinical screening tested positive for malaria by the NAT PLUS HIV/HCV/HBV/Malaria assay. Among them, one donor (12.5%) developed symptomatic *P. falciparum* malaria, confirmed by thick blood smear approximately one month after donation. Previous studies have shown that *P. falciparum* is the most frequently associated species with TTM, accounting for 73-98% of cases in sub-Saharan Africa and 43.1% in Latin America[4,40]. A recent systematic review of studies conducted in India identified that the main species involved in TTM are *P. falciparum*

(21-71% of positive cases) and the *P. vivax* (28 to 68% two positive cases), using MO, RTDs or ELISA for detection[5]. Another study, conducted in China, a non-endemic area of malaria, reported three non-native cases from Indonesia, Ghana and Equatorial Guinea, detected by PCR and MO, where the species involved was *P. falciparum*[41]. Notably, five of the eight positive donors (62.5%) were detected in January and February. Although malaria transmission peaks between June and September, high rainfall in the early months of the year alters river levels and, combined with social factors, promotes *Anopheles* proliferation and increases malaria incidence[42]. In Brazil, 30,654 cases were reported in the first trimester of 2023, and 34,539 in the same period of 2024 timeframes coinciding with most of the NAT PLUS assay positive detections in this study[1].

For seven donors, no records of case notification were found in SIVEP-MALARIA, either by active or passive search. It is believed that, due to the absence of symptoms, infected individuals, even when referred by the Blood Center, do not seek care at a health unit, becoming potential reservoirs of the parasite and maintaining the transmission of the disease, in addition to the probability of presenting relapses of the disease and, consequently, longer inability to donate blood. Therefore, it is essential to adopt strategies for monitoring these asymptomatic individuals, facilitating their access to health units for treatment, and ensuring adequate reporting of these cases.

Donor deferrals due to travel (1.6%), infections or temporary conditions (6.6%), and malaria-related criteria (2.6%) were also observed. A previous report from the Amazonas blood center showed that 99.5% of deferrals attributed to "risk exposure" (8%) were linked to visits to malaria-endemic areas, making it the third most common cause[39]. Over the years, with the rural exodus and urbanization, malaria in the state of Amazonas has reached peri-urban areas, bringing exposed individuals closer to hospitals and blood centers[43]. For this reason, it is essential to strengthen malaria hemovigilance, combining adequate epidemiological screening with a sensitive molecular method for the detection of *Plasmodium* spp.

The HEMOAM is the local referral center for hematologic diseases and the blood bank for the state of Amazonas, recruiting blood donors and directing the supply of blood component bags according to demand. After identifying positive malaria cases through the NAT-PLUS platform, the donor is contacted and referred to a reference unit for malaria diagnosis, where they are included in the malaria outpatient routine and receive antimalarial treatment. The current treatment regimen indicated for uncomplicated malaria is *P. vivax*. In Brazil, it is Chloroquine, administered for 3 days, and Tafenoquine (single dose) or Primaquine (7 days) to prevent relapses[44]. However, there is a limitation regarding the traceability of information on whether the asymptomatic positive donor detected at the blood center actually seeks the reference unit later to receive antimalarial treatment, which constitutes a limitation in the control of TTM and can contribute to the maintenance of transmission or for the development of clinical episodes of malaria. Improvements in the protocols for reporting infection by *Plasmodium* spp. in Brazilian blood banks and the development of a system to track the antimalarial treatment carried out by the positive donor.

This study has some limitations. Its retrospective design, based on secondary data, may affect data completeness and accuracy. Malaria case tracking in SIVEP was restricted to notifications from health units in Manaus but enabled confirmation of post-donation symptomatic malaria among NAT PLUS-positive donors. Missing exposure data in HemoSys likely reflect gaps in clinical screening or underreporting. Species identification was not possible, as the NAT PLUS assay does not differentiate *Plasmodium* species. Nonetheless, the findings underscore that asymptomatic infections at donation can evolve into symptomatic malaria. Enhancing NAT assays to allow species-level detection could improve transmission control and guide early treatment.

CONCLUSIONS

This study described the epidemiological profile of blood donors screened for malaria using the NAT PLUS HIV/HBV/HCV/Malaria assay in the state of Amazonas, Brazil. Among donors evaluated, malaria positivity was 0.01%, and all reactive donors were asymptomatic at the time of clinical screening. Most malaria-positive donors were male, self-identified as mixed race, and resided in Manaus, reflecting the overall donor population profile.

The findings demonstrate the contribution of molecular

Blood Transfus. 2026; 24(1): 65-76 doi: 10.2450/BloodTransfus.1168

screening to transfusion safety in malaria-endemic regions, enabling the detection of asymptomatic low-density infections that may not be identified through conventional screening. Strengthening hemovigilance strategies through the integration of molecular assays such as NAT PLUS can enhance the reliability of blood safety programs and support malaria control efforts in endemic areas.

ACKNOWLEDGMENTS

We thank the NAT PLUS laboratory team for their support in identifying positive cases. We also acknowledge the Information Technology Department at HEMOAM for their assistance in data retrieval and spreadsheet preparation. We are also grateful to all professionals who contributed to the development of this study. We thank the XXVIII Laveran & Deane Seminar on Malaria for the valuable discussions and contributions. This work was supported by student scholarships granted by the Foundation for the Support of Research in the State of Amazonas (*Fundação de Amparo à Pesquisa do Estado do Amazonas [FAPEAM]*) and the National Council for Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico ([CNPq]*).

FUNDING

This study was supported by the Laboratory of Experimental Therapeutics and Pharmacogenetics of Antimalarials (LATEFA), under the coordination of Dr. Gisely Cardoso de Melo. Financial resources were allocated to support study execution, data analysis, and dissemination of findings, reflecting the group's commitment to advancing scientific knowledge in transfusion-transmitted diseases and malaria diagnostics. This project also received funding through the 2nd Participatory Call for Budget Amendments promoted by Federal Deputy Amom Mandel.

AUTHOR'S CONTRIBUTION

ACGA, ACSL, MGR, VGCG, FBMB, JSMC: methodology, investigation. ACGA, ACSL: formal analysis. ACGA, ACSL: writing-original draft preparation. GCM, AMT: project administration. JMHC, CMG, SRLA: validation. GCM, AMT, YOC, SRLA, MFFC, PAB, MAC: writing-review. GCM, AMT, YOC: supervision. GCM, AMT: funding acquisition.

The Authors declare no conflicts of interest.

REFERENCES

1. Ministério da Saúde. Boletim Epidemiológico. Secretaria de Vigilância em Saúde e Ambiente. 2024. [Malaria Day in the Americas – an overview of malaria in Brazil in 2022 and the first half of 2023.] Available at: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/epidemiologicos/edicoes/2024/boletim-epidemiologico-volume-55-no-01/. Accessed on 26/12/2024. [in Portuguese.]
2. Matlani M, Kojom LP, Mishra N, Dogra V, Singh V. Severe vivax malaria trends in the last two years: a study from a tertiary care centre, Delhi, India. Ann Ann Clin Microbiol Antimicrob. 2020; 19(1): 49. doi: 10.1186/s12941-020-00393-9.
3. Abdullah S, Karunamoorthi K. Malaria and blood transfusion: Major issues of blood safety in malaria-endemic countries and strategies for mitigating the risk of Plasmodium parasites. Parasitol Res. 2016; 115(1): 35-47. doi: 10.1007/s00436-015-4808-1.
4. Alho RM, Machado KVA, Val FFA, Fraiji NA, Alexandre MAA, Melo GC et al. Alternative transmission routes in the malaria elimination era: An overview of transfusion-transmitted malaria in the Americas. Malar J. 2017; 16(1): 78. doi: 10.1186/s12936-017-1726-y.
5. Kojom Foko LP, Sharma S, Sharma A. Transfusion-transmitted Plasmodium spp. infections and safety challenges for malaria in the Indian subcontinent: A systematic review. Lancet Reg Health Southeast Asia. 2025; 40: 100641. doi: 10.1016/j.lansea.2025.100641.
6. Rocha D, De Melo GC, Carneiro JMH, Ribeiro M, Ribeiro S, De Godoy DT et al. Use of a NAT-based assay to improve the surveillance system and prevent transfusion-transmitted malaria in blood banks. Malar J. 2020; 19(1): 275. doi: 10.1186/s12936-020-03345-y. Erratum in: Malar J. 2020; 19(1): 325. doi: 10.1186/s12936-020-03396-1.
7. Bousema T, Okell L, Felger I, Drakeley C. Asymptomatic malaria infections: detectability, transmissibility and public health relevance. Nat Rev Microbiol. 2014; 12(12): 833-840. doi: 10.1038/nrmicro3364.
8. Nguitragool W, Mueller I, Kumpitak C, Saeseu T, Bantuchai S, Yorsaeng R et al. Very high carriage of gametocytes in asymptomatic low-density Plasmodium falciparum and P. vivax infections in western Thailand. Parasit Vectors. 2017; 10(1): 512. doi: 10.1186/s13071-017-2407-y.
9. Almeida ACG, Kuehn A, Castro AJM, Vitor-Silva S, Figueiredo EFG, Brasil LW et al. High proportions of asymptomatic and submicroscopic Plasmodium vivax infections in a peri-urban area of low transmission in the Brazilian Amazon. Parasit Vectors. 2018; 11(1): 194. doi: 10.1186/s13071-018-2787-7.
10. Ministério da Saúde. [Consolidation Ordinance No. 05, of September 28, 2017.] Available at: https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/s/saude-da-pessoa-com-deficiencia/legislacao/portaria-de-consolidacao-no-05-de-28-de-setembro-de-2017.pdf/view. Accessed on 02/04/2025. [in Portuguese.]
11. Ministério da Saúde. [Technical Note No 49/2023-CGSH/DAET/SAES/MS.] Available at: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/notas-tecnicas/2023/nota-tecnica-no-49-2023-cgsh-daet-saes-ms. Accessed on 8/04/2025. [in Portuguese.]
12. Ahmadpour E, Foroutan-Rad M, Majidiani H, Moghaddam SM, Hatam-Nahavandi K, Hosseini SA et al. Transfusion-Transmitted Malaria: A systematic review and meta-analysis. Open Forum Infect Dis. 2019; 6(7): ofz283. doi: 10.1093/ofid/ofz283. Erratum in: Open Forum Infect Dis. 2020; 7(1): ofz540. doi: 10.1093/ofid/ofz540.
13. Tetteh AK, Arthur S, Bram P, Baffe C, Aglagoh G. Prevalence of asymptomatic Malaria Parasitemia among blood donors in Cape Coast, Ghana: A cross-sectional study. J Trop Med. 2023; 2023: 8685482. doi: 10.1155/2023/8685482.
14. Alharazi T, Alzubiery TK, Alcantara JC, Qanash H, Bazaid AS, Altayar MA et al. Prevalence of transfusion-transmitted infections (HCV, HIV, Syphilis and Malaria) in blood donors: A large-scale cross-sectional study. Pathogens. 2022; 11(7): 726. doi: 10.3390/pathogens11070726.

15. Pinheiro TCP, Santos SS, Simião FMEB, Pimentel C de B, Mello AR de L, Lomonaco LA et al. Molecular test for screening malaria-infected blood donors to maximise recipient safety in Acre State, a Brazilian endemic area. Mem Inst Oswaldo Cruz. 2024; 119: e240109. doi: 10.1590/0074-02760240109.
16. Costa E, Rocha D, Lopes JIF, Andrade E, Cardoso P, Ribeiro M et al. Detection of Plasmodium spp. in asymptomatic blood donors by the new Brazilian NAT PLUS HIV/HBV/HCV/Malaria Bio-Manguinhos kit. Transfusion. 2024; 64(3): 501-509. doi: 10.1111/trf.17726.
17. Carlos BC, Rona LDP, Christophides GK, Souza-Neto JA. A comprehensive analysis of malaria transmission in Brazil. Pathog Glob Health. 2019; 113(1): 1-13. doi: 10.1080/20477724.2019.1581463.
18. Martins-Campos KM, Pinheiro WD, Vítor-Silva S, Siqueira AM, Melo GC, Rodrigues ÍC et al. Integrated vector management targeting Anopheles darlingi populations decreases malaria incidence in an unstable transmission area, in the rural Brazilian Amazon. Malar J. 2012; 11:351. doi: 10.1186/1475-2875-11-351.
19. Monteiro W, Karl S, Kuehn A, Almeida A, White M, Vitor-Silva S et al. Prevalence and force of Plasmodium vivax blood-stage infection and associated clinical malaria burden in the Brazilian Amazon. Mem Inst Oswaldo Cruz. 2022; 117: e210330. doi: 10.1590/0074-02760210330.
20. Barros LB, Calil PR, Rodrigues PT, Tonini J, Fontoura PS, Sato PM et al. Clinically silent Plasmodium vivax infections in native Amazonians of northwestern Brazil: acquired immunity or low parasite virulence? Mem Inst Oswaldo Cruz. 2022; 117: e220175. doi: 10.1590/0074-02760220175.
21. Moreno JE, Rubio-Palis Y, Páez E, Pérez E, Sánchez V, Vaccari E. Malaria entomological inoculation rates in gold mining areas of Southern Venezuela. Mem Inst Oswaldo Cruz. 2009; 104(5): 764-768. doi: 10.1590/s0074-02762009000500017.
22. Bio-Manguinhos. [Kit NAT PLUS HIV/HBV/HCV/Malária: instructions for use.] Available at: https://www.bio.fiocruz.br/index.php/br/produtos/reativos/testes-moleculares/nat-hivhcv-malaria-nat-plus. Accessed on 21/07/2025. [in Portuguese.]
23. Lacerda MVG, Monteiro WM, Alexandre MAA, Alho RRM, Kiesslich D, Fraiji NA. We need to talk more about transfusion-transmitted malaria in Plasmodium vivax endemic areas. Rev Bras Hematol Hemoter. 2014; 36(6): 385-387. doi: 10.1016/j.bjhh.2014.09.005.
24. Markus MB. Biological concepts in recurrent Plasmodium vivax malaria. Parasitology. 2018; 145(13): 1765-1771. doi: 10.1017/S003118201800032X.
25. Word Health Organization (WHO). Global Technical Strategy for Malaria 2016-2030. 2015. Available at: https://www.who.int/docs/default-source/documents/global-technical-strategy-for-malaria-2016-2030.pdf. Accessed on 09/09/2025.
26. Zucoloto ML, Gonçalez T, Custer B, McFarland W, Martinez EZ. Comparison of the demographic and social profile of blood donors and nondonors in Brazil. Health Soc Care Community. 2019; 27(2): 330-336. doi: 10.1111/hsc.12650.
27. Carneiro-Proietti AB, Sabino EC, Sampaio D, Proietti FA, Gonçalez TT, Oliveira CDL et al. Demographic profile of blood donors in Brazil: Results from the International REDS II Study, 2007-2008. Transfusion. 2009; 50(4): 918-925. doi: 10.1111/j.1537-2995.2009.02529.x.
28. Martins-Campos KM, Kuehn A, Almeida A, Duarte APM, Sampaio VS, Rodriguez ÍC et al. Infection of Anopheles aquasalis from symptomatic and asymptomatic Plasmodium vivax infections in Manaus, western Brazilian Amazon. Parasit Vectors. 2018; 11(1): 288. doi: 10.1186/s13071-018-2749-0.
29. Almeida GG, Costa PAC, Araujo M da S, Gomes GR, Carvalho AF, Figueiredo MM et al. Asymptomatic Plasmodium vivax malaria in the Brazilian Amazon: Submicroscopic parasitemic blood infects Nyssorhynchus darlingi. PLoS Negl Trop Dis. 2021; 15(10): e0009077. doi: 10.1371/journal.pntd.0009077. Erratum in: PLoS Negl Trop Dis. 2023; 17(6): e0011429. doi: 10.1371/journal.pntd.0011429.
30. Kojom Foko LP, Moun A, Singh V. Addressing low-density malaria infections in India and other endemic part of the world-the opportune time? Crit Rev Microbiol. 2025; 51(2): 229-245. doi: 10.1080/1040841X.2024.2339267.
31. Howes RE, Battle KE, Mendis KN, Smith DL, Cibulskis RE, Baird JK et al. Global epidemiology of Plasmodium vivax. Am J Trop Med Hyg. 2016; 95(6 Suppl): 15-34. doi: 10.4269/ajtmh.16-0141.
32. Barbosa LRA, da Silva EL, de Almeida ACG, Salazar YEAR, Siqueira AM, Alecrim MDGC et al. An ultra-sensitive technique: Using pv-mtcox1 qpcr to detect early recurrences of plasmodium vivax in patients in the brazilian amazon. Pathogens. 2020; 10(1): 19. doi: 10.3390/pathogens10010019.
33. FVS. Fundação de Vigilância em Saúde do Amazonas Dra. Rosemary Costa Pinto. [Epidemiological Panel - Malaria.] Available at: https://www.fvs.am.gov.br/indicadorSalaSituacao_view/124/2. Accessed on 13/10/2025. [in Portuguese.]
34. Tadei WP, Thatcher BD. Malaria vectors in the Brazilian Amazon: Anopheles of the subgenus Nyssorhynchus. Rev Inst Med Trop Sao Paulo. 2000; 42(2): 87-94. doi: 10.1590/s0036-46652000000200005.
35. Costa MRF, Vieira PPR, Ferreira CDO, De Lacerda MVG, Alecrim WD, Alecrim MDGC. [Molecular diagnosing of malaria in a tertiary care center in the Brazilian Amazon region.] Rev Soc Bras Med Trop. 2008; 41(4): 381-385. Portuguese. doi: 10.1590/s0037-86822008000400011. [in Portuguese.]
36. Torres KL, Figueiredo DV, Zalis MG, Daniel-Ribeiro CT, Alecrim W, De Fátima Ferreira-da-Cruz M. Standardization of a very specific and sensitive single PCR for detection of Plasmodium vivax in low parasitized individuals and its usefulness for screening blood donors. Parasitol Res. 2006; 98(6): 519-524. doi: 10.1007/s00436-005-0085-8.
37. Torres KL, dos Santos Moresco MN, Sales LR, da Silva Abranches J, Araújo Alexandre MA, Malheiro A. Transfusion-transmitted malaria in endemic zone: epidemiological profile of blood donors at the Fundação HEMOAM and use of rapid diagnostic tests for malaria screening in Manaus. Rev Bras Hematol Hemoter. 2014; 36(4): 269-274. doi: 10.1016/j.bjhh.2014.05.002.
38. Verra F, Angheben A, Martello E, Giorli G, Perandin F, Bisoffi Z. A systematic review of transfusion-transmitted malaria in non-endemic areas. Malar J. 2018; 17(1): 36. doi: 10.1186/s12936-018-2181-0.
39. Rivera Chavez GM, Barbosa AN, Pontes GS. Trends in unsuitability for blood donation in the Brazilian Amazon. Front Public Health. 2022; 10:1056332. doi: 10.3389/fpubh.2022.1056332.
40. Owusu-Ofori AK, Parry C, Bates I. Transfusion-transmitted malaria in countries where malaria is endemic: A review of the literature from sub-Saharan Africa. Clin Infect Dis. 2010; 51(10): 1192-1198. doi: 10.1086/656806.
41. Zhang MH, Xu S, Gu YP, Liu YB, Lin H, Xie CY et al. Classification of induced malaria case in an elimination setting: Investigation of transfusion-transmitted malaria cases. Malar J. 2020; 19(1): 136. doi: 10.1186/s12936-020-03203-x.
42. Wolfarth-Couto B, Filizola N, Durieux L. Seasonal pattern of malaria cases and the relationship with hydrologic variability in the Amazonas State, Brazil. Rev Bras Epidemiol. 2020; 23: e200018. Portuguese, English. doi: 10.1590/1980-549720200018.
43. Sampaio VS, Siqueira AM, Alecrim M das GC, Mourão MPG, Marchesini PB, Albuquerque BC et al. Malaria in the State of Amazonas: a typical Brazilian tropical disease influenced by waves of economic development. Rev Soc Bras Med Trop. 2015; 48 Suppl 1: 4-11. doi: 10.1590/0037-8682-0275-2014.
44. Ministério da Saúde. [Malaria treatment guide in Brazil - 2nd Edition 2021.] Available at: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/svsa/malaria/tratamento/guia_tratamento_malaria_2nov21_isbn_site.pdf/view. Accessed on 13/10/2025. [in Portuguese.]