Malaria: Current epidemiology, laboratory diagnosis, and challenges in the prevention of transfusion-transmitted malaria

Malária: Epidemiologia atual, diagnóstico laboratorial e desafios na prevenção da malária transfusional

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Abstract

Malaria remains one of the infectious diseases with the greatest global impact, especially in tropical countries, where it represents an important cause of morbidity and mortality in vulnerable populations. In 2024, the World Health Organization estimated approximately 282 million cases and more than 610,000 deaths, with about 94% of cases concentrated in the African Region. In Brazil, transmission is predominantly restricted to the Legal Amazon, with predominance of *Plasmodium vivax*, although *Plasmodium falciparum* is still associated with severe disease and fatal outcomes. In addition to classical vector-borne transmission, malaria may be transmitted through transfusion of blood components containing viable parasite forms, which represents a relevant risk to non-immune recipients and in situations involving submicroscopic parasitemia in asymptomatic donors. Laboratory diagnosis has evolved from exclusively microscopic methods to the incorporation of rapid immunochromatographic tests, high-sensitivity molecular techniques, and, more recently, nucleic acid amplification tests (NAT) specifically used for transfusion screening. In this narrative review, current epidemiological data are presented, the main diagnostic methodologies are discussed, and the challenges and perspectives for the prevention of transfusion-transmitted malaria in Brazil are analyzed, with emphasis on the importance of continuous surveillance and the adoption of more sensitive laboratory strategies.

Keywords: malaria; laboratory diagnosis; transfusion-transmitted malaria; NAT; transfusion safety.

Resumo

A malária permanece como uma das doenças infecciosas de maior impacto global, sobretudo em países tropicais, onde representa importante causa de morbidade e mortalidade em populações vulneráveis. Em 2024, a Organização Mundial da Saúde estimou cerca de 282 milhões de casos e mais de 610 mil óbitos, com aproximadamente 94% dos casos concentrados na região africana. No Brasil, a transmissão encontra-se predominantemente restrita à Amazônia Legal, com predomínio de *Plasmodium vivax*, embora o *Plasmodium falciparum* ainda esteja associado a quadros graves e desfechos fatais. Além da transmissão vetorial clássica, a malária pode ser transmitida por transfusão de hemocomponentes contendo formas parasitárias viáveis, o que constitui um risco relevante em receptores não imunes e em contextos de parasitemias submicroscópicas em doadores assintomáticos. O diagnóstico laboratorial evoluiu de métodos exclusivamente microscópicos para a incorporação de testes rápidos imunocromatográficos, técnicas moleculares de alta sensibilidade e, mais recentemente, ensaios de amplificação de ácidos nucleicos (NAT) específicos para triagem transfusional. Nesta revisão narrativa, são apresentados dados epidemiológicos atuais, discutidas as principais metodologias diagnósticas e analisados os desafios e perspectivas para a prevenção da malária transfusional no Brasil, com ênfase na importância da vigilância contínua e da adoção de estratégias laboratoriais mais sensíveis.

Palavras-chave: malária; diagnóstico laboratorial; malária transfusional; NAT; segurança transfusional.

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Received on 02/24/2026 | Approved on 02/26/2026 | DOI: 10.21877/2448-3877.202600290.en

INTRODUCTION

Malaria is an infectious disease caused by protozoa of the genus *Plasmodium*, transmitted mainly through the bite of infected female *Anopheles* mosquitoes. Despite the availability of effective therapies and successive control initiatives, the disease remains one of the major public health challenges in tropical and subtropical regions, particularly in contexts of social vulnerability, inadequate sanitary infrastructure, and limited access to health services.[1-3]

The most recent data from the World Health Organization (WHO) indicate that, in 2024, approximately 282 million malaria cases and more than 610,000 deaths were reported in 80 countries, with a strong concentration in sub-Saharan Africa.[1,4] In the same year, five countries — Nigeria (24.3%), Democratic Republic of the Congo (12.5%), Uganda (4.7%), Ethiopia (4.4%), and Mozambique (3.6%) — accounted for nearly half of all cases.[4] In the Americas, the prevalence of the disease is lower in relative terms but remains relevant in countries such as Brazil, Venezuela, Colombia, Peru, Haiti, and Bolivia.[5] In Brazil, most cases are concentrated in the Legal Amazon, with predominance of *P. vivax*, although *P. falciparum* is still responsible for part of the severe cases and for deaths. Environmental changes, human mobility, and the expansion of activities such as illegal mining have contributed to the persistence of transmission and to the risk of reintroduction in non-endemic areas.[6,7]

Traditionally, malaria transmission is associated with the vector cycle, in which the parasite alternates developmental stages in the mosquito and in the human host. However, other routes of transmission have gained importance, particularly in hospital settings and transfusion medicine. Among these, transfusion-transmitted malaria stands out, and occurs when blood components containing viable blood-stage parasites are transfused into susceptible recipients. In such cases, the absence of the pre-erythrocytic hepatic phase may shorten the incubation period and favor more severe clinical presentations, particularly in individuals without previous immunity.[8,9]

In this context, laboratory diagnosis plays a central role, both in the clinical management of suspected cases and in the prevention of transfusion-transmitted malaria through adequate screening of blood donors. The evolution of diagnostic techniques, from conventional microscopy to rapid tests, molecular methods, and nucleic acid amplification tests (NAT), has expanded the ability to detect low parasitemia and subclinical infections, but has also introduced new challenges related to cost, operational complexity, and the need for specialized infrastructure.[10,11]

This narrative review aims to integrate updated epidemiological data, critically describe the main available diagnostic strategies, and discuss current challenges and future perspectives for the prevention of transfusion-transmitted malaria in Brazil, with focus on the reality of transfusion services and clinical laboratories. The search was conducted in the PubMed and SciELO databases, as well as in national and international guidelines on malaria and blood transfusion, without date restrictions, using the descriptors, in Portuguese, English, and Spanish: malaria; laboratory diagnosis of malaria; transfusion-transmitted malaria; NAT for malaria. Articles containing data and information consistent with the objectives of this study were included.

EPIDEMIOLOGY

Global epidemiology

Malaria remains one of the leading causes of morbidity and mortality in low- and middle-income countries. In 2024, global estimates indicated 282 million cases and approximately 610,000 deaths attributed to malaria.[4] The distribution of the disease remains strongly concentrated in sub-Saharan African countries, which account for approximately 94% of cases and 95% of deaths, with notable impact on children under five years of age, who account for about 80% of deaths.[1,12]

Several factors contribute to the persistence of this high burden, including social determinants (poverty, inadequate housing, low educational level), environmental factors (climate favorable to the vector, deforestation, use of unsafe water), and structural factors (fragility of health systems, limited access to diagnosis and treatment). The emergence of insecticide resistance in vectors and antimalarial drug resistance in some regions also represents an additional threat to control programs.[13,14]

Epidemiology in the Americas

In the Region of the Americas, malaria incidence has shown a declining trend over the past decades, although with important variations among countries. Between 2000 and 2022, the incidence rate decreased from approximately 13.1 to 3.6 cases per 1,000 population at risk, while mortality declined from 0.7 to 0.2 deaths per 100,000 inhabitants. This reduction reflects the intensification of vector control measures, the incorporation of effective therapeutic regimens, and expanded access to diagnosis.[5]

Despite this overall downward trend, areas of intense transmission still persist in the Amazon Basin and in regions with longstanding difficulties in access to health services. In 2024, the Americas reported more than 537,000 malaria cases, representing a 6% increase compared with 2023 (505,000 cases). Bolivia, Peru, Colombia, Haiti, and Panama showed increased malaria transmission in 2024 due to factors related to internal migration, gold mining, climatic events such as El Niño, and limited access to health services in remote or conflict-affected areas. Indigenous peoples remain the most affected group, accounting for more than one third of reported cases and nearly 30% of deaths.[15]

Fifteen countries in the Region of the Americas still report areas with malaria transmission, with Brazil (30%), Colombia (24%), and Venezuela (19%) accounting for nearly three quarters of all cases. However, countries such as Suriname have shown progress toward elimination.[15]

Most transmission remains concentrated in the Amazon Basin and along the Pacific coast of Colombia (88%),[15] where *Plasmodium vivax* is the predominant species, followed by *P. falciparum*, while *P. malariae* and *P. ovale* are less frequent but may contribute to persistent infections and prolonged clinical manifestations, representing challenges for surveillance and elimination, especially in remote areas with limited access to health services.[4,16]

Epidemiology in Brazil

According to WHO data, the estimated number of malaria cases in Brazil in 2024 was 162,000.[4] In Brazil, malaria is a notifiable disease, and almost all autochthonous cases occur in the Legal Amazon (99%), encompassing the states of Acre, Amapá, Amazonas, part of Maranhão, part of Mato Grosso, Pará, Rondônia, Roraima, and Tocantins. In this region, *P. vivax* accounts for most notifications (approximately 84%), whereas *P. falciparum* (approximately 16%) is more frequently associated with severe disease and risk of death.[5,17]

Factors such as deforestation, expansion of agricultural frontiers, illegal mining, construction of large infrastructure projects, and seasonal migration of workers have contributed to the persistence of transmission hotspots. Population mobility between endemic and non-endemic areas increases the risk of imported cases in regions where the vector is present, creating a scenario of possible reintroduction of transmission.[6,7]

In parallel, previously exposed individuals migrating from endemic to non-endemic regions may maintain low and asymptomatic parasitemia for long periods, including potential blood donors. This epidemiological aspect is particularly relevant for the discussion of transfusion-transmitted malaria and justifies the need for more sensitive screening strategies in transfusion services.[18]

LABORATORY DIAGNOSIS

Microscopy

Light microscopy of peripheral blood, using stained thick and thin smears, remains the classical method for malaria diagnosis and is widely considered the reference standard in many clinical settings. The thick smear increases sensitivity, whereas the thin smear allows better species identification and morphological evaluation of parasitic forms.[19]

When performed by experienced microscopists, with careful examination of a large number of fields, microscopy may detect parasitemia in the range of 5 to 50 parasites/µL. However, under routine conditions, with limited time for examination and lower examiner experience, sensitivity decreases significantly, frequently exceeding detection thresholds of 200 parasites/µL. This limitation makes microscopy inadequate as a standalone method for screening asymptomatic donors, especially in endemic areas, where subclinical infections with low parasitemia are common.[20,21]

Microscopy plays an important role in parasite detection, species identification, stage differentiation, and estimation of parasite density, providing comprehensive data for clinical management. However, in the context of clinical research, the study by Dhorda et al. (2020) emphasizes the need for harmonization of microscopic methods through more rigorous standards to ensure consistency and reliability of results. Such standards require high specificity (≤2.5% false positives) and detailed procedures, including standardization of blood volume on the slide and examination of at least 200 high-power fields or 2,500 leukocytes before reporting a negative slide.[22]

The review by Coro et al. (2025) highlighted that microscopy presents implementation challenges in resource-limited settings due to the need for equipment, electricity, and trained microscopists, making it less compatible with the ASSURED criteria, a set of WHO specifications used to evaluate the suitability of diagnostic technologies, particularly for use in developing countries. However, despite these limitations, microscopy has significant potential for advancement in point-of-care applications through the incorporation of low-cost high-magnification lenses, automated image processing, and artificial intelligence, aiming to improve speed and reduce dependence on highly trained personnel.[23]

Rapid tests

Rapid diagnostic tests (RDTs) for malaria, generally based on immunochromatography, allow detection of specific *Plasmodium* antigens in peripheral blood samples, such as histidine-rich protein 2 (HRP2) and parasite lactate dehydrogenase (pLDH). These assays have the advantage of being simple, easy to perform, and not dependent on sophisticated laboratory infrastructure, which makes them particularly useful in remote or resource-limited settings.[11,24]

A systematic review and meta-analysis that evaluated the performance of malaria RDTs in sub-Saharan Africa demonstrated high overall diagnostic accuracy, with pooled sensitivity of 0.830 (95% CI: 0.752 to 0.887) and pooled specificity of 0.949 (95% CI: 0.913 to 0.971), reflected by a diagnostic odds ratio of 91.196. However, the study revealed significant variability in performance across the 48 included studies, with notable heterogeneity (bivariate I^2 of 0.733), highlighting the importance of region-specific diagnostic strategies. The main challenges identified included persistence of the HRP2 antigen after treatment, causing false-positive results, and genetic deletions in the *pfhrp2* and *pfhrp3* genes, which lead to false-negative results in HRP2-based tests. Additionally, current rapid tests show reduced sensitivity for detection of low-density parasitemia and asymptomatic infections, a critical limiting factor for malaria elimination goals. When post-treatment performance was compared, non-HRP2 RDTs, such as those detecting pan-pLDH, showed greater reliability and stability in sensitivity and specificity, in contrast to the variable specificity observed with HRP2-based RDTs.[25]

Despite their practicality, it is clear that RDT sensitivity depends on parasite density and may vary according to the manufacturer and antigenic target. Furthermore, deletions in the *pfhrp2* and *pfhrp3* genes, already described in several countries, may compromise the performance of tests based exclusively on HRP2, resulting in false-negative results, as previously described.[25-29]

A cross-sectional observational study analyzed 365 *P. falciparum* samples collected between 2016 and 2018 in the state of Roraima, Brazil, mostly originating from mining areas in Venezuela and Guyana, in order to characterize the prevalence of *pfhrp2* and *pfhrp3* gene deletions that affect HRP2-based RDTs. The main finding was the markedly low prevalence, only 1%, of parasites with confirmed deletion of the *pfhrp2* gene among isolates circulating in the Central-Northern Guiana Shield, contrasting with higher rates reported at the border with Peru. Although the rate of false-negative results in HRP2-based RDTs was 6% in this study, most discordant results (RDT−/PCR+) were attributed to low parasitemia. Consequently, as the prevalence of *pfhrp2* deletion is below the 5% threshold established by WHO for replacement of RDTs, the study concluded that there is no recommendation to change HRP2-based tests in the investigated area, while emphasizing the need for continuous surveillance due to intense cross-border movement.[30]

These findings reinforce the importance of continuous molecular surveillance to monitor the occurrence of these deletions in different regions, as well as the need, whenever possible, to combine RDTs with other diagnostic methods, especially in critical settings.[31]

Molecular tests

Molecular tests represent the most sensitive group of diagnostic methods for *Plasmodium* detection, allowing identification of very low parasitemia, often undetectable by microscopy or RDTs. Among the available approaches are conventional PCR (polymerase chain reaction), nested PCR, quantitative real-time PCR (qPCR), and isothermal amplification techniques, such as loop-mediated isothermal amplification (LAMP).[32,33]

Assays based on amplification of conserved regions of the 18S rRNA gene have been widely used for detection and species differentiation of *Plasmodium* species due to the presence of multiple copies of this gene in the parasite genome (5 to 8 copies per genome). In comparative studies, qPCR has demonstrated superior performance compared with microscopy and RDTs in detecting asymptomatic infections, revealing a large number of submicroscopic carriers in endemic areas.[32]

LAMP techniques generally show slightly lower sensitivity than qPCR but have the advantage of not requiring thermocyclers and can be performed with simpler infrastructure, making them attractive for field use. On the other hand, standardization across different platforms remains a challenge, and cost may represent a barrier to large-scale use.[33,34]

A field evaluation conducted by Viana et al. (2018) in Cruzeiro do Sul, Acre, Brazil, aimed to determine the performance of the RealAmp molecular assay (real-time LAMP), a LAMP-based tool, for malaria diagnosis in a remote area with limited laboratory infrastructure. The cross-sectional study included 1,000 patients with fever or history of fever, using a combination of real-time PCR and nested PCR as the reference test. RealAmp was performed by personnel

without previous experience in molecular techniques, using a simplified DNA extraction protocol based on thermal treatment (boil-and-spin). Compared with the reference test, RealAmp showed high sensitivity, 94.1% (95% CI: 90.1 to 96.8), and specificity of 83.9% (95% CI: 81.1 to 86.4) in the field laboratory. The findings demonstrated that RealAmp performance was comparable to microscopy performed by experienced professionals (sensitivity of 96.4%), supporting the feasibility of implementing simplified molecular tests in resource-limited settings. However, the study showed limitations in detecting mixed infections with *P. falciparum* and *P. vivax*, since neither RealAmp nor microscopy identified 20 cases confirmed by the reference test.[35]

Ultrasensitive molecular methods allow detection of DNA or RNA with detection limits as low as 0.1 parasite/µL or lower. Although techniques such as ultrasensitive qPCR require highly equipped laboratories, alternative approaches such as LAMP offer more portable solutions while maintaining high analytical sensitivity. Implementation of these ultrasensitive assays is most relevant in three contexts: detection of asymptomatic infections associated with low parasitemia, diagnosis of malaria in pregnancy, and strategic use in malaria elimination campaigns. Low-density infections, which are missed by conventional methods, account for 30% to 50% of ongoing transmission and contribute to unfavorable long-term clinical outcomes, such as chronic anemia and cognitive impairment in children. Although these molecular methods may not be feasible in primary care settings in low- and middle-income countries, there is consensus that implementation policies should be adapted to the local epidemiological context, particularly targeting pregnant women, asymptomatic carriers, and populations living in areas approaching malaria elimination.[36]

Molecular tools, including nucleic acid tests (NAT), allow sensitive and specific detection of these cases, in addition to enabling characterization of *Plasmodium* genetic diversity, drug-resistance markers, and transmission patterns, information essential for surveillance, targeted interventions, and evaluation of control programs. Systematic incorporation of these methods may transform epidemiological surveillance, guide focused interventions, and accelerate elimination in low-transmission settings, although implementation barriers in endemic environments still need to be overcome.[37]

In transfusion medicine, implementation of NAT for malaria, similarly to what already occurs for HIV (human immunodeficiency virus), HCV (hepatitis C virus), and HBV (hepatitis B virus), represents an important step toward reducing transfusion risk. These tests, when performed in pools of six samples followed by individual testing of reactive donors, allow identification of subclinical infections with very low parasitemia, significantly increasing transfusion safety.[38]

A retrospective observational evaluation conducted at the Amazonas Hematology and Hemotherapy Foundation (HEMOAM), in Manaus, Brazil (the main endemic area in the country), aimed to describe positivity rates and the epidemiological profile of individual blood donors (N = 67,114) submitted to molecular screening for *Plasmodium* spp. using the NAT PLUS HIV/HBV/HCV/Malaria assay between January 2023 and June 2024. The main outcome was the low frequency of malaria positivity (0.01%; 8/67,114), with the particularly relevant finding that all reactive donors were asymptomatic at the time of clinical screening.

The predominant epidemiological profile among positive donors included male individuals (75%), mixed-race individuals (87.5%), and residents of Manaus, reflecting a profile consistent with the local donor population. The study reinforces the importance of molecular screening by demonstrating that one of the positive donors later developed symptomatic *P. falciparum* malaria, subsequently confirmed by blood smear and reported shortly after donation. This finding highlights the ability of NAT PLUS HIV/HBV/HCV/Malaria to detect low-density asymptomatic infections that represent a potential risk to transfusion safety.[39]

Serological tests

Serological tests for malaria, such as indirect immunofluorescence and ELISA (enzyme-linked immunosorbent assay), detect antibodies against *Plasmodium* antigens, reflecting previous exposure to infection. Although useful in epidemiological and surveillance studies, conventional serology does not allow distinction between active and past infections, since antibodies may persist for months or years after clinical resolution.[40,41] Because of this limitation, serological tests are not recommended as the primary diagnostic method in acute cases nor as a standalone tool for donor screening in endemic areas, where previous exposure is common. Their use tends to be more appropriate in non-endemic areas, in combination with detailed epidemiological interviews, or in investigations of exposure in travelers and specific populations.[42]

A study conducted in Brazil aimed to establish appropriate screening criteria for blood donors in Brazilian regions with different epidemiological profiles for malaria,

including areas without vector transmission (São Paulo, SP), areas with low active transmission (Belém, PA), and areas with high active transmission (Matupá and Peixoto de Azevedo, MT). The adopted strategy combined clinical-epidemiological screening with direct laboratory methods for detection of *Plasmodium* or parasitic antigens — thick smear, QBC Test®, and immunofluorescence — in addition to serology by indirect immunofluorescence (IFA-IgG anti-*Plasmodium vivax* and *Plasmodium falciparum*) for detection of antiplasmodial antibodies. All direct methods for parasite or antigen detection were negative in all analyzed samples. In contrast, serology showed wide variation in positivity among regions, reaching 1.98% in eligible donors in São Paulo, 17.2% in Belém, and 80.6% in Matupá/Peixoto de Azevedo. The significant difference in seropositivity between eligible and deferred donors in São Paulo and Belém ($p < 0.01$) demonstrated the effectiveness of clinical-epidemiological screening for exclusion of individuals at risk in areas with low or absent transmission. Based on these findings, the authors concluded that donor screening should be guided by clinical-epidemiological criteria adjusted to the epidemiological reality of each region and that, from a laboratory perspective, the most appropriate methods are those aimed at direct detection of *Plasmodium* or parasitic antigens. In areas of high endemicity, serology is poorly informative due to high positivity rates, making the inclusion of direct tests such as thick smear and/or the QBC Test® essential tools for malaria screening in blood banks.[43]

A systematic review analyzed malaria seroprevalence in the Brazilian Amazon. The qualitative synthesis of 23 studies identified IgG antibodies directed against the circumsporozoite protein (CSP) of *P. falciparum* and *P. vivax*, as well as against the 19-kDa C-terminal region of the *P. vivax* merozoite surface protein 1 (PvMSP1-19), as potential biomarkers of malaria exposure in areas with variable and unstable transmission. However, the review demonstrated considerable heterogeneity regarding the evaluated antigens and lack of standardization of serological assays, concluding that harmonization of target antigens and analytical methods is an essential step for effective incorporation of seroprevalence as a surveillance tool in the Brazilian Malaria Control Program.[44]

Helb et al. (2015) evaluated antibody responses to 856 *P. falciparum* antigens using a protein microarray in 186 Ugandan children with well-characterized exposure history. Using adaptive statistical methods, the main finding was the identification of a limited set of biomarkers in which responses to only three novel *P. falciparum* antigens were able to accurately classify whether an individual had been infected within the previous 30, 90, or 365 days, with area under the curve (AUC) ranging from 0.86 to 0.93. In addition, measurement of responses to six antigens allowed accurate estimation of individual malaria incidence in the previous year. This new method demonstrates the potential to generate precise quantitative estimates of *P. falciparum* exposure at individual and community levels from cross-sectional samples, overcoming the limitations and lower resolution of traditional serological assays based on a small number of conventional antigens.[41]

Another study evaluated the feasibility of using a multiplex serological assay for serosurveillance of malaria exposure in U.S. military personnel deployed to endemic regions. The methodology involved simultaneous measurement of antibody responses against *Anopheles* vector antigens (such as gSG6P1 and gSG6P2, indicative of mosquito bites) and *P. falciparum* parasite antigens, including sporozoite-stage proteins (such as CSP) and blood-stage proteins (such as MSP-1). The results demonstrated the feasibility of using an electrochemiluminescence multiplex platform in samples collected after deployment to African countries. Among military personnel routinely receiving chemoprophylaxis, some individuals showed anti-CSP antibody levels comparable to those observed in populations living in endemic areas, suggesting exposure to sporozoites through bites from infected mosquitoes despite effective prophylaxis. In addition, isolated cases of high anti-MSP-1 antibody levels suggested possible symptomatic or subclinical infections. Overall, these findings demonstrate that multiplex serosurveillance may act as a complementary tool to passive surveillance by allowing assessment of malaria risk and exposure, as well as providing information to evaluate the effectiveness of chemoprophylaxis in preventing blood-stage infection.[45]

Table 1 summarizes a comparative overview of malaria diagnostic methods.

Table 1

Comparison of diagnostic methods for malaria.

Method	Estimated sensitivity	Advantages	Limitations
Microscopy	5–50 parasites/µL; routine detection >200 parasites/µL	Classical reference standard; low cost; widely available	Low sensitivity in asymptomatic donors; operator-dependent
Rapid diagnostic tests (RDT)	100–200 parasites/µL; sensitivity 70–100%	Rapid; does not require laboratory infrastructure	False negatives due to *pfhrp2/pfhrp3* deletions; performance variability
Conventional PCR / Nested PCR	0.1 to 1 parasite/µL	High sensitivity	High cost; requires laboratory infrastructure
qPCR	<0.1 parasite/µL	Higher sensitivity; allows quantification	High cost
LAMP	2 to 8 parasites/µL	No thermocycler required; suitable for field use; higher sensitivity than microscopy	Standardization issues; cost; lower sensitivity than qPCR
Serology	Antibody detection	Useful for surveillance / epidemiological studies	Does not distinguish active from past infection
NAT	<0.1 parasite/µL	High level of transfusion safety	Cost; laboratory infrastructure required

Legend: PCR: polymerase chain reaction; qPCR: real-time polymerase chain reaction; LAMP: loop-mediated isothermal amplification; NAT: nucleic acid amplification test.

Source: Adapted from[22,23,32-35,40,42].

TRANSFUSION-TRANSMITTED MALARIA

Transfusion-transmitted malaria occurs when blood components containing viable blood-stage parasites of *Plasmodium* are transfused into susceptible recipients. The blood components most frequently implicated in transfusion-transmitted malaria are whole blood, red blood cell concentrates, and platelet concentrates, due to the presence of residual erythrocytes. Fresh frozen plasma, because it contains few erythrocytes, is rarely associated with transmission.[38,46,47]

Unlike vector-borne transmission, transfusion-transmitted infection does not involve the pre-erythrocytic hepatic phase, during which innate immune responses are activated to protect the host. Parasites are introduced directly into the bloodstream, which may shorten the incubation period and favor more abrupt and severe clinical presentations, especially in individuals without previous immunity.[8,46] These conditions reinforce the need for diagnostic methods that are more sensitive than conventional microscopy and rapid tests.[48]

Transfusion-transmitted malaria remains a critical challenge for transfusion services worldwide, particularly in countries with active circulation of *Plasmodium* or intense population mobility between endemic and non-endemic areas. Factors such as partial immunity, prolonged parasite persistence, low parasite density, and a high proportion of asymptomatic donors directly contribute to the risk of transfusion transmission.[49,50] Historically, countries have adopted different approaches to mitigate the risk of transfusion-transmitted malaria, combining standardized interviews, temporary deferral criteria based on travel or residence in endemic areas, microscopy, and, in some cases, serological testing. However, these strategies have clear limitations in settings with high endemicity or high population mobility.[8,51]

Table 2 presents a comparative summary of the main strategies adopted for transfusion-transmitted malaria screening in different regions of the world, including laboratory methods, regulatory approaches, limitations, and recent evidence, with emphasis on Brazil.

Donors originating from endemic areas, with repeated prior exposure, may develop partial immunity and maintain very low parasitemia, often asymptomatic and submicroscopic. Nevertheless, these parasitemias are sufficient to cause infection in non-immune recipients. Classical studies have shown that infusion of approximately 10 *P. vivax* parasites may be sufficient to establish infection. In practical terms, this implies that a donor with parasitemia of 1 to 2 parasites/µL may contain hundreds of thousands of parasites in a single unit of blood.[49,56]

Another relevant aspect is the ability of the parasite to persist. *P. malariae* is known to maintain long-lasting infections, remaining in the host for decades. *P. vivax* and *P. ovale* may persist for more than three years, whereas *P. falciparum* may also be detected for prolonged periods in some individuals. This long persistence means that donors who left endemic areas many years earlier may still represent a transfusion risk.[56-59].

Table 3 highlights the main risk factors for transfusion-transmitted malaria.

Table 2

Screening strategies for prevention of transfusion-transmitted malaria in different regions of the world.

Region / Country	Screening strategy	Laboratory methods	Regulatory basis	Main limitations	Notes / Evidence
Sub-Saharan Africa	Clinical and epidemiological screening associated with microscopy/RDT or absent	Microscopy, RDT; PCR in research settings	National guidelines + WHO	High endemicity; asymptomatic carriers; *pfhrp2/3* deletions	Significant residual risk even after conventional screening procedures [a,b]
Western Europe	Temporary deferral based on travel or residence in endemic areas	Epidemiological interview; serology; PCR/NAT in specialized centers	National policies and ECDC	Reduction of donor pool; imported cases	Detection of asymptomatic donors only by PCR in the United Kingdom has been reported [c]
United States	Temporary deferral based on epidemiological history	Epidemiological interview; PCR/NAT in specific protocols	CDC / FDA	Excessive dependence on interview; residual risk	Documented cases of transfusion-transmitted malaria in asymptomatic donors [d]
Asia / Southeast Asia	Combined screening with expansion of molecular methods	Microscopy, RDT, PCR	Variable national policies	High population mobility; epidemiological heterogeneity	Increasing use of PCR in elimination settings [e]
Brazil	Integrated screening with mandatory NAT	Epidemiological interview + NAT PLUS HIV/HBV/HCV/Malaria kit testing	Ministry of Health Technical Note No. 49/2023	Cost, infrastructure, and training requirements	National regulatory milestone aligned with international best practices [f,g]

Legend: RDT: rapid diagnostic test; PCR: polymerase chain reaction; NAT: nucleic acid amplification test; WHO: World Health Organization; ECDC: European Centre for Disease Prevention and Control; CDC: Centers for Disease Control and Prevention; FDA: Food and Drug Administration; NAT PLUS HIV/HBV/HCV/Malaria: Bio-Manguinhos (Fiocruz) diagnostic kit. Notes: [a]African studies demonstrate residual risk of transfusion-transmitted malaria even with microscopy and RDT. [b]The World Health Organization recommends progressive molecular surveillance in regions with high endemicity. [c]Studies in the United Kingdom identified *Plasmodium* DNA in asymptomatic donors with negative conventional tests. [d]Historical cases in the United States demonstrate limitations of screening based only on interview. [e]Asian countries in the elimination phase have expanded the use of PCR for surveillance. [f]Implementation of the NAT PLUS kit in Brazil represents a regulatory advance. g National studies demonstrate molecular positivity in donors from endemic and non-endemic areas.

Source: Adapted from [38,39,48,52–55].

Table 3

Main risk factors for transfusion-transmitted malaria.

Factor	Characteristic	Estimated probability	Associated severity	Detectable by	References
Asymptomatic donors	Submicroscopic carriers who may remain with low parasitemia for months to years	High	Moderate to high, depending on species and recipient	PCR, qPCR, NAT	(46,49)
Prolonged persistence	Long-lasting infections due to species-specific biology (e.g., hypnozoites, low parasitic density)	Moderate	Moderate; prolonged risk for transfusion services	PCR, NAT	(46,59)
High infectivity	Minimal inoculum sufficient to cause infection even at parasitemia <1 parasite/μL	Very high	High in non-immune recipients	qPCR, NAT	(8,51)
Species involved	Species with longer persistence or specific tropism increase the risk of transfusion transmission	Variable	High for *P. falciparum*; prolonged risk for *P. malariae*	Microscopy, PCR, NAT	(46)
Vulnerable recipients	Non-immune individuals, pregnant women, older adults, immunosuppressed patients, and critically ill patients	High	Very high; higher documented lethality	All diagnostic methods	(38,46)

Legend: PCR: polymerase chain reaction; qPCR: real-time polymerase chain reaction; NAT: nucleic acid amplification test.

The most vulnerable recipients include children, pregnant women, older adults, immunosuppressed individuals, and patients with severe comorbidities, in whom a malaria episode may trigger significant clinical decompensation. Evidence shows that severe cases of transfusion-transmitted malaria occur predominantly in recipients without previous immunity, with lethality ranging from 3% to 12%.[50,51]

A systematic review of transfusion-transmitted malaria in the Americas identified a total of 422 reported cases between 1971 and 2016, of which approximately 40.3% occurred in the United States of America. *P. malariae* was responsible for most cases, and the observed lethality was 5.3%.[60]

Given this scenario, transfusion-transmitted malaria should be regarded as a potentially severe but preventable event, provided that appropriate donor selection strategies and sensitive diagnostic methods are adopted in transfusion services.[38]

DONOR SCREENING AND IMPLEMENTATION OF NAT IN BRAZIL

Transfusion-transmitted malaria represents a significant risk for transfusion services worldwide due to the possibility of asymptomatic infections in donors. Blood donor screening to prevent transfusion-transmitted malaria involves a combination of clinical, epidemiological, and laboratory evaluation. In endemic areas, where exposure to malaria is frequent, simple deferral of donors based on recent fever or travel history may not be sufficient, since many individuals present asymptomatic infections with low parasite density.[51]

Traditionally, screening in blood banks, including in Brazil, has been based on microscopy, despite its limitations in detecting low-density parasitemia, or on geographic risk assessment of donors. However, the need for a highly sensitive screening tool for prevention of transfusion-transmitted malaria in endemic areas of Brazil, such as the Amazon region, led to the validation of molecular methods. In 2020, the study by Rocha et al. evaluated the inclusion of a NAT assay targeting the *Plasmodium* spp. 18S rRNA gene in the Brazilian NAT platform already mandatory for screening of HIV, HCV, and HBV. Implementation of this NAT HIV/HCV/HBV/malaria prototype proved effective in detecting infected donation candidates, revealing a prevalence of 0.06% in a pilot study in Manaus (HEMOAM). These findings demonstrated the potential of the assay to reduce the risk of transfusion-transmitted malaria by discarding reactive units, significantly contributing to improved transfusion safety.[48]

Table 4 summarizes the main strengths and limitations of NAT for malaria in blood donor screening, considering technical, operational, and epidemiological aspects, based on recent scientific evidence and international guidelines.

Table 4

Strengths and limitations of NAT for malaria in transfusion screening.

Evaluated dimension	Strengths of NAT	Limitations of NAT	Implications for transfusion services
Diagnostic sensitivity	High sensitivity for detecting very low parasitemia and submicroscopic infections, including in asymptomatic donors.	Possible false-negative results at extremely low parasitemia below the analytical limit.	Marked reduction of transfusion risk compared with microscopy and RDT [a,b]
Specificity	High specificity when primers/probes are properly designed and validated	Theoretical risk of cross-contamination if good laboratory practices are not strictly followed in the laboratory	Need for continuous quality control and validation [c]
Species coverage	Ability to detect multiple *Plasmodium* species in multiplex assays	Dependence on assay design for detection of rare species	Important in areas with circulation of multiple species [d]
Applicability in asymptomatic donors	Identifies asymptomatic and subclinical carriers not detected by conventional methods	Does not differentiate viable infection from residual parasite DNA in rare cases	Improves safety in both endemic and non-endemic areas [e]
Operational aspects	Possibility of pooled testing, optimizing costs and turnaround time	Requires technological infrastructure and trained personnel	Requires initial investment but cost-effective in the medium term [f]
Integration with other methods	Complements epidemiological interview, microscopy, and RDT	Does not completely replace clinical and epidemiological evaluation	A layered approach increases the robustness of the screening system [g]

Legend: NAT — nucleic acid amplification test; RDT — rapid diagnostic test. Notes: [a] NAT detects submicroscopic parasitemia associated with transfusion risk. [b] Studies demonstrate sensitivity greater than 95% in validated commercial assays. [c] Quality assurance programs are essential to prevent contamination. [d] Multiplex assays expand coverage of *Plasmodium* species. [e] Asymptomatic carriers represent an important source of transfusion risk. [f] Economic evaluations indicate cost-effectiveness of NAT in endemic areas. g Integrated strategies are recommended by international guidelines.

Source: Adapted from [8,32,38,39,46,48–50,53,61].

The study by Costa et al. (2024) evaluated the performance and applicability of the Brazilian NAT PLUS HIV/HBV/HCV/Malaria kit (Bio-Manguinhos) for detection of *Plasmodium* spp. in asymptomatic blood donors, reinforcing its strategic role for transfusion safety in Brazil. In an endemic setting, the authors demonstrated that inclusion of molecular screening for malaria in a NAT platform already established in routine testing allowed the identification of subclinical infections with low parasitemia that would not be detected by conventional clinical screening. The results showed high analytical sensitivity of the assay for *P. falciparum* and *P. vivax*, confirming its ability to prevent potential transfusion-transmitted malaria events through discard of reactive blood components. The study also highlights the operational feasibility of implementing malaria NAT in the routine of Brazilian blood centers, since the test is integrated into a system already used nationwide, providing a technically robust, scalable solution aligned with surveillance needs in endemic areas and in regions approaching malaria elimination.[53]

The Technical Note No. 49/2023, published in Brazil by the Ministry of Health, represented an important milestone in the approach to transfusion-transmitted malaria by establishing the use of NAT PLUS HIV/HBV/HCV/Malaria for donor screening.[54] In practice, screening is performed in pooled plasma samples, and when a reactive result is obtained, individual testing of the donors included in the pool is carried out. Donors with positive results must have all blood components blocked, be called for counseling, referred to a specialized service, undergo diagnostic confirmation, and receive appropriate treatment. In addition, lookback procedures are recommended for recipients who received red blood cells, platelets, or granulocytes from previous donations by the same donor within a defined time window, in order to investigate possible transmission.[39,48,54]

The adoption of NAT for malaria places the Brazilian transfusion system in line with transfusion safety practices of countries that already use molecular testing for other agents, such as HIV, HBV, and HCV. Although challenges related to cost, technological infrastructure, and staff training exist, the benefits in terms of reduction of transfusion risk are substantial, especially in a country with active endemic areas.[54]

Prevention of transfusion-transmitted malaria requires an integrated approach combining epidemiological interview, sensitive diagnostic methods, and continuous molecular surveillance. In the medium and long term, it is plausible that the combination of NAT with improved epidemiological screening strategies and, when appropriate, with other diagnostic methods will result in a more robust screening system, further reducing the probability of transfusion-transmitted malaria.[50,51,62]

FINAL CONSIDERATIONS

Malaria remains a relevant public health problem at global and regional levels, despite therapeutic advances and the implementation of control strategies. In addition to vector-borne transmission, the possibility of transmission through blood transfusion poses additional challenges, especially in contexts characterized by endemicity and high population mobility.[1,4]

Laboratory diagnosis plays a central role both in clinical management and in prevention of transfusion-transmitted malaria. Microscopy remains fundamental in clinical practice, but it is not sufficient for donor screening in endemic areas due to its inability to consistently detect very low parasitemia. Rapid diagnostic tests provide speed and ease of use, but their performance is affected by intrinsic variability and by deletions in target genes such as *pfhrp2* and *pfhrp3*. In this scenario, molecular methods, particularly qPCR and NAT, emerge as strategic tools for identifying submicroscopic infections, providing a higher level of transfusion safety.[11,24,25,63]

The implementation of NAT for malaria in routine donor screening in Brazil represents an important advance in transfusion safety, aligning the country with international best practices and addressing the epidemiological particularities of the Amazon region. Nevertheless, the effectiveness of this strategy depends on maintaining active surveillance systems, adequate training of laboratory and transfusion personnel, and continuous investment in technological infrastructure.[54]

It is recommended that each country establish national-level logistics to properly implement molecular screening for malaria, preferably integrated with NAT testing already used in blood banks. At the same time, it is essential that laboratories responsible for screening adopt internal and external quality control measures for molecular malaria testing.

The prevention of transfusion-transmitted malaria, therefore, does not rely on the choice of a single test, but requires an integrated approach involving coherent public health policies, robust epidemiological surveillance, sensitive and accessible laboratory diagnosis, and continuous education of health professionals.[8,32,50] As new technologies emerge, prevention strategies can be further refined, ensuring greater protection for blood recipients and contributing effectively to reduction of the impact of malaria in the population.

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