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Evaluating Diagnostic Methods for Transfusion-Transmitted Malaria: RDT vs. Microscopy in Blood Donation Screening

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Abstract:

Background: Transfusion-transmitted malaria (TTM) remains a significant public health concern in malaria-endemic regions such as Ghana. The persistence of *Plasmodium falciparum* in donated blood, often undetected, poses a risk to transfusion recipients. This study evaluates the diagnostic accuracy of Rapid Diagnostic Tests (RDTs) and microscopy in detecting *P. falciparum* in donor blood.

Aim: To compare the diagnostic performance of RDTs and microscopy in detecting *P. falciparum* in stored blood samples at SDA Hospital, Accra.

Methods: A cross-sectional study was conducted on 115 blood samples donated to SDA Hospital. The samples were screened using First Response RDT kits and Giemsa-stained microscopy (gold standard). Sensitivity, specificity, and parasite density were analyzed using SPSS version 26.

Results: Of the 115 samples, microscopy detected *P. falciparum* in 8.7% of the cases, while RDTs detected 6.1%. Microscopy showed 100% sensitivity and specificity, while RDTs demonstrated 100% sensitivity and 99.1% specificity. Blood group O positive was most frequently associated with parasitemia. All infected donors were male, with parasite densities ranging from 600 to 1200 parasites/µL.

Conclusion: Both RDTs and microscopy showed high diagnostic performance, but microscopy detected more positive cases. Given the residual risk of TTM, integrating both diagnostic methods in screening protocols is recommended to improve transfusion safety in malaria-endemic regions.

Keywords: *Plasmodium falciparum, Malaria Diagnosis, Rapid Diagnostic Tests (RDTs), Microscopy, Blood Donor Screening.*

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Introduction

According to the latest data from the World Health Organization (WHO), in 2023, there were an estimated 263 million cases of malaria and 597,000 deaths worldwide (WHO, 2023). The WHO African Region continues to bear the brunt of the global malaria burden, accounting for 94% of malaria cases (246 million) and 95% of malaria deaths (569,000) in 2023 (World Health Organization, 2024). Malaria remains one of the most pressing global health concerns, particularly in sub-Saharan Africa, where Plasmodium falciparum accounts for the majority of malaria-related morbidity and mortality (Kolawole *et al*., 2023).

Transfusion-transmitted malaria (TTM) is an unintended yet serious consequence of blood transfusion when blood donors harbor undetected *Plasmodium* parasites. TTM can lead to severe complications in recipients, including fever, chills, anemia, jaundice, and organ failure, particularly in immunocompromised individuals. Severe cases may progress to cerebral malaria, respiratory distress, or death (Bansal *et al.*, 2024). This mode of transmission is especially concerning in malaria-endemic regions, where a significant proportion of the population may carry asymptomatic infections (Niederhauser & Galel, 2022). In non-endemic countries, blood donor screening policies have been established to mitigate the risks associated with TTM. However, in endemic regions, the implementation of effective diagnostic strategies for donor screening remains an ongoing challenge (O'Brien *et al*., 2019). The accurate detection of *Plasmodium falciparum* in blood donors is therefore crucial to preventing the transmission of malaria through transfusions and ensuring blood safety.

Historically, the gold standard for malaria diagnosis has been microscopy using Giemsa-stained thick and thin blood films. This method allows for both parasite identification and quantification, making it highly informative for malaria diagnosis (Kotepui *et al*., 2020). However, microscopy has several limitations, including the requirement for highly trained personnel, time-consuming procedures, and variability in sensitivity based on the skill of the microscopist (Hamid *et al*., 2024). Microscopy's sensitivity typically ranges between 5 and 100 parasites per microliter (µl) of blood, meaning that low-density infections may go undetected. Given these limitations, alternative diagnostic

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approaches have been explored to enhance the reliability and efficiency of malaria screening (WHO, 2023; Opoku Afriyie *et al*., 2023).

Rapid diagnostic tests (RDTs) have emerged as a popular alternative to microscopy, particularly in resource-limited settings where laboratory infrastructure may be lacking. RDTs are immunochromatographic assays designed to detect malaria antigens, such as histidine-rich protein 2 (PfHRP2), Plasmodium lactate dehydrogenase (pLDH), and aldolase (Zeleke *et al*., 2023 ; Mortazavi *et al*., 2025). These tests offer several advantages, including ease of use, rapid turnaround time, and minimal technical expertise requirements. However, their performance varies depending on the manufacturer, the target antigen, and the prevalence of antigen deletions within specific populations. Despite these advantages, RDTs also present challenges, such as reduced specificity at low parasite densities and the potential for false-negative results due to PfHRP2 deletions (Poti *et al*., 2020).

Beyond RDTs and microscopy, molecular diagnostic techniques, such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), have demonstrated superior sensitivity in detecting Plasmodium parasites, particularly in cases of low parasitemia (Slater *et al*., 2022).

Blood transfusion is an essential medical intervention, often required in cases of severe anemia, surgical procedures, trauma, and pregnancy complications. However, in malaria-endemic areas, the risk of transfusion-transmitted infections, including malaria, necessitates the need for stringent donor screening protocols (Ahmadpour *et al*., 2019). Studies conducted in Nigeria have reported varying malaria prevalence rates among blood donors, with microscopy-based detection yielding higher prevalence rates than RDTs (Adebusuyi *et al*., 2024). A 2024 study comparing RDTs, microscopy, and PCR found 21% positivity by microscopy vs. 8.9% by RDT among blood donors, with microscopy demonstrating superior sensitivity (65.3% vs. 51.4%) and specificity (98.2% vs. 69.5%) (Adebusuyi *et al*., 2024). This discrepancy highlights the need for comparative studies to assess the sensitivity and specificity of different diagnostic methods in donor screening.

The current study seeks to compare the effectiveness of rapid diagnostic tests against the gold standard microscopic examination in detecting *Plasmodium falciparum* infections in stored blood at SDA Hospital. Given the public health implications of transfusion-transmitted malaria, it is essential to determine the reliability of both RDTs and microscopy in detecting malaria infections among donors. This study will provide valuable insights into the strengths and limitations of both microscopy and RDTs in donor screening and inform policies regarding the most appropriate diagnostic approach to minimize the risk of transfusion-transmitted malaria.

Material and Method

Study design

This study employed a descriptive cross-sectional laboratory-based design, conducted at the transfusion laboratory of SDA Hospital in Accra, Ghana, from March to April 2025. The study aimed to evaluate the diagnostic accuracy of Rapid Diagnostic Tests (RDTs) in comparison with the gold standard microscopy for detecting *Plasmodium falciparum* in stored blood samples intended for transfusion.

Study site

The study was carried out at the blood bank of the Seventh-Day Adventist (SDA) Hospital located at Gbawe CP in the Weija–Gbawe Municipality of the Greater Accra Region, Ghana. The hospital serves Gbawe and surrounding communities by providing comprehensive healthcare services.

SDA Hospital is a secondary healthcare facility offering both outpatient and inpatient care. It comprises several departments, including the Outpatient Department (OPD) for routine consultations and follow-up services, inpatient wards for male, female, and pediatric patients, an Emergency Unit for critical and urgent cases, an operating theatre for surgical procedures, an Antenatal Clinic (ANC) for maternal healthcare services, and a medical laboratory responsible for diagnostic investigations, including blood screening.

The hospital records an average daily outpatient attendance of 80–150 patients, with malaria, respiratory tract infections, gastrointestinal infections, hypertension, and diabetes being the most common conditions treated. The ANC and maternity units receive consistent patronage from pregnant women within Gbawe and nearby communities. Due to its location near a major highway, the Emergency Unit frequently manages road traffic accident cases alongside other acute medical and surgical emergencies.

Study population

The target population for this study comprised donor blood units collected at the SDA Hospital for transfusion purposes. A total of 115 donor blood units constituted the study population.

Sample Size Determination

One hundred and fifteen (115) blood samples was used for the study, although the minimum sample size was determined to be 113.09 using the Cochran formula below:

$$N = \frac{Z^2(P)(1-P)}{D^2}$$

Where:

N = Sample size,

Z = A constant of 1.96,

E/D = Error of 5% (0.05),

P = Previous prevalence rate 8.0% (Adusei & Owusu-Ofori, 2018).

$n = z^2p(1-p)/d^2$

$n = (1.96)^2 \times 0.080 (1-0.080) / (0.05)^2$

$n = 3.8416 (0.080) \times (1-0.080) / 0.0025$

$n = 3.8416 (0.080) \times (0.920) / 0.0025$

$n = 0.2827 / 0.0025$

$n = 113.09$

However, the total number of blood samples was increased to 115 to maintain the proportionality of the sample size.

Inclusion Criteria

The study included whole blood samples from voluntary donors at SDA Hospital that met the following criteria:

1. Blood samples that had been screened and passed the necessary tests required for blood transfusion.
2. Samples stored under appropriate conditions for transfusion purposes.
3. Donors who completed the required data forms regarding their medical history, including malaria infection, symptoms, and recent use of anti-malarial drugs.

Only these samples, deemed eligible for transfusion, were included in the analysis.

Exclusion criteria

The study excluded whole blood samples that have not been screened for malaria. No fresh frozen plasma was used

Sample collection

Blood samples were obtained from voluntary donors at SDA Hospital, Accra, who had completed data forms regarding their medical history, including details of previous malaria infections, symptoms, and any recent use of anti-malarial drugs. Two blood sample tubes containing EDTA (anti-coagulating agent) were used to collect blood from each donor. The samples were drawn into the tubes and gently mixed to prevent clotting.

The collected blood was immediately transferred to the laboratory for analysis. The samples were processed within hours of collection to maintain the integrity of the malaria parasites. Any remaining samples were stored at -20°C for later analysis.

Rapid Diagnostic Test (RDT)

The First Response rapid diagnostic test kit (Premier Medical Corporation, Ltd, India) was used to screen all blood samples for malaria. The test kit is used as a qualitative screening in vitro diagnostic test for detection of *P. falciparum* specific Histidine Rich Protein 2 (HRP2) antigen. The content of the kit includes a test cassette sealed in an aluminum pouch, assay diluents (buffer), and a pipette. The cassette was removed from the pouch and placed on a plain surface. In performing the test and with the use of a pipette in the kit about 5µl of blood was taken from the EDTA container and transferred into the sample well in the cassette. Three drops (15µL) of buffer were added to the blood in the sample well after which it was left for 1minutes and was allowed to flow to the result window on the cassette. After 15 minutes, the cassette was checked for the appearance of colored lines on the result window. The test was interpreted to be positive when the colored line appeared at the control region of the cassette and at the test region, while the test was interpreted to be negative where only a single-colored line appeared at the control region of the cassette and none at the test region.

Estimation of Parasite Density

The parasite density was estimated by counting the number of trophozoites observed per 200 white blood cells (WBCs) in the thick smear. To estimate the parasite density per microliter (µL) of blood, a standard WBC count of 8,000 cells/µL was assumed.

Data Processing & Analysis

Data were analyzed using standard software Statistical Package for Social Sciences (SPSS) version 26.0 and Microsoft Excel. Descriptive analysis was done using frequencies and percentages and were presented as charts and graphs.

Ethical consideration

Approval for this study were obtained from the Ethical Review Committee of the School of Biomedical and Allied Health Sciences, Baldwin University College. Permissions were also obtained from the Medical Directors of the SDA Hospitals.

Results

A total of 115 blood samples were collected from voluntary blood donors at SDA Hospital, Accra, for analysis. These samples were screened using both Rapid Diagnostic Tests (RDTs) and microscopy to detect *Plasmodium falciparum*. The diagnostic performance of the two methods was compared based on sensitivity, specificity, and parasite density.

Prevalence of Malaria Infections

Out of the 115 blood samples tested, *P. falciparum* was detected in 8.7% (10/115) of the samples by microscopy, while RDTs identified 6.1% (7/115) as positive for malaria. The difference in detection rates between microscopy and RDTs can be attributed to the higher sensitivity of microscopy in identifying *P. falciparum* infections, particularly in cases with low parasitemia.

Diagnostic Performance

Microscopy demonstrated a sensitivity of 100% and specificity of 100%. Conversely, RDTs exhibited a sensitivity of 100%, with a specificity of 99.1%. These results highlight that both diagnostic methods are highly reliable; however, microscopy had a slight advantage in detecting more positive cases, as indicated by the higher prevalence of *P. falciparum* detected by microscopy (8.7% vs. 6.1% for RDTs).

Blood Group Distribution of Malaria Cases

The prevalence of malaria varied across blood groups. Blood group O positive was most frequently associated with parasitemia, accounting for 50% (5/10) of the malaria-positive cases. Blood group A positive followed with 40% (4/10), while blood group B positive accounted for 10% (1/10). These findings are consistent with previous studies indicating a higher susceptibility to malaria in individuals with blood group O, likely due to the greater affinity of *Plasmodium falciparum* for O-positive red blood cells (Oluwafemi et al., 2024; Adejoh et al., 2023).

Parasite Density

The parasite density in the infected blood samples ranged from 600 to 1200 parasites/µL. All malaria-positive samples were from male donors, and all were Rh-positive, with blood groups O, A, and B representing the infected samples. These findings further reinforce the association between blood group O and higher parasitemia levels, as well as the male predominance observed in this study.

Species Identification

Microscopic examination revealed that *Plasmodium falciparum* was the only species identified in all malaria-positive samples. No other *Plasmodium* species, such as *P. vivax*, *P. ovale*, or *P. malariae*, were detected in the blood samples. This is consistent with global reports indicating *P. falciparum* as the dominant species responsible for malaria transmission in sub-Saharan Africa (Adebusuyi et al., 2024; Kolawole et al., 2023).

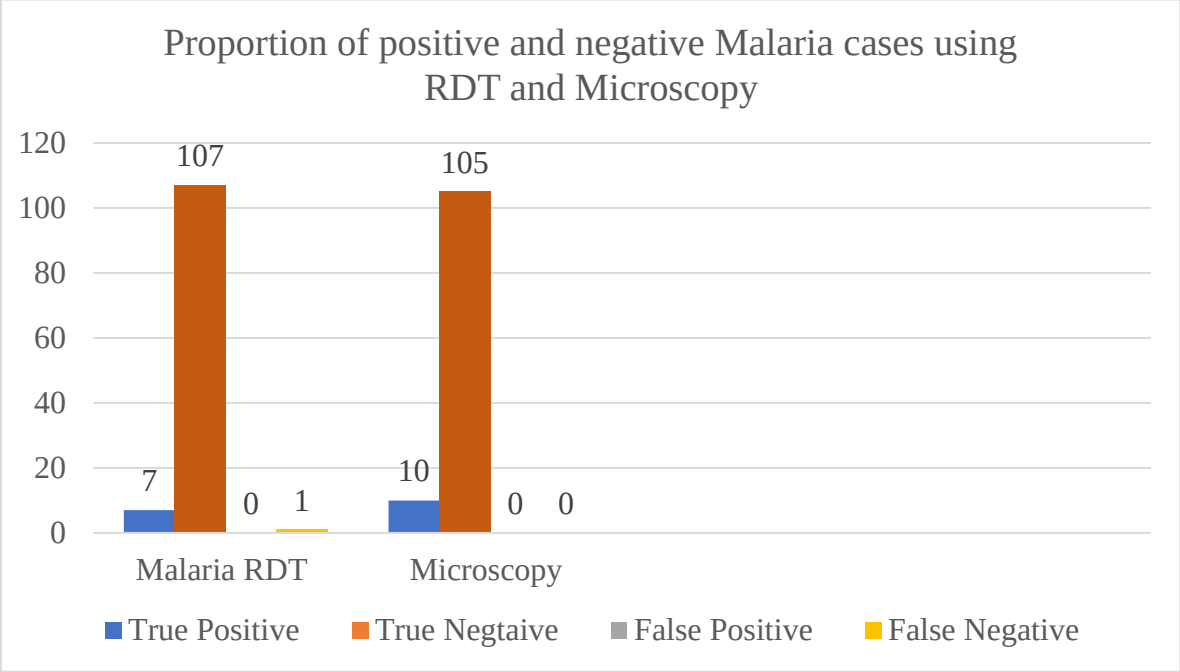

Figure 1 Proportion of positive and negative malaria cases using RDT and Microscopy

Figure 1. demonstrates the diagnostic performance of the Rapid Diagnostic Test (RDT) and microscopy in detecting *Plasmodium falciparum* infections in blood samples intended for transfusion. Of the 115 blood samples tested, RDT detected 6.1% (7/115) true positive cases, 0.9% (1/115) false positives, and 93% (107/115) true negatives, with a 0% false negative rate. Conversely, microscopy detected 8.7% (10/115) true positive cases, with no false positive cases, and 91.3% (105/115) true negatives, also with a 0% false negative rate. These results indicate that while both RDT and microscopy show excellent sensitivity and specificity, microscopy slightly outperforms RDTs in terms of detecting positive cases, corroborating findings from previous studies that microscopy is considered the gold standard for malaria detection (Adebusuyi et al., 2024; Bansal et al., 2024). Moreover, while RDTs have the advantage of faster results, they are subject to limitations, particularly in cases of low parasitemia and genetic variations in the malaria parasite (Zeleke et al., 2023; Mortazavi et al., 2025).

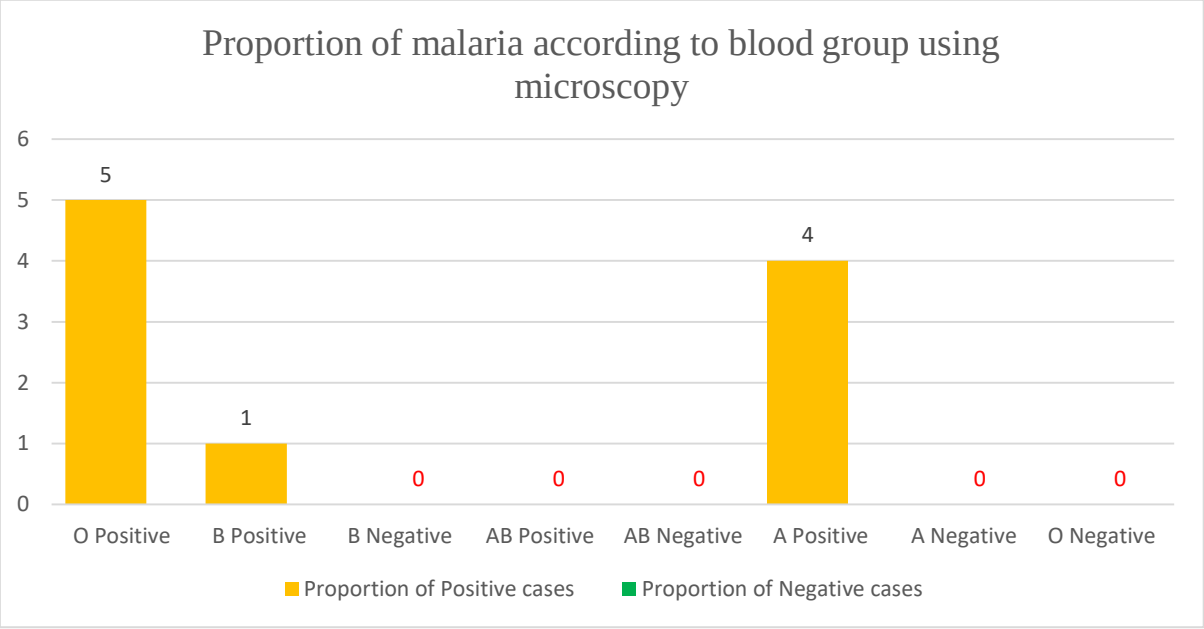

Figure 2 Proportion of malaria cases according to blood groups (Field data, 2025)

This above figure 2 shows the distribution of malaria cases among different blood groups in the study population. Blood group O positive dominated with parasitemia, accounting for 50% (5/10) of the infected samples, followed by blood group A positive with 40% (4/10), and blood group B positive with 10% (1/10). These findings are consistent with previous research indicating a higher prevalence of malaria in individuals with blood group O, due to the greater affinity of *Plasmodium falciparum* for O-positive red blood cells (Oluwafemi et al., 2024; Adejoh et al., 2023). Blood group O's higher susceptibility to malaria parasitemia may be linked to the absence of A and B antigens on red blood cells, which could allow the parasite to bind more readily to O-positive red blood cells (Rattanapan et al., 2023).

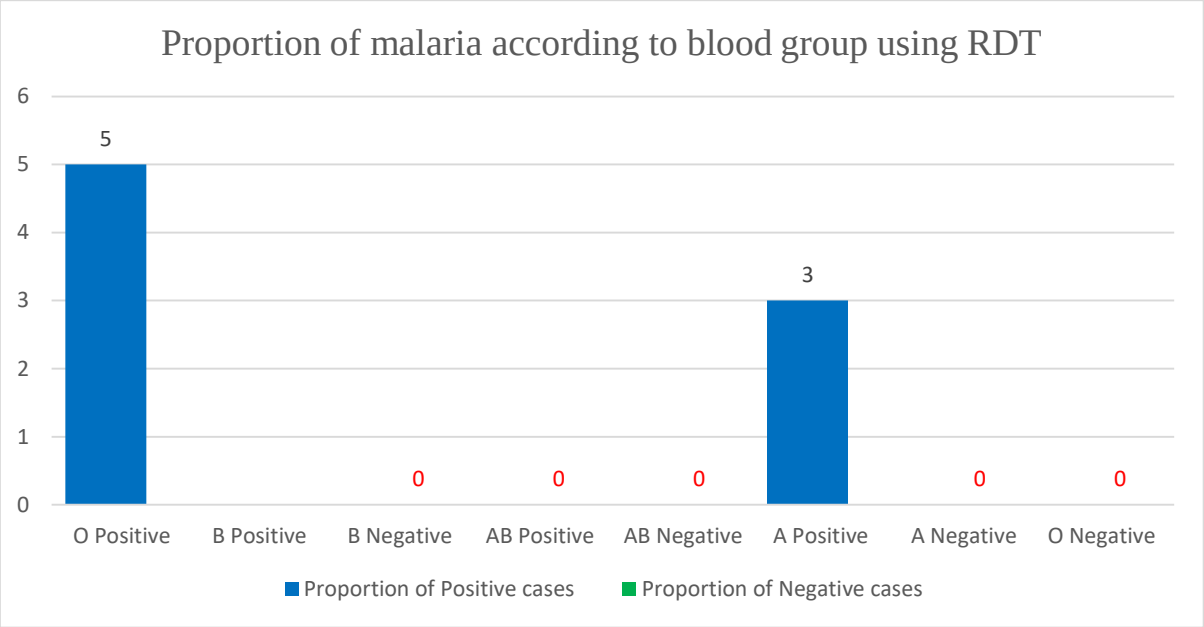

Figure 3 Proportion of malaria cases according to blood groups (Field data, 2025)

Figure 3 illustrates the prevalence of malaria among different blood groups based on Rapid Diagnostic Test (RDT) results. Blood group O positive showed the highest prevalence, accounting for 62.5% (5/8) of the positive cases, followed by blood group A positive with 50% (4/8). The data aligns with studies showing that individuals with blood group O tend to have higher susceptibility to *Plasmodium falciparum* due to the lack of A and B antigens on red blood cells, which allows the parasite to more easily bind and infect the cells (Oluwafemi et al., 2024; Rattanapan et al., 2023). Additionally, genetic and immune response factors have been found to influence the distribution of malaria across different blood groups (Adejoh et al., 2023). These findings emphasize the need for further investigation into the role of ABO blood groups in the susceptibility to malaria and their implications for blood donor screening (Gomerep et al., 2017).

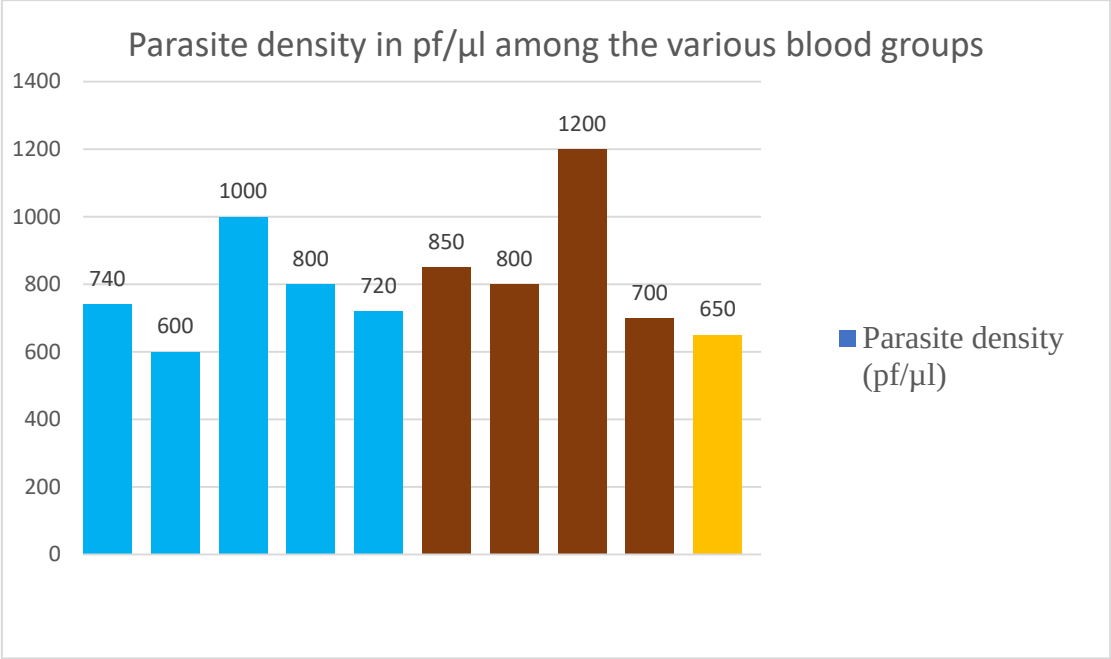

Figure 4 Parasite density among the various blood group (Field data, 2025).

This figure 4 presents the distribution of parasite densities across different blood groups among malaria-positive donors. Blood group O positive recorded the highest parasite density, ranging from 600 to 1000 parasites/µL, followed by blood group A positive, which had a parasite density ranging from 700 to 1200 parasites/µL. Blood group B positive showed the least density. These results support previous studies indicating that blood group O individuals may experience higher parasitemia levels due to a greater number of receptors available for *Plasmodium falciparum* binding (Oluwafemi et al., 2024). Additionally, studies have noted that individuals with blood group A may exhibit higher parasitemia than those with other blood types, likely due to specific interactions between the parasite and the red blood cell antigens (Opi et al., 2023).

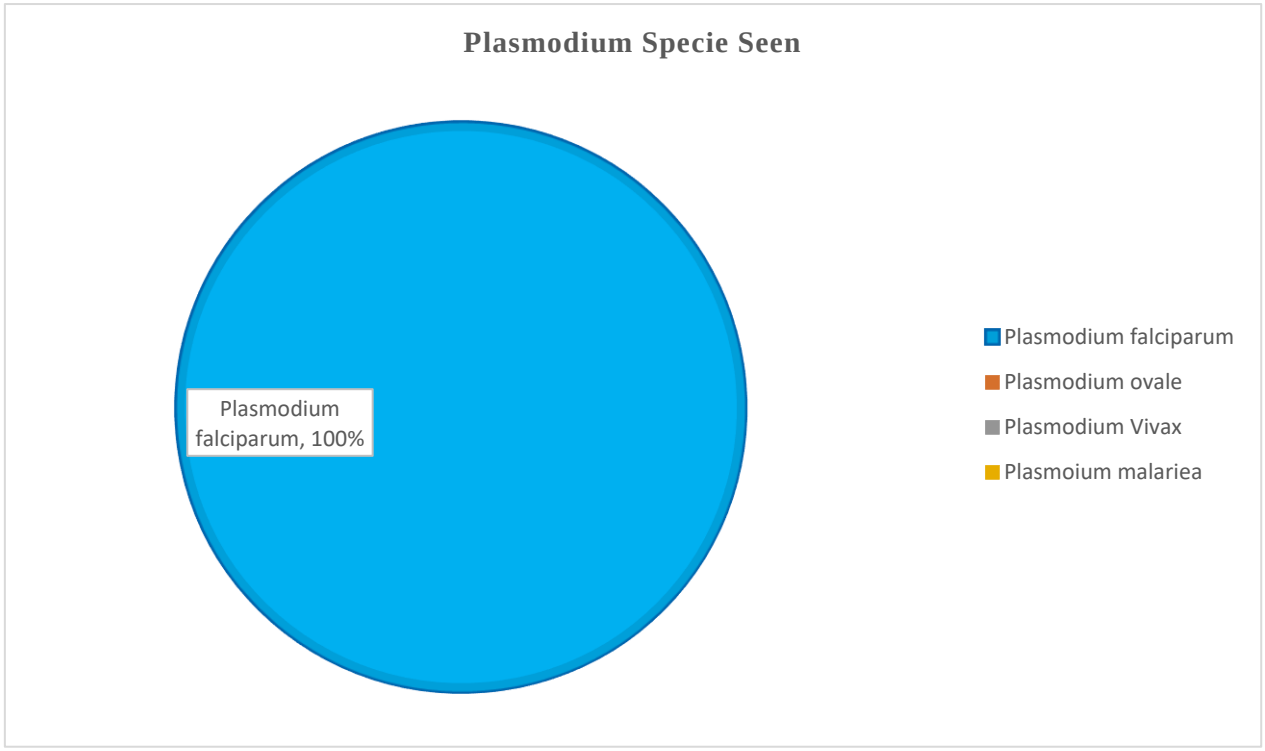

Figure 5 Type of malaria parasite specie seen.

Figure 5 displays the species of *Plasmodium* parasites identified in the blood samples. The study exclusively detected *Plasmodium falciparum* in all the malaria-positive samples. No other *Plasmodium* species, such as *P. vivax*, *P. ovale*, *P. malariae*, or *P. knowlesi*, were observed. These findings are consistent with

global reports, as *P. falciparum* is the predominant cause of malaria in sub-Saharan Africa and is particularly prevalent in malaria-endemic regions like Ghana (Adebusuyi et al., 2024; Kolawole et al., 2023). Additionally, *P. falciparum* is known for its ability to cause severe malaria, especially in cases of high parasitemia (Bansal et al., 2024).

Discussion

Blood transfusions are a cornerstone of modern medical treatment, offering life-saving support to patients. However, transfusion-transmitted infections (TTIs), particularly transfusion-transmitted malaria (TTM), pose significant risks, especially in malaria-endemic regions like sub-Saharan Africa (Olaniyi, 2019). The persistence of malaria parasites in blood stored for transfusion is a crucial public health concern, highlighting the importance of effective screening methods to ensure transfusion safety (Adusei & Owusu-Ofori, 2018).

The present study conducted at SDA Hospital in Accra aimed to assess the diagnostic performance of Rapid Diagnostic Tests (RDTs) and microscopy in detecting *Plasmodium falciparum* infections in blood samples intended for transfusion. The results revealed a 10% prevalence of *P. falciparum* among the tested blood samples, with a range of parasite densities from 600 to 1200 parasites/µL. These findings underscore the significant risk posed by asymptomatic infections in blood donors, as the majority of infected individuals in the study were males, and all positive samples were from Rh-positive blood groups, primarily O positive (Oluwafemi et al., 2024).

Notably, the study observed that *P. falciparum* was the sole malaria species detected in the blood samples, consistent with previous reports that *P. falciparum* remains the dominant species in malaria-endemic regions (Kolawole et al., 2023). This finding is particularly concerning as *P. falciparum* is associated with severe malaria, particularly in individuals with high parasitemia levels (Bansal et al., 2024). The study's results are further corroborated by Adebusuyi et al. (2024), who found higher prevalence rates of *P. falciparum* among asymptomatic blood donors in malaria-endemic areas.

The analysis of diagnostic methods revealed that both microscopy and RDTs demonstrated high sensitivity. Microscopy detected 8.7% of positive cases, while RDTs detected 6.1%. Both methods showed 100% sensitivity, but RDTs had a specificity of 99.1%, while microscopy achieved 100% specificity. These findings align with those of Bansal et al. (2024), who reported that microscopy remains the gold standard due to its ability to identify parasites at various stages of the life cycle, though it is limited by inter-observer variability and the need for highly trained personnel. The high sensitivity of both methods emphasizes the effectiveness of microscopy in identifying malaria in stored blood, but also highlights the potential of RDTs as a rapid and reliable alternative, particularly in resource-limited settings (Zeleke et al., 2023).

In terms of blood group susceptibility, the study found that blood group O positive had the highest prevalence of malaria parasitemia, followed by blood group A positive, and blood group B positive recorded the least. Previous studies have indicated that blood group O may be more susceptible to *P. falciparum* infection due to the absence of A and B antigens, which increases the number of receptors available for the parasite to bind (Oluwafemi et al., 2024). This is consistent with the findings of Adejoh et al. (2023), who found that blood group O individuals had a higher parasite load compared to other blood groups.

Additionally, the findings from this study contribute to the growing body of literature that explores the relationship between blood group and malaria susceptibility. Studies in Nigeria and Ethiopia have also indicated that blood group O individuals tend to have a higher prevalence of *P. falciparum* infections (Gomerep et al., 2017; Asmerom et al., 2023). However, the relationship between blood group and malaria susceptibility remains complex and may involve genetic factors, immune responses, and environmental influences (Rattanapan et al., 2023).

The findings of this study have important implications for transfusion safety in malaria-endemic regions. While both RDTs and microscopy demonstrated high diagnostic performance, the slightly higher sensitivity of microscopy suggests that it remains the preferred method for detecting *P. falciparum* in blood donor screening. However, given the advantages of RDTs, including faster results and ease of use, they may be a valuable tool in settings with limited resources, where microscopy may not always be feasible (Opi et al., 2023).

Despite the promising results of both methods, the study highlights the ongoing risk of transfusion-transmitted malaria, particularly in asymptomatic blood donors. The detection of *P. falciparum* in stored blood, even with negative RDT results, emphasizes the need for enhanced screening protocols and potentially the integration of molecular diagnostic techniques, such as PCR, which has higher sensitivity for detecting low-density infections (Dahal et al., 2021). The combination of RDTs and microscopy, along with the use of PCR, could further improve the detection of *P. falciparum* and enhance the safety of blood transfusions in endemic regions.

In conclusion, this study confirms that both RDTs and microscopy are effective in detecting *P. falciparum* infections in blood samples intended for transfusion. However, the slightly superior performance of microscopy highlights its importance in transfusion safety. The study also underscores the need for continued efforts to improve screening methods, including the potential integration of PCR, to reduce the risk of transfusion-transmitted malaria (TTM) and ensure the safety of blood transfusions in malaria-endemic regions (Olaniyi, 2019; Bansal et al., 2024).

Conclusion

This study underscores the critical importance of reliable diagnostic methods in ensuring the safety of blood transfusions, particularly in malaria-endemic regions. Both microscopy and Rapid Diagnostic Tests (RDTs) demonstrated robust performance in detecting *Plasmodium falciparum* in blood samples, with microscopy revealing slightly superior sensitivity. Specifically, microscopy detected 8.7% of malaria-positive samples, while RDTs identified 6.1%. Both methods exhibited excellent specificity, with microscopy achieving 100% specificity and RDTs showing 99.1%. These findings align with previous studies, affirming that while RDTs offer the advantages of rapid results and ease of use, microscopy remains the gold standard for accurate

detection, particularly in high-risk settings where precise identification of *P. falciparum* is paramount (Zeleke et al., 2023; Bansal et al., 2024).

Furthermore, the study revealed that blood group O positive exhibited the highest prevalence of *P. falciparum* parasitemia, consistent with earlier reports indicating a higher susceptibility of blood group O individuals to malaria due to the greater availability of receptors for parasite binding (Oluwafemi et al., 2024; Adejoh et al., 2023). This highlights the need for tailored screening strategies that consider blood group variations in malaria detection and transfusion safety.

Despite the effectiveness of both diagnostic approaches, this study highlights the persistent risk of transfusion-transmitted malaria (TTM), especially in asymptomatic donors. The detection of *P. falciparum* in stored blood emphasizes the need for enhanced donor screening protocols that integrate multiple diagnostic modalities. While molecular methods such as PCR offer higher sensitivity, their cost and infrastructure requirements may limit their widespread use in resource-limited settings (Dahal et al., 2021). Thus, a combination of RDTs, microscopy, and PCR could optimize malaria detection in blood donor screening, mitigating the risk of TTM and improving transfusion safety.

In conclusion, this study provides compelling evidence for the continued use of microscopy in blood donor screening, with RDTs serving as a viable supplementary tool in resource-constrained settings. To further reduce the risk of TTM, we advocate for the integration of advanced diagnostic techniques, alongside the development of standardized, cost-effective screening protocols that can be implemented globally, particularly in malaria-endemic regions. These measures are essential to safeguarding transfusion recipients from the potentially severe consequences of transfusion-transmitted malaria, ensuring a safer and more effective healthcare system.

Recommendations

Given the findings of this study, we propose the following recommendations to enhance blood transfusion safety and mitigate the risk of transfusion-transmitted malaria (TTM) in malaria-endemic regions:

1. It is crucial to adopt a dual approach combining Rapid Diagnostic Tests (RDTs) and microscopy for the detection of *Plasmodium falciparum* in blood donor screening. While microscopy remains the gold standard for its sensitivity and ability to identify parasite species, RDTs provide a rapid and accessible alternative, particularly in resource-constrained settings where laboratory infrastructure may be limited. This combination can enhance the reliability and efficiency of malaria screening.
2. While PCR-based techniques offer superior sensitivity, especially in detecting low-density parasitemia, their high cost and infrastructural demands remain significant barriers to their widespread adoption. However, given the ongoing risk of TTM from asymptomatic malaria carriers, we recommend integrating PCR-based diagnostics as a confirmatory method, particularly for blood donations that test positive by microscopy or RDTs. This would ensure more accurate detection and reduce the risk of undiagnosed cases.
3. Our findings suggest that blood group O positive individuals are more susceptible to malaria infection, a factor that may influence parasitemia levels. Blood group-based screening strategies could be incorporated into malaria detection protocols, with more frequent monitoring or enhanced diagnostic procedures for donors with blood group O, as well as those from high-risk regions.
4. To maintain the high-quality standards of diagnostic methods, continuous training of laboratory personnel is essential, particularly in malaria-endemic regions where microscopy remains a critical diagnostic tool. Training should emphasize quality control, accurate interpretation of test results, and the management of false positives and negatives in both RDTs and microscopy.
5. The study further highlights the need for policy reforms that mandate universal malaria screening of blood donors in endemic regions. Given the residual risk of TTM, we urge health authorities to consider the implementation of mandatory malaria testing in blood banks across sub-Saharan Africa and other malaria-endemic regions. Policy changes should focus on integrating modern diagnostic tools, such as multiplex RDTs and molecular diagnostics, into standard blood screening protocols.
6. Further research is needed to explore the long-term survival of malaria parasites in stored blood and to evaluate the effectiveness of emerging diagnostic tools. Investment in the development of affordable, point-of-care diagnostic technologies is critical to improving detection rates and reducing the global burden of TTM.

By adopting these recommendations, healthcare systems can significantly enhance the safety of blood transfusions, reduce the risk of TTM, and ensure better health outcomes for recipients of transfused blood, particularly in malaria-endemic regions.

Limitations of the Study

While this study provides valuable insights into the diagnostic performance of Rapid Diagnostic Tests (RDTs) and microscopy for detecting *Plasmodium falciparum* in blood donor samples, several limitations must be acknowledged:

1. The study utilized a total of 115 blood samples from voluntary blood donors at SDA Hospital. While this sample size is sufficient for preliminary analysis, a larger, multicenter study involving more diverse populations would provide a more comprehensive understanding of malaria prevalence and diagnostic accuracy across different demographic groups.
2. The study employed a cross-sectional design, which limits the ability to assess the long-term viability of *Plasmodium falciparum* in stored blood or to establish causal relationships between diagnostic methods and transfusion outcomes. Longitudinal studies are needed to track malaria transmission and infection dynamics over time in blood donations.

3. Although microscopy was used as the gold standard in this study, molecular methods, such as Polymerase Chain Reaction (PCR), were not employed to confirm the presence of *Plasmodium falciparum* in all samples. PCR offers superior sensitivity for detecting low-density parasitemia, which could potentially have been missed by both RDTs and microscopy, especially in cases of submicroscopic infections (Dahal et al., 2021).
4. The performance of RDTs can vary depending on several factors, including the quality of the test kits, the storage conditions, and the antigenic variations of *Plasmodium falciparum* strains. In this study, we used a single brand of RDT (First Response RDT kit), which may limit the generalizability of the findings to other RDT brands or formulations. Future studies should consider comparing multiple RDTs to assess their reliability across different settings.
5. The study was conducted at a single institution in Accra, Ghana, which may not fully represent the malaria burden or blood donation practices across the entire country or in other malaria-endemic regions. Regional variations in malaria prevalence, blood group distribution, and diagnostic capabilities could influence the results.
6. Although microscopy remains the gold standard for malaria diagnosis, its accuracy is highly dependent on the skill and experience of the microscopist. In this study, there was potential for observer bias, as different laboratory technicians may have had varying levels of expertise in interpreting blood films. To reduce this limitation, a standardized protocol and double-reading of blood smears could be implemented in future studies.
7. This study focused exclusively on *Plasmodium falciparum*, the most common and dangerous species of malaria in sub-Saharan Africa. However, there are other *Plasmodium* species, such as *P. vivax*, *P. ovale*, and *P. malariae*, which were not observed in the blood samples. The inclusion of other species in the diagnostic analysis could provide a more comprehensive understanding of malaria diversity in blood donations.
8. The study was conducted over a relatively short period (March to April 2025), which may not have captured seasonal variations in malaria prevalence. Malaria transmission dynamics in endemic areas often vary by season, with higher transmission rates during the rainy season. A longer study duration would allow for a more accurate representation of malaria prevalence throughout the year.

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