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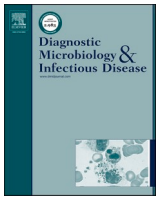

Original Article

Performance of microscopy and rapid test for the diagnosis of mixed malaria in an endemic area of Colombia.

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ABSTRACT

Background: Mixed infection by *Plasmodium* (MIP) occurs when more than one species are infecting one individual at the same time. Methods: Frequency of MIP was determined in a cohort of patients with acute febrile syndrome. Diagnostic performance of microscopy (MI) and a rapid diagnostic test (RDT) was evaluated against real-time PCR (qPCR), as reference method. Results: The frequency of MIP was 3% by qPCR, 1.4% by MI and 0.4% by RDT. The sensitivity of MI and RDT for detecting MIP was 21.43% and 15.25%, respectively, and specificity was over 99% for both techniques. Conclusion: The currently available tests for the diagnosis of *Plasmodium* in the field, showed poor performance for detecting MIP in this study. It is necessary to improve diagnostic tests sensitivity for the correct detection of these coinfections and to monitor the elimination of parasitemia to detect possible recurrences due to inadequate treatments.

1. Introduction

Mixed infection by *Plasmodium* (MIP[1]) is defined as the presence of more than one of its species in the same individual. The species most frequently responsible for MIP worldwide are *P. falciparum* and *P. vivax* [1,2] and is demonstrated that patients with MIP had a higher proportion of severe anemia and pulmonary complications than those with only *P. falciparum* infection [3].

Studies in Colombia and other countries show frequencies of MIP detected by PCR from 1.1% to 43% [4–8] By 2023, MIP corresponded to 1.1% (1078/102457) of all malaria cases microscopically diagnosed in Colombia [9].

Field diagnostic methods such as MI[2] and RDT[3] fail to detect coinfections [10,11]. Sensitivity of these techniques when compared to qPCR, for detection of monoinfections, ranges from 50% to 82% for MI, depending on the observer expertise, and 6% to 77% for RDT [12–14]. These techniques have several limitations for malaria diagnosis in the field such as lack of microscopists training to recognized low parasitemia infections, antigen detection limits and lack of parasite antigen expression in RDTs [15,16]. In recent years, molecular diagnostic methods such as PCR have improved their sensitivity to detect *Plasmodium* infections [17], becoming the best alternative for the detection of MIP.

The inability to detect MIP with field methods, leads to incorrect treatments and persistent infections [18–20]. In such case, patients receive medication for only one of the infecting species, which increases the risk of recurrences [21] therapeutic failures and drug resistance [22]. This is critical in countries with significant prevalence of *P. vivax*, where no universal treatment guideline is followed [23] and MIP misdiagnose would affect radical cure treatment.

In the present study, the frequency of MIP was determined in febrile patients with suspected malaria, who consulted at a first-level hospital in Quibdó, Chocó, Colombia. The performance of MI and RDT for the diagnosis of MIP was evaluated against a reference molecular test (qPCR).

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[1] MIP: mixed infection by *Plasmodium*.
[2] MI: microscopy.
[3] RDT: rapid diagnostic test.

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2. Methodology

2.1. Study site

The study was conducted in the municipality of Quibdó (Chocó, Colombia), located on the pacific coast, with an area of 3337.5 km^2, 100000 inhabitants approximately, mostly Afro-Colombians [24].

2.2. Study design

A cross-sectional study of diagnostic accuracy was carried out within a macro-study designed to evaluate the performance of a hematological analyzer for the detection of *Plasmodium*. A sample size of 2326 participants was considered assuming an expected sensitivity of 60% and specificity of 90% for MI or RDT, relative to qPCR for diagnosis of MIP [25], a prevalence of 4% of MIP in Quibdó, 95% confidence interval and 10% error [26]. Individuals who consulted with suspected malaria at the first-level hospital were enrolled consecutively between 2018 and 2019 [27]. The Standards for Reporting Diagnostic Accuracy Studies (STARD) guidelines were used [28]

2.3. Eligibility criteria

Presence of fever (axillary temperature $\geq 37.5°C$) or history of fever in the last 72 h, with less than 7 days of evolution, absence of a localized infection and underlying chronic disease-causing fever at enrollment. Individuals >2 years old, weighing >12 kg, residing in Quibdó or coming from malaria transmission areas, who can give informed consent/assent were included.

2.4. Blood samples

Two blood samples were taken from each participant by capillary puncture and by venous puncture in an EDTA tube. The first was used for malaria diagnosis by MI and the second was used for RDT, automated blood count using an XN-450 (Sysmex, Kobe, Japan) and 200ul aliquot for molecular diagnosis.

2.5. Microscopic diagnosis

Two sets of thick and thin blood films were made for each patient and stained with 10% Giemsa [29]. One set was used for patient́s microscopic diagnosis in the field. The second set was read latter by an independent expert microscopist, who was evaluated in their performance by local and international evaluators (WHO Quality Control). This analysis was blind to the results of the field diagnosis and patients clinical information. Thick film was classified as negative after observing 500 microscopic fields without finding parasites. When thick film was positive, *Plasmodium* species was determined by analyzing parasite morphology and counting parasitic stages on thin blood film. Total parasitemia was calculated according to a previous study [27].

MIP diagnosis was performed following WHO criteria [30]. Final report was confirmed with a second slides reading by an expert microscopist. When there was a discrepancy between the two microscopists, a third expert microscopist evaluated the slide and the final result was taken as coincident in at least two readings.

2.6. Rapid diagnostic test

The SD BIOLINE Pf/Pv (HRP2[4]/pLDH[5]) test, Reference 05FK80, was used, following manufacturer's instructions. This test allows for diagnosis of *P. falciparum, P. vivax* and MIP, detecting the *P. falciparum* antigen histidine rich protein 2 (HRP2) and P. vivax lactate dehydrogenase (pLDH).

2.7. DNA extraction and molecular diagnosis

A QIAmp DNA Blood Midi Kit, Qiagen, Germany (Invitrogen) was used to extract DNA from 160 uL of cryopreserved whole blood following the manufacturer's instructions. Detection of *Plasmodium spp* genus was performed by a modified qPCR method based on Rougemont *et al.* [31]; (detection limit 0.5 parasites/μL). A second qPCR was performed only on genus-positive samples to determine species (*P. falciparum, P. vivax* and *P. malariae*), using a modified protocol from Singh et al. [32]. A sample with a CT value ≤ 39 was considered positive and a CT value between 36 and 39 was interpreted as high. Samples processing was performed blindly to field test results.

Positive samples for Plasmodium spp and negative for species were processed with a modified nested PCR (nPCR) method, based on Singh et al. [32]. When these results were negative, samples were classified as Plasmodium spp.

MIP was determined when there were two positive results in the PCR for each species independently. Species predominance in MIP was analyzed for each sample based on Ct difference between *P. falciparum* and *P. vivax* tests difference $\leq 33\%$, was interpreted as presence of both species in equal proportion, and >33% corresponded to MIP with predominance of the species with lowest Ct[6] The cut-off value of 33% difference was adapted from electropherogram analysis to define the presence of more than one clone [33]

2.8. Data collection and analysis

Clinical and sociodemographic information was collected on each participant, and data was entered on a Microsoft Access 2010 database. A descriptive analysis of the sociodemographic, clinical, laboratory and malaria history variables was performed. Performance of MI and RDT for the diagnosis of *Plasmodium* infection and species detection (*P. falciparum, P. vivax* and MIP), was evaluated by calculating sensitivity (SE[7]), specificity (SP[8]), positive predictive value (PPV[9]), negative predictive value (NPV[10]), positive likelihood ratio (PLR[11]) and negative likelihood ratio (NLR[12]), with their respective 95% confidence intervals (CI 95%), using qPCR as reference. The MacNemar test was used to compare MI and RDT SE and SP. IBM SPSS statistics program version 24 with a license from Universidad de Antioquia was used for descriptive analyses. The R and rstudio cloud programs with epiR version 1.0-14 for SE, SP, PPV, NPV, PLR and NLR; DTComPair version 1.0.3 were used for comparison of SE and SP by McNemar test and diagnostic test performance analyses [34].

3. Results

A total of 2327 febrile patients were enrolled in the macro project and included for current study, with a median age of 27 years, 51.4% were men and 88.8% were Afro-Colombians. The presence of fever prior to consultation had a median of three days and the most frequent hematological alteration was anemia (30.3%) (Table 1).

[4] HRP2: histidine rich protein 2.
[5] pLDH: Plasmodium lactate dehydrogenase.
[6] Ct: cycle threshold value.
[7] SE: sensitivity Ct value: cycle threshold value.
[8] SP: specificity.
[9] PPV: positive predictive value.
[10] NPV: negative predictive value.
[11] PLR: positive likelihood ratio.
[12] NLR: negative likelihood ratio.

Table 1
Baseline demographic and clinical characteristics of participants included ($n = 2327$). IQR: Interquartile range, WBC: White blood cells, RBC: Red blood cells. Hematological parameters were collected from an XN-450. The sex was assigned at the discretion of the medical doctor who admitted the patient, according to the observed characteristics.

Characteristic		
Age; median (IQR)	27	(14 - 42)
Sex: man; n (%)	1194	(51.4)
Previous days with fever; n (%)	3	(3 - 5)
Pattern of fever, n (%)		
Daily	2054	(88.6)
Every two days	91	(3.9)
Irregular	172	(7.4)
Afro-Colombians; n (%)	2058	(88.8)
Pregnant; n (%)	20	(0.9)
Main occupation; n (%)		
Farmer	50	(2.2)
Miner	218	(9.4)
Housewife	458	(19.7)
Student	756	(32.5)
Other	678	(29.2)
Any	164	(7.1)
Place of origin; n (%)		
Quibdó city, Chocó	2186	(94.1)
Other places in Chocó	133	(5.7)
Outside Chocó	4	(0.2)
Rural area; n (%)	1961	(84.5)
Residence time in the endemic region (years); median (IQR)	7	(2 - 16)
Travel to other endemic region (last month); n (%)	645	(27.8)
Previous diagnosed malaria episodes (self-report); n (%)	1378	(59.3)
Intake of antimalarials drugs on last 4 weeks; n (%)	31,0	(1.3)
Haematological parameters		
WBC(10^3/uL); median (IQR)	6.41	(4.91 - 8.61)
RBC(10^6/uL); median (IQR)	4.54	(4.18 - 4.93)
Hemoglobin (g/dL); median (IQR)	12.9	(11.7 - 14.2)
Hematocrit (%)	36.8	(33.6 - 40.2)
Platelets (10^3/uL); median (IQR)	229,0	(168 - 289)
Anemia (<11 g/dL); n (%)	596	(30.3)
Thrombocytopenia (<150000 plat/uL); n (%)	384	(19.5)
Leucocytosis (>15.5 × 10^3/uL); n(%)	93	(4.7)

3.1. Frequency of Plasmodium spp and mixed infection

The frequency of *P. falciparum* infection was very similar by all three techniques (qPCR 24%, MI 24.2% and RDT 23.2%) and for *P. vivax* there was slightly higher detection by molecular test (qPCR 9.5%, MI 8.9% and RDT 8.3%). As shown in Table 2, the frequency of MIP in febrile patients was 3% by qPCR, 1.4% MI and 0.4% by RDT. These values corresponded to proportions of 4.1%, 1.2% and 8.7% respectively of total of participants positive for *Plasmodium spp* infection.

Table 2
Frequency of *Plasmodium spp* infection and MIP according to diagnostic tests. MI: microscopy. RDT: rapid diagnostic test. qPCR: real time PCR. Plasmodium spp: DNA detected but at levels lower than the LoQ was reported. IQR Interquartile range. % iRBC: percentage of red blood cells infected.

	MI ($n = 2327$)		RDT ($n = 2282$)		qPCR ($n = 2325$)	
***Plasmodium* positive; n (%)**	803	(34.3)	728	(31.9)	882	(37.9)
Mixed Pf/Pv	33	(1.4)	10	(0.4)	70	(3.0)
P. falciparum	564	(24.2)	529	(23.2)	559	(24.0)
P. vivax	206	(8.9)	189	(8.3)	221	(9.5)
P. malariae	0	(0)	0	(0)	1	(0)
Plasmodium spp	0	(0)	0	(0)	31	(1.3)
Parasitaemia (parasites/uL); median (IQR)	3504	(960 - 10048)				
Parasitaemia (%iRBC); median (IQR)	0.07	(0.02 - 0.24)				

3.2. Performance of MI and RDT for diagnosis of Plasmodium infection

Table 3 describes the samples included in the performance analysis of MI and RDT for the detection of *Plasmodium spp.* infections, comparing them with the reference test (qPCR). MI had good performance (SE 90.7%, SP 100%, PPV 100% and NPV 94.6%). Regarding RDT, although SE was lower (85.7%) than MI, other parameters were similar (SP 99.4%, PPV 98.7 and NPV 92.3%) (Table 3).

The frequency of false negatives was 9.3% for MI (82/882) (submicroscopic infections), out of which 84% (69/82) had high Ct values, close to the cutoff point, indicating that most of those infections had very low parasitemias. The frequency of false negatives for RDT was higher compared to MI, 14.3% (120/839), and only 57% of these (68/120) had high Ct values, close to the threshold for considering a sample as positive. Out of the 120 samples not being diagnosed by RDT, 34% (44/120) were detected by MI and had parasitemias between 11 and 6192 parasites/uL.

There were no false positives on microscopic examination. In the case of RDT there were nine samples with this diagnostic error (0.6%) (Table 3), eight of those corresponded to patients who had a malaria episode in the last two months (self-reported), and one sample from a patient who had taken antibiotics in the last two weeks.

The performance of the test for detecting mono-infections of each species of *Plasmodium,* MI sensitivity being higher for *P. falciparum* detection compared to RDT (94.1% vs 91.06); while for *P. vivax* infection, SE was similar for both techniques (MI 83.7% and RDT 82.5%) (see Table S1).

3.3. Performance of MI and RDT for diagnosis of MIP

MI and RDT performed in the field, had low SE for detecting MIP, 21.4% and 15.2% respectively; however, SP was good for both methods (99.2% and 99.9% respectively) (Fig. 1 and Table 4).

Compared to molecular test, MI failed to detect 78.6% (55/70) of coinfections identified by qPCR. These false negatives were diagnosed as *P. falciparum* monoinfection in 61.8% (34/55) (parasitemia between 16 and 45,920 parasites/uL), *P. vivax* monoinfection 36.4% (20/55) (parasitemias between 213 and 38,208 parasites/uL), and one sample was reported as negative (1.8%) (Table S2).

The RDT failed to detect 84.7% (50/59) of MIP by qPCR. The false negatives were 58% (29/50) *P. falciparum* monoinfection, 28% (14/50) *P. vivax* monoinfection and 14% (7/50) negative results. Only one sample was detected as MIP by RDT, corresponding to *P. vivax* monoinfection by qPCR (Table 4).

3.4. Microscopic characteristics of MIP

Microscopic characteristics and parasitemia of the 70 MIP detected by qPCR are shown in Table S2. Most of these infections had parasitemias greater than 500 parasites/uL. Only 21.4% (15/70) met the microscopic criteria for MIP described by WHO and 10 of these samples (66.7%) did not have a predominant species, according to the Ct value obtained in the molecular test.

4. Discussion

In this study, a frequency of 3% MIP (*P. vivax, P. falciparum*) was found by qPCR in patients with malaria symptoms. This frequency is comparable with published data from other regions in Colombia: 3% in northwest transmission focus [6] and other endemic countries (1.1% Ethiopia and 5% in Angola [7,8].

Field diagnostic methods used in this study had poor sensitivity for detecting MIP (21.4% MI and 15.2% RDT); however, specificity was good for both (99.2% MI and 99.9% RDT). Nevertheless, these tests had better performance for the diagnosis of single *Plasmodium* infections. An explanation could be that in many cases of MIP there is difference in the

Table 3
Performance MI and RDT vs qPCR for detection of *Plasmodium spp* infection . 95% CI: confidence intervals 95%.. MI: microscopy. RDT: rapid diagnostic test. PPV: positive predictive value. NPV: negative predictive value, PLR: positive likelihood ratio. NLR: negative likelihood ratio.

		MI (n: 2324)		RDT (n: 2282)		McNemar test	
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	Difference	P value
PCR	POSITIVE	800	82	719	120		
	NEGATIVE	0	1442	9	1434		
		Value	95% CI	Value	95% CI		
	Sensitivity	90.70	(88.59 - 92.54)	85.70	(83.14 - 88)	4.53	<0.0001
	Specificity	100	(99.74 - 100)	99.38	(98.82 - 99.71)	0.05	0.0047
	PPV	100	(99.54 - 100)	98.76	(97.67 - 99.43)		
	NPV	94.62	(93.37 - 95.7)	92.28	(90.84 - 93.56)		
	PLR	-		137.40	(71.60 - 263.69)		
	NLR	0.09	(0.08- 0.11)	0.14	(0.12 - 0.17)		

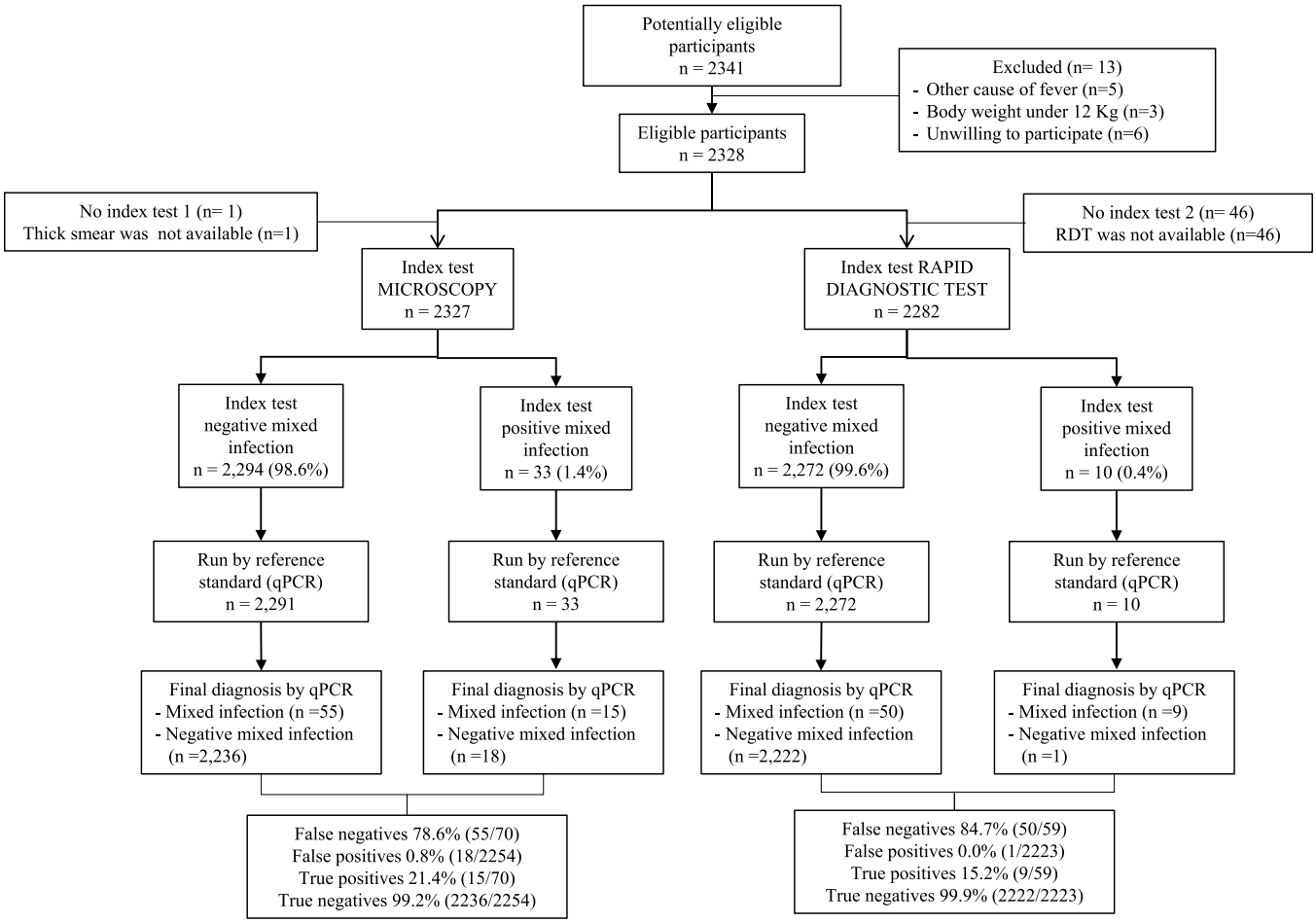

Fig. 1. Flow chart showing performance of MI/RDT vs qPCR for mixed infection detection.

proportion of parasite biomass of each specie, and only one of them is above the threshold of detection of these methods [35]. As a consequence, these samples are classified as monoinfections.

According to the diagnostic guidelines published by WHO, when *Plasmodium* is detected in a thick smear, it is required to rule out the possibility of MIP [10]. One challenge is the morphological differentiation of immature trophozoites (ring forms) characteristic of each species, guided by parameters like ring size (small - *P. falciparum*, large - *P. vivax*). In this study, we confirmed previous observations of a particular morphology of *P. falciparum* trophozoites in patients from Choco region, characterized by larger rings than what is commonly reported in the literature, and some of them with malarial pigment (Fig. 2). This characteristic could contribute to the difficulties for accurate diagnosis of MIP in this region. Although WHO diagnostic guidelines give a general framework for endemic countries, it is important to note that there may be morphological variations in the circulating strains which makes it challenging to apply a standardized guideline.

Personnel expertise is recognized as a determining factor in the sensitivity of malaria diagnostic by MI [36], this factor could also explain why RDT exhibited higher false-negative rates compared to MI (14.3% vs. 9.3%) in *Plasmodium spp* infections. Microscopy can outperform RDT in many settings, according to microscopist expertise. Literature refers to an average detection limit of 100 parasites/uL in non-expert microscopists and 10 parasites/uL in expert microscopists. In contrast, RDT can only detect close to 200 parasites/uL [27,37,38].

Table 4
Performance of MI and RDT vs qPCR for detection of MIP. 95% CI: confidence intervals 95%. MI: microscopy. RDT: rapid diagnostic test. qPCR: real time PCR. PPV: positive predictive value. NPV: negative predictive value, PLR: positive likelihood ratio. NLR: negative likelihood ratio.

		MI (n:2324)		RDT (n: 2282)		McNemar test	
		POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	Difference	P value
qPCR	POSITIVE	15	55	9	50		
	NEGATIVE	18	2236	1	2222		
		Value	95% CI	Value	95% CI		
	Sensitivity	21.43	(12.52 - 32.87)	15.25	(7.22- 26.99)	5.10	0.366
	Specificity	99.20	(98.74 - 99.53)	99.96	(99.75 - 100)	0.67	<0.001
	PPV	45.4	(28.11 - 63.65)	90.00	(55.50- 99.75)		
	NPV	97.60	(96.89 - 98.19	97.80	(97.11 - 98.36)		
	PLR	26.8	(14.11 - 51.02)	339.10	(43.67 - 2633.43)		
	NLR	0.79	(0.7 - 0.9)	0.85	(0.76 - 0.94)		

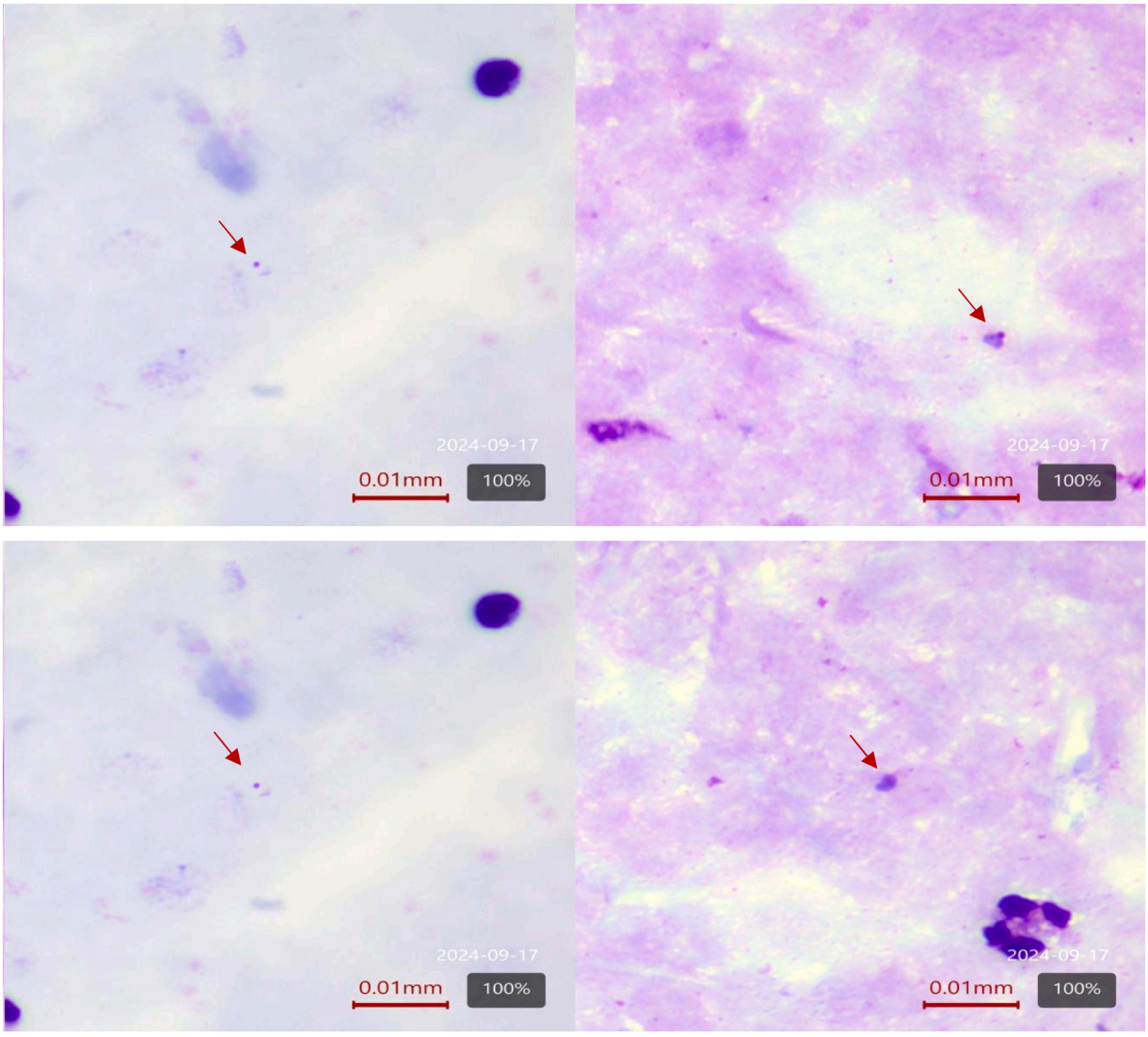

Fig. 2. Comparison of shape and size of immature trophozoites (rings) of *Plasmodium falciparum*. Left: Typical rings from a sample obtained from WHO for microscopy quality control. **Right**: Common ring morphology observed in some of the patient included in this study.

Additionally, PfHRP2 protein can persist in the blood 1–5 weeks after effective treatment, which complicates results interpretation in clinical settings [39]. In this study 9 false positive cases for *Plasmodium spp* infection and one for MIP by RDT were found. False negative samples by RDT associated with parasitemias above detection limit, such those with up to 6192 parasites/ul found in this study could be explained by the deletion of the pfHRP2 gene previously reported in Colombia [40].

Out of all the MIP detected by qPCR and microscopy in this study, 66.7% (10/15) did not show any predominant species by the molecular method. Absence of a predominant species in MIP, could improve chances of its detection by microscopist. In addition, it was observed that when *P. falciparum* was predominant in MIP according to qPCR Ct, MI incorrectly diagnosed these samples as monoinfection, which supports the difficulty for microscopist to identify the second parasite

species. In MIP misdiagnosed as *P. vivax* by MI, 75% (15/20) of them did not show a predominant species. In this case, simultaneous circulation of several parasitic forms, could affect the ability of the microscopist to discriminate specific morphology of each *Plasmodium* species.

A low number of publications show the performance of MI and RDT compared to a molecular test [6,33]. In Iran, a study reported SE of 16.6% (2/12) of thick smear and 58% (7/12) for CareStart Pv/Pf® RDT for detecting MIP. Another study in Ethiopia reported a SE 74.0% (95% CI 66.2 - 80.6%) and SP 87.4% (95% CI 80.6 - 92.2%) of MI for MIP. It should be noted that the heterogeneity between the studies makes it difficult to compare them, since different molecular tests of reference were used, RDTs tested were different as well and the microscopist expertise was variable.

The reasons for low MIP detection by field tests in symptomatic individuals may be related with the fact that one of the infecting species may have relatively low parasitic biomass, due to one of the following reasons: 1) Several infective mosquito bites occurring in different moments, which could lead to one of the species initiating erythrocytic phase earlier than the other one. Although *P. falciparum* infections can have higher parasitemias compared to *P. vivax*, it may happen that, at the time of MIP diagnosis, *P. vivax* parasitemia could be higher because of earlier erythrocytic initiation. 2) Competition between parasite species for the host blood cells [41]. 3) Previous specific immunity for one of the species, that may control parasitemia to submicroscopic levels [35]. All these possibilities could contribute to MIPs with higher proportion of one of the parasite species in peripheral blood, which makes the diagnosis more challenging.

Field tests failed to diagnose a high number of MIPs compared to molecular test. Consequently, 54 MIP cases were not adequately classified and incomplete treatment was provided, 20 at risk of recrudescence due to potential *P. falciparum* resistance to CQ, 34 did not receive radical cure treatment. A metanalysis (15341 patients) shows higher risk of *P. vivax* parasitemia following *P. falciparum* malaria, suggesting that some of these cases could be misdiagnosed MIP [42]. Diagnosing and treating MIPs properly would be very important to prevent potential complications and prevent transmission. Failing to treat hypnozoites can lead to increased number of relapses and new infections due to gametocytes early circulation in *P. vivax* infection. Accurate MIP diagnosis will help surveillance systems, by revealing the true extent of this problem and promote informed decisions on control measures.

The evidence presented in the article on failure to detect MIP using routine methods could support the incorporation of universal treatments with artemisinin combination therapies, preventing resistance and relapses. This approach aims to contribute to malaria control and create scenarios that facilitate its elimination.

4.1. Limitations

The low frequency of MIP in this study, led to wide confidence intervals for estimates of the diagnostic tests performance and did not allow complementary analyzes based on parasitemia count. The results are only generalizable to clinical settings of outpatients with uncomplicated malaria, in our context. The comparison of diagnostics methods using different targets (antigens, DNA, complete parasites), have limitations, because each of them have different kinetics (reaching detection limit/elimination). However, the inclusion of symptomatic cases gives more value to these methods for malaria case diagnostics.

5. Conclusion

In this study MI and RDT detected a low percentage of MIPs compared to qPCR, an event that could be related with the detection limits of these techniques and the predominance of one of the parasite species in many of these infections. Microscopy was performed by highly qualified personnel, following WHO recommendations, was not a good tool for discriminating parasite species in MIP. In contexts with transmission of several *Plasmodium* species, where MI or RDT are used as routine diagnostic methods, combined and unified treatment and patient's follow-up after treatment should be a strategy to monitor parasite clearance and prevent recurrences, drug-resistant strains recrudescence and clinical complications, which are all consequences of undetected MIP.

Sources of funding

Sysmex Corporation and University of Antioquia (Vice-Rectory of Research) funded the design of the study, field work, laboratory tests and data analysis

Ethical Approval

This study was approved by the ethics committee of the Faculty of Medicine at Universidad de Antioquia (Record No. 007 of May 11, 2017).

Data availability

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Alexandra Rios-Orrego: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Tatiana M. Lopera-Mesa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Veronica Sierra Cifuentes:** Writing – review & editing, Investigation, Conceptualization. **Lina Zuluaga-Idárraga:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2025.116799.

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