

Diagnostic accuracy of ICT- rapid diagnostic tests versus microscopy for malaria: A City-wide study from Karachi

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ABSTRACT

Objective: To assess the diagnostic accuracy of an Immunochromatographic Rapid Diagnostic Test (ICT-RDT) for the diagnosis of malaria, by comparing it with microscopy as the gold standard.

Material and Methods: A cross-sectional diagnostic accuracy study was conducted from July 20th to November 5th, 2025, at Karachi Institute of Medical Sciences and the Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Karachi. Suspected malaria patients were enrolled. Venous blood samples were used for microscopy (thick and thin smears) and ICT-based RDT (HRP2/pLDH). Diagnostic indices (sensitivity, specificity, PPV, NPV) were calculated and compared with microscopy.

Results: Of the 341 enrolled patients, 218 (63.9%) had malaria parasite on microscopy. Majority of cases showed *Plasmodium vivax* (*P. vivax*) 195 (89.4%), followed by *Plasmodium falciparum* (*P. falciparum*) 18(8.2%) and mixed infections 5(2.2%). The ICT-RDT demonstrated a high diagnostic performance with a sensitivity of 94.49%, a specificity of 95.93%, and an accuracy of 95.0%. Sensitivity did not differ significantly between *P. vivax* and *P. falciparum* (92.8% vs. 95.6%). However, specificity (96.5% vs. 99.3%, $p=0.022$) and diagnostic accuracy (94.4% vs. 99.1%, $p=0.0005$) were significantly higher for *P. falciparum*. Mixed infections frequently appeared as single-species bands on RDTs.

Conclusion: In this study, the ICT-RDT demonstrated excellent overall performance with high diagnostic accuracy and proved to be a reliable frontline tool for routine clinical practice. However, the tendency to misclassify mixed infections as single-species bands requires careful interpretation.

Keywords: Immunohistographic test, Malaria rapid diagnostic test, Microscopy

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INTRODUCTION

Malaria remains a leading cause of morbidity and mortality worldwide, and continues to impede elimination efforts. The World Malaria Report 2024 estimated approximately 263 million cases and about 597,000 deaths in 2023, highlighting that progress has slowed mainly in several endemic areas [1]. Within the WHO Eastern Mediterranean Region, malaria transmission persists with around 10.2 million cases reported in 2023. Most of these cases were concentrated in Pakistan and other high-burden countries, including Afghanistan, Somalia, and Sudan, emphasizing that malaria poses a significant threat to public health in the region [1,2]. In Pakistan, a marked resurgence occurred after the 2022 floods, resulting in approximately 2.73 million confirmed cases by 2023. This corresponds to nearly 11-12

cases per 1,000 population and is influenced by climatic changes, population displacement, and uneven vector control strategies [3-5]. The national epidemiology is characterized by malaria transmission involving two major species, predominantly *Plasmodium vivax* (*P. vivax*), while *Plasmodium falciparum* (*P. falciparum*) continues to cause significant morbidity [5].

Accurate and timely diagnosis of malaria is crucial for effective treatment, case management, and disease control. At the same time, preventing overdiagnosis and avoiding the injudicious use of anti-malarial drugs is critical. Microscopy remains the gold standard due to its ability to identify species and to assess parasite density. However, it requires skilled personnel, quality reagents, and functional laboratories, which are often lacking in resource-limited areas, leading to delayed or inappropriate treatment [6,7]. Rapid diagnostic tests (RDTs), especially immunochromatographic tests (ICTs), detect specific parasite antigens such as histidine-rich protein 2 (HRP2) and *Plasmodium* lactate dehydrogenase (pLDH). They provide rapid, point-of-care results that improve diagnostic accessibility and guide timely treatment decisions [8-10].

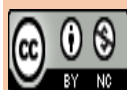
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Molecular assays, such as polymerase chain reaction (PCR), offer greater analytic sensitivity and can detect submicroscopic infections. However, the reliance on specialized laboratories and the slow turnaround time generally limit the routine use of PCR for immediate clinical management in endemic, under-resourced settings [10]. Given these diagnostic limitations and the evolving malaria situation in Pakistan, it is essential to generate local evidence by comparing microscopy with RDT performance to refine diagnostic algorithms and optimize control strategies. Unlike earlier studies from Karachi that were based on single centers, our city-wide design captures a broader socioeconomic representation.

MATERIAL AND METHODS

We conducted a cross-sectional diagnostic accuracy study from July 20th to November 5th, at Karachi Institute of Medical Sciences, Karachi, and the Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences (LUMHS), Karachi, under IRB approval No. LUMHS/rec/-867. The laboratory serves as the university's central diagnostic hub, receiving specimens from 13 collection centers located across the city of Karachi. The consecutive non-probability sampling technique was used. Patients of all ages and both sexes with suspected malaria, defined by a history of fever and axillary temperature $>37.5^{\circ}\text{C}$ at presentation, were included. Common accompanying symptoms were chills, myalgia, headache, and fatigue. Patients who had received antimalarial treatment within the preceding two weeks, or who had an alternative established cause of fever, such as productive cough, diarrhoea, odynophagia, and skin rashes.

The sample size was calculated using Buderer's method for diagnostic accuracy studies [11], incorporating expected sensitivity (74%), specificity (85.5%), and prevalence (17.5%) from a prior Karachi study (4). The Z value of 1.96 and desired precision levels of 0.11 and 0.10 for sensitivity and specificity, respectively, were used. The minimum required numbers were 139 diseased and 73 non-diseased ($n = 212$). We enrolled 341 patients (218 microscopy-positive, 123 microscopy-negative), significantly exceeding these requirements.

Written informed consents were obtained from all participants or from parents/guardians for minors. Demographic and clinical data (age, sex, prior use of antimalarials, and duration of fever) were recorded on a standardized case record form.

A 5mL venous blood sample was collected from each participant into an EDTA anticoagulant tube. Two

drops of blood were used to prepare thick and thin peripheral blood smears, and one drop was reserved for ICT-RDT. Standardized volumes of 10 μL and 2 μL were used to prepare thick and thin smears, respectively. The smears were air-dried and stained with Leishman stain. Thick films were examined for the presence of malarial parasites, while thin films were used for species identification [4,12]. If no parasites were observed after examination of 100 oil immersion fields, the smear was considered negative. Two experienced microscopists, blinded to the RDT results, independently reviewed all slides. A third senior microscopist resolved any discrepancies. The commercially available SD Malaria Ag P.f/Pan (Sun Diagnostics, Inc.), an ICT assay that detects *P. falciparum*-specific HRP2 and pan-plasmodium pLDH antigens, was used as the index test. The assay was performed according to the manufacturer's instructions, and the results were recorded as *P. falciparum* positive, Pan positive, Mixed infection, Negative, or Invalid.

Patient information and laboratory results were documented on a structured proforma. Data was entered and analyzed using SPSS version 26. Mean $\pm$ Standard Deviation was calculated for age, while categorical variables were summarized as frequencies and percentages. For analytical purposes, age was categorized into predefined groups to facilitate comparison across demographic strata. Diagnostic indices, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy, were calculated for RDTs against microscopy. 95% confidence intervals were derived using the Wilson score method. Species-wise comparisons were performed using the chi-square test, with a p-value ≤ 0.05 considered statistically significant.

RESULTS

A total of 341 patients met the inclusion criteria. The mean age of the study population was 29.8 years (SD = 18.1). Most of our patients, 208/341 (61%) were males, resulting in a male to female ratio of 1.6:1. Most patients belonged to the younger age groups, with 202 (59.3%) individuals aged 1–30 years, indicating a higher burden of malaria in children, adolescents, and young adults. The gender and age-group distribution details are presented in Table-I. Microscopy identified 218 (63.9%) malaria-positive and 123 (36.1%) malaria-negative specimens, with *P. vivax* as the predominant species. Asexual parasite stage was dominantly seen in the microscopy positive cases, whereas few *P.*

falciparum cases showed gametocytes only. ICT-RDT performed well for detecting *P. falciparum*, while some disagreements were seen in *P. vivax*, and microscopy- negative cases. No association was seen between asexual or asexual parasite stages on microscopy, and ICT-RDT performance. The false-negative results were also not confined to any specific parasite stage. The details are shown in Table-II. The ICT-RDT demonstrated high diagnostic accuracy for overall malaria detection, as well as for both *Plasmodium* species. The specificity and overall diagnostic accuracy were significantly higher for *P.*

falciparum than for *P. vivax* ($p = 0.022$ and 0.0005 respectively), whereas the sensitivity did not differ significantly between species (Table-III).

Receiver Operating Characteristic (ROC) curves were plotted to ascertain the diagnostic performance of ICT-RDT. The AUC for overall malaria, *P. vivax*, and *P. falciparum* detection were 0.952, 0.935, and 0.975 respectively, indicating high diagnostic accuracy of the test (Figure-I). Mixed infections identified by microscopy appeared as single-species bands on the RDT.

Table-I: Demographic characteristics of patients evaluated for malaria (n = 341).

Variable	Number of Cases n (%)	
Gender	Male	Female
	208 (61.0%)	133 (39.0%)
Age distribution (Years)		
	1-15	78 (22.9%)
	16-30	124 (36.4%)
	31-45	72 (21.1%)
	46-60	45 (13.2%)
	>60	22 (6.5%)

Table-II: Comparison of microscopy findings with ICT-RDT results.

MICROSCOPY RESULTS			ICT-RDT RESULTS		
Microscopy Findings	Parasite Stages	No. of Samples n (%)	ICT- RDT: <i>P. falciparum</i>	ICT- RDT: <i>P. vivax</i>	ICT- RDT: Negative
Total <i>P. vivax</i>	asexual (+ sexual)	195 (89.4%) *	3	181	11
<i>P. falciparum</i>	asexual (+ sexual)	15	15	0	0
	sexual only	03	03	0	0
Total <i>P. falciparum</i>	--	18 (8.2%) *			
Mixed infection	<i>P. falciparum</i> + <i>P. vivax</i> (+ sexual)	05 (2.2%) *	04	01	0
Microscopy Positive Subtotal	--	218 (63.9%) **	25	182	11
Microscopy Negative	--	123 (36.1%) **	0	05	118
Total	--	341 (100%)	25	187	129

* Percentage of microscopy positive cases (n=218), **Percentage of total study cases (n=341)

Table-III: Diagnostic performance of ICT-RDT compared with microscopy for overall malaria and for species-specific detection.

Group	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy %
Overall malaria	94.49% (90.63-96.82)	95.93% (90.84-98.25)	97.64% (94.60-98.99)	91.47% (85.38-95.17)	95.0%
<i>Plasmodium vivax</i>	92.82% (88.31-95.68)*	96.58% (92.23-98.53)**	97.31% (93.86-98.85)***	90.97% (85.41-94.54)	94.4%
<i>Plasmodium falciparum</i>	95.65% (79.01-99.23)*	99.37% (97.74-99.83)**	91.67% (74.15-97.68)***	99.68% (98.23-99.94)	99.1%

*Sensitivity *P. vivax* vs *P. falciparum*: NS, **Specificity *P. vivax* vs *P. falciparum*: $p = 0.022$, ***Accuracy *P. vivax* vs *P. falciparum*: $p = 0.0005$

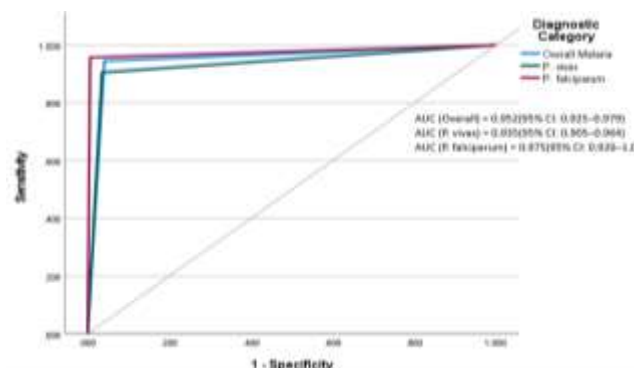


Figure-I: ROC Curve: ICT- RDT diagnostic accuracy for overall malaria, *Plasmodium vivax*, and *Plasmodium falciparum*.

DISCUSSION

In this study, we evaluated the diagnostic performance of an ICT-RDT compared with microscopy, the reference standard for malaria diagnosis in routine clinical practice. The demographic profile of our study population with a predominance of young adults and a higher proportion of males, is consistent with previously reported local studies [3,12]. This distribution can be explained by societal gender roles, where males have greater occupational and outdoor exposure in malaria-prone settings [2,3]. Similarly, higher burden in adolescents and young adults aligns with regional

trends and could be due to increased mobility for education and work [2,3,12].

Most of the malaria-positive patients in our series were infected with *P. vivax*, followed by *P. falciparum*, and mixed infections. This reflects the species distribution pattern reported in Pakistan [3,12,13]. The performance of antigen-based tests varies for different *Plasmodium* species; therefore, knowledge of specie distribution is essential to improve diagnostic practices in malaria-endemic regions.

The RDT that we evaluated demonstrated high overall sensitivity and specificity when compared with microscopy. Our findings are consistent with previous studies from Karachi and Rawalpindi, which reported diagnostic accuracies of 93.1% and 94% respectively, supporting the reliability of RDTs as practical and dependable tools in routine clinical use [14, 15]. However, Shah *et al.* demonstrated a diagnostic accuracy of 80.5%. This difference in performance is likely due to variations in parasite burden at presentation, RDT brands used, and level of operator expertise [4].

Internationally, studies from several endemic regions in Africa have confirmed high diagnostic accuracy of RDTs, supporting their use as frontline diagnostic tools in resource-limited settings [16, 17]. Our findings are in concordance with Ayogu *et al.*, who reported higher RDT specificity than sensitivity, particularly at low parasite burden [18]. We did not directly measure parasite density in the present study, the observed high specificity with comparatively variable sensitivity suggests that cases with low parasitemia may have caused false-negative RDT results.

Literature from South-east Asia supports the variable performance of RDTs for *P. vivax* and *P. falciparum* detection, when compared against RT-PCR, emphasizing their limitations for detecting low-level infections [19]. Similarly, Watson *et al.*, in a large community-based survey, reported that nearly 20% of microscopy-confirmed cases were missed by RDTs, in low-transmission African settings [20]. In contrast, we observed only 11 false-negatives out of 341 participants, most likely due to our facility-based sampling of symptomatic patients with higher parasite densities at presentation.

International studies have established deletions of the *pfhrp2* and *pfhrp3* genes as another established cause of reduced performance of HRP2-based RDTs, leading to false-negative *P. falciparum* results [21]. But information on these deletions is deficient in the local literature. Our study recorded only one *P. falciparum* false-negative case, which occurred in a

mixed infection detected as *P. vivax* on RDT. This could be either due to gene deletions or low *P. falciparum* density masked by high *P. vivax* burden.

Species-specific analysis in our study revealed slightly lower sensitivity for *P. vivax* (92.4%) than for *P. falciparum* (95.7%), though not statistically significant. A similar trend was reported by Harani *et al.*, with slightly reduced sensitivity for *P. vivax* infections [13]. In contrast, Rafique *et al.* reported reduced RDTs' sensitivity for *P. falciparum* in serial-dilution experiments, especially at lower parasite concentrations [22]. This is most likely due to methodological differences and inter-brand variability in RDT performance.

We observed high specificity for both species in the study, particularly with higher *P. falciparum* specificity. Comparable findings have been reported from Faisalabad, where RDTs performed well in excluding malaria infection [12]. Molecular comparative studies further support these findings. Khan *et al.* reported that PCR was more sensitive than both microscopy and antigen-based RDTs in detecting malaria, particularly at low densities, highlighting the limitations of conventional diagnostic approaches [23]. Importantly, despite lower sensitivity, RDTs in that study exhibited excellent specificity, a pattern also observed in our findings.

In the present study, we observed the reduced ability of RDTs to accurately identify mixed infections, which were frequently interpreted as single-species bands. Of our five mixed infections, four were identified as *P. falciparum* and one as *P. vivax*. This limitation has been documented in studies from Pakistan and Tanzania, where mixed infections were often under-detected or misclassified relative to microscopy or molecular methods [6,8,23]. Such misclassification is primarily due to unequal antigen expression, whereby dominance of one species suppresses detection of the other.

Although earlier literature has suggested that parasite developmental stage influences antigen-based RDTs performance, we did not observe a significant stage-dependent effect. Our findings are consistent with current WHO guidelines which indicate that malaria RDT performance is primarily affected by parasite density and antigen availability rather than developmental stage [1, 10, 24].

A key strength of our study is its wide catchment area, supported by 13 geographically distributed collection centers linked to a central laboratory. This approach enhances sample representativeness and improves generalizability beyond findings from single-center Karachi-based studies. Additional strengths of

this study include species-stratified analysis, and sample collection during the post-monsoon peak transmission season in Karachi, when malaria detection is highest, thereby enhancing the relevance of the findings to routine clinical practice.

LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The unavailability of PCR verification for low parasitemia cases and absence of parasite density analysis remain important limitations. Future multi-center studies with longer follow up are needed integrating existing local molecular and parasitological data and incorporating multi-season surveillance. This would help refine diagnostic estimates and support malaria control strategies in Pakistan.

CONCLUSION

ICT-based RDTs are a rapid and reliable first-line tool for malaria diagnosis. Microscopy remains essential for confirmation and detection of mixed infections. PCR, though very sensitive, has limited routine use due to its cost and infrastructure requirements. An RDT-first strategy with microscopy confirmation is therefore the most practical and WHO-recommended approach.

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CONFLICT OF INTEREST

None

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Declared none

AUTHORS CONTRIBUTION

Umer Adnan: Conceptualization, acquisition of data, interpretation of data, manuscript drafting, final approval, accountable for all aspects of publication.

Mahadev Harani: Conceptualization, supervision, data acquisition support, critical review of manuscript, final approval.

Sadia Talib: Manuscript writing, final approval, accountable for all aspects of publication.

Maliha Saad: Substantial contributions to study design and concept, final approval, accountable for all aspects of publication.

Tahera Kadir: Substantial contributions to analysis and interpretation of data, final approval, accountable for all aspects of publication.

Bakhtawar Akbar: Substantial contributions to study design and concept, final approval, accountable for all aspects of publication

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