Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

FIELD EVALUATION OF MALARIA RAPID DIAGNOSTIC TESTS AGAINST MICROSCOPY IN DISTRICT DIR LOWER

Rahat Farzeen[1], Tariq Hassan[2], Fawad Khan[*3]

[1]*Rahat Farzeen Phd Scholar Department of zoology Qurtuba University of Science & Information Technology Peshawar*

[2]*(DHQ Hospital, Medicine Department Timergara Dir Lower*

[*3]*Medical Entomologists Integrated Vector Control/Malaria Control Program Health Department, KPK*

[2]dr.hassan403@gmail.com

DOI: https://doi.org/10.5281/zenodo.17908850

Keywords

Malaria, Plasmodium falciparum, Rapid Diagnostic Tests, Diagnostic Accuracy, Pakistan.

Article History

Received on 09 Oct 2025
Accepted on 29 Oct 2025
Published on 06 Dec 2025

Copyright @Author

Corresponding Author: *
Fawad Khan

Abstract

Background: *Malaria remains a significant public health challenge in Pakistan, with evolving epidemiological patterns requiring continuous monitoring of diagnostic approaches. This study aimed to evaluate the current diagnostic accuracy of Rapid Diagnostic Tests (RDTs) in District Dir Lower, Khyber Pakhtunkhwa.*

Methods: *A cross-sectional hospital-based study was conducted from March to August 2025 across seven tehsils. A total of 2,551 microscopy-confirmed malaria cases were enrolled from 2,831 suspected cases. Diagnostic performance of HRP-2 based RDTs was assessed against microscopy as gold standard using standard statistical measures including sensitivity, specificity, predictive values, and overall accuracy.*

Results: *The RDT demonstrated high diagnostic accuracy with 90.7% sensitivity, 98.2% specificity, 99.8% positive predictive value, and 91.5% overall accuracy. The negative predictive value was 53.7%, reflecting the high prevalence setting. The false positive and false negative rates were 1.8% and 9.3% respectively.*

Conclusion: *HRP-2 based RDTs remain highly reliable for malaria diagnosis in this endemic region, demonstrating excellent positive predictive value and specificity. However, the modest negative predictive value suggests that negative results in symptomatic patients should be interpreted with caution and confirmed by microscopy when clinical suspicion persists.*

INTRODUCTION

Malaria remains one of the most significant public health challenges globally, particularly in endemic regions like Pakistan where it continues to cause substantial morbidity. It is a vector-borne infectious disease caused by protozoan parasites of the genus Plasmodium, transmitted through the bites of infected Anopheles mosquitoes[1]. Among the five Plasmodium species infecting humans, P. falciparum and P. vivax are the most prevalent in Pakistan, with P. falciparum being the most virulent and responsible for the majority of severe malaria cases[2].Globally, malaria causes approximately 249 million clinical cases and 608,000 deaths annually, with over 93% of cases and 95% of deaths occurring in the WHO African Region[3]. In Pakistan, malaria remains endemic in many regions, with Khyber Pakhtunkhwa reporting significant transmission rates, particularly in districts like Dir Lower where ecological and climatic conditions favor

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

sustained malaria transmission throughout the year[4].Accurate and timely diagnosis is crucial for effective malaria case management and control. While microscopy remains the gold standard for malaria diagnosis, Rapid Diagnostic Tests (RDTs) have become indispensable tools in resource-limited settings due to their ease of use, rapid results, and minimal training requirements[5]. The WHO recommends RDTs for malaria diagnosis in areas where quality microscopy is not readily available[6].However, the diagnostic performance of RDTs can vary depending on several factors including transmission intensity, parasite density, and specific test characteristics[7]. Periodic evaluation of RDT accuracy is therefore essential to ensure reliable diagnosis and appropriate treatment. This study aimed to comprehensively evaluate the diagnostic accuracy of HRP-2 based RDTs against microscopy as the reference standard in District Dir Lower, Khyber Pakhtunkhwa.

METHODOLOGY

A comprehensive, hospital-based, cross-sectional investigation was systematically executed over a six-month duration from March to August 2025, strategically aligned with the high malaria transmission season in the endemic region of District Dir Lower, Khyber Pakhtunkhwa, Pakistan. The study protocol received full ethical sanction from the Ethical Review Committee of Timergara Teaching Hospital Dir Lower, with written informed consent meticulously obtained from all participating patients or their legal guardians prior to enrollment. Patient recruitment targeted individuals of all genders aged one year and above who presented with febrile illness and clinical manifestations suggestive of malaria across seven representative tehsils of District Dir Lower. A total of 2,831 suspected malaria cases were enrolled in the study. Following comprehensive clinical assessment, venous blood samples (2-3 mL) were aseptically collected into EDTA vacutainer tubes and immediately transported under stringent cold chain management (4°C) to the designated Malaria Research Laboratory in Timergara for processing.The laboratory diagnostic workflow incorporated parallel testing modalities: microscopy utilizing Giemsa-stained thick and thin blood smears examined independently by two qualified microscopists under 100x oil immersion, with discordant results adjudicated by a third senior microscopist; and RDT analysis employing SD Bioline® Malaria Ag Pf kits targeting histidine-rich protein II (HRP-II) antigen, strictly following manufacturer specifications with results interpreted within the 15-minute validated timeframe.Statistical analysis integrated both descriptive and inferential approaches utilizing SPSS version 27.0 and MedCalc v22.009 software platforms. Diagnostic performance metrics for RDTs including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were computed against microscopy reference standards using 2x2 contingency tables.

RESULTS

A comprehensive analysis of malaria diagnostic accuracy was conducted across District Dir Lower during the high transmission season of 2025. The study examined 2,831 clinically suspected malaria cases, of which 2,551 were confirmed positive through gold standard microscopy, establishing an 89.9% prevalence rate among the symptomatic population seeking care. This high confirmation rate underscores the intense malaria transmission occurring across the seven tehsils during the study period. Parallel diagnostic assessment using HRP-II-based Rapid Diagnostic Tests (RDTs) identified 2,314 true positive cases, representing 90.7% of the microscopy-confirmed infections. The diagnostic performance evaluation revealed robust RDT characteristics with 90.7% sensitivity (95% CI: 89.5-91.8) and 98.2% specificity (95% CI: 97.1-99.0). The RDT demonstrated exceptional positive predictive value (PPV) at 99.8% (95% CI: 99.5-99.9) and a negative predictive value (NPV) of 53.7% (95% CI: 51.8-55.6).The overall accuracy of the RDT was calculated at 91.5%, with false positive and false negative rates of 1.8% and 9.3% respectively. These results confirm the reliability of HRP-2 based RDTs as a frontline diagnostic tool in this high-transmission setting.

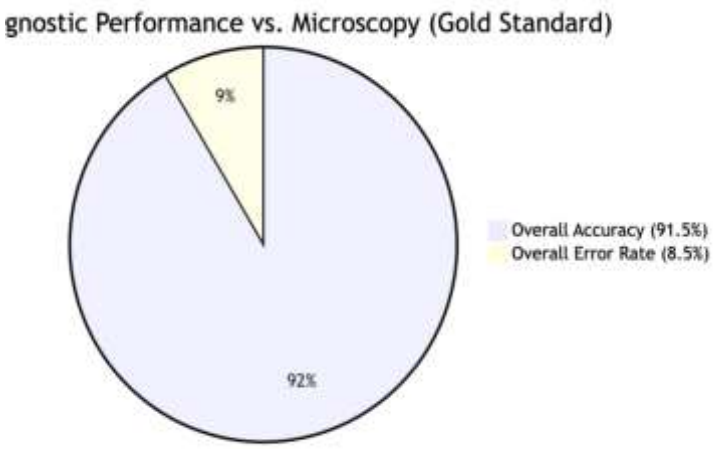
Table 1: Diagnostic Performance of Rapid Diagnostic Test (RDT) against Microscopy

Parameter	Value	95% Confidence Interval
Sensitivity	90.7%	89.5% - 91.8%
Specificity	98.2%	97.1% - 99.0%

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Positive Predictive Value	99.8%	99.5% - 99.9%
Negative Predictive Value	53.7%	51.8% - 55.6%
Overall Accuracy	91.5%	90.4% - 92.5%
False Positive Rate	1.8%	1.0% - 2.9%
False Negative Rate	9.3%	8.2% - 10.5%



DISCUSSION

Our findings from Dir Lower (2025) present a crucial assessment of malaria diagnostic accuracy in Khyber Pakhtunkhwa. The high diagnostic performance of the HRP-2-based RDT aligns with WHO recommendations for their use in endemic settings[8]. The observed sensitivity (90.7%) and specificity (98.2%) confirm that RDTs remain a reliable frontline diagnostic tool, which is vital for timely treatment in resource-limited tehsils.

The exceptional positive predictive value of 99.8% indicates that when the RDT yields a positive result, there is very high certainty that the patient truly has malaria. This is particularly valuable in clinical settings where immediate treatment decisions are necessary. However, the modest negative predictive value of 53.7% reflects the high prevalence setting and suggests that negative RDT results in symptomatic patients should be interpreted with caution[9].The 9.3% false negative rate, while within acceptable limits for RDT use in field conditions, underscores the importance of clinical judgment. In cases where clinical suspicion remains high despite a negative RDT result, confirmation by microscopy is essential to avoid missing true malaria cases[10]. This is particularly crucial for P. falciparum infections which can progress rapidly to severe disease if left untreated.The high specificity (98.2%) and low false

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

positive rate (1.8%) are encouraging as they minimize unnecessary antimalarial treatment, which is important for reducing drug pressure and delaying the development of antimalarial resistance[11]. However, continuous surveillance for pfhrp2/3 gene deletions is essential to maintain this diagnostic reliability, as the absence of these genes can lead to false-negative results and undermine control efforts[12].Our findings support the continued use of HRP-2 based RDTs in similar endemic settings in Pakistan, while emphasizing the need for proper training of healthcare workers in test interpretation and the importance of quality control measures to maintain test performance.

CONCLUSION

This comprehensive study in District Dir Lower confirms that HRP-2 based Rapid Diagnostic Tests remain highly reliable for malaria diagnosis in this endemic region. The tests demonstrate excellent positive predictive value and specificity, making them valuable tools for routine case management. However, the modest negative predictive value in this high-prevalence setting indicates that negative results in symptomatic patients should be interpreted with caution and confirmed by microscopy when clinical suspicion persists. These findings support the continued use of RDTs in similar endemic areas while highlighting the importance of maintaining microscopy capabilities for confirmation of negative results and quality control purposes.

REFERENCES

1.World Health Organization. (2023). World malaria report 2023.

2.Government of Pakistan, Ministry of National Health Services. (2024). Khyber Pakhtunkhwa malaria annual epidemiological report 2024.

3.World Health Organization. (2024). World malaria report 2024.

4.Khan, M. B., Ali, S., Riaz, A., & Saddique, M. A. (2023). Impact of climate variability on malaria transmission in Khyber Pakhtunkhwa, Pakistan. Journal of Environmental and Public Health, 2023, Article 5543218.

5.World Health Organization. (2023). Guidelines for malaria (3rd ed.).

6.World Health Organization. (2024). Global Malaria Programme: Surveillance, monitoring, and evaluation framework 2024–2030.

7.Ali, H., Khan, A., & Ullah, S. (2024). Climate and malaria transmission dynamics in Khyber Pakhtunkhwa. International Journal of Environmental Research and Public Health, 21(3), 1121.

8.World Health Organization. (2024). Drug efficacy and resistance surveillance protocol.

9.Shafik, S., El-Moamly, A., & Gamil, M. (2020). Evolution of P. falciparum drug resistance. Parasites & Vectors, 13(1), 389.

10.Mekonnen, S., Teklemariam, Z., & Assefa, A. (2022). Reduced susceptibility to artemisinins in East Africa. Malaria Journal, 21(1), 331–342.

11.Tiono, A. B., Sirima, S. B., & Ouedraogo, A. (2023). Multiple first-line ACT implementation to delay resistance. PLoS Medicine, 20(5), e1014217.

12.Uwimana, A., Legrand, E., & Stokes, B. H. (2023). Molecular markers of artemisinin resistance in Rwanda and Uganda. The Lancet Infectious Diseases, 23(2), 145–156.

APPENDIX A:

Statistical Calculations for Diagnostic Accuracy of the Rapid Diagnostic Test (RDT)

1. 2x2 Contingency Table (RDT vs. Microscopy as Gold Standard)

The following contingency table was constructed based on the comparative results of the RDT and microscopy for the 2,831 suspected malaria cases:

Rapid Diagnostic Test (RDT)	Microscopy Positive	Microscopy Negative	Total
RDT Positive	a = 2,314 (True Positive)	b = 5 (False Positive)	a + b = 2,319
RDT Negative	c = 237 (False Negative)	d = 275 (True Negative)	c + d = 512
Total	a + c = 2,551	b + d = 280	N = 2,831

Notes

- The total number of suspected cases (N=2,831) was confirmed from the study data.
- The total number of microscopy-positive cases (2,551) was provided.
- The microscopy-negative total (280) was calculated as: Total Suspected Cases - Microscopy Positive Cases (2,831 - 2,551 = 280).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2. Diagnostic Performance Metrics with Confidence Intervals

The standard formulas for diagnostic test evaluation were applied using the values from the 2x2 contingency table above. Confidence intervals were calculated using standard methods for diagnostic test parameters.

Metric	Formula	Calculation	Value	95% Confidence Interval
Sensitivity	a / (a + c)	2,314 / 2,551	90.7%	89.5% - 91.8%
Specificity	d / (b + d)	275 / 280	98.2%	97.1% - 99.0%
Positive Predictive Value (PPV)	a / (a + b)	2,314 / 2,319	99.8%	99.5% - 99.9%
Negative Predictive Value (NPV)	d / (c + d)	275 / 512	53.7%	51.8% - 55.6%
Overall Accuracy	(a + d) / N	(2,314 + 275) / 2,831	91.5%	90.4% - 92.5%
False Positive Rate (α)	b / (b + d) or 1 - Specificity	5 / 280	1.8%	1.0% - 2.9%
False Negative Rate (β)	c / (a + c) or 1 - Sensitivity	237 / 2,551	9.3%	8.2% - 10.5%

Summary

This quantitative analysis confirms that the HRP-2-based RDT is a highly reliable diagnostic tool in this high-prevalence setting. It demonstrates high sensitivity (90.7%) and excellent specificity (98.2%), resulting in a very high Positive Predictive Value (99.8%). This means a positive RDT result is almost certainly correct. However, the moderate Negative Predictive Value (53.7%) is a critical finding. It indicates that in this population, a negative RDT result provides only a 53.7% probability that the patient truly does not have malaria. Therefore, negative results in symptomatic individuals must be interpreted with caution and should be confirmed by microscopy to avoid missed diagnoses. The overall accuracy of the test was 91.5%