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Evaluation of MAL Card Rapid Test Versus Microscopic Methods for Malaria Diagnosis in Resource-Limited Rural Settings of India

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ABSTRACT

Background: Malaria remains a major public health concern in resource-limited rural settings, where access to reliable diagnostic tools is often constrained. The MAL Card rapid diagnostic test (RDT) offers a potential alternative to traditional microscopic methods, but its diagnostic accuracy in rural healthcare settings needs further evaluation.

Objective: This study aims to compare the performance of the Advantage MAL Card rapid test with conventional microscopy for malaria diagnosis in a rural hospital in India.

Methods: A retrospective study was conducted among suspected malaria patients. Blood samples were tested using both the MAL Card rapid test and microscopy (gold standard). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the MAL Card were assessed against microscopy.

Results: The MAL Card rapid test demonstrated a sensitivity of 94.12% and specificity of 99.54%, with a PPV of 80% and NPV of 99.88%. While the rapid test provided quicker results, microscopy remained more accurate in detecting malaria cases.

Conclusion: The MAL Card test proves to be a reliable and efficient diagnostic tool for malaria in resource-limited rural settings, offering high sensitivity, specificity, and ease of use. Its rapid turnaround time makes it a valuable first-line diagnostic method, particularly where microscopy is inaccessible. However, the risk of false positives necessitates confirmatory testing in certain cases to ensure accurate diagnosis and treatment. Despite this limitation, integrating the MAL Card into malaria control programs can enhance surveillance, support timely intervention, and contribute to reducing the malaria burden in endemic regions.

KEYWORDS: Malaria, MAL Card, Microscopy, Rapid Diagnostic Test, Rural Healthcare

INTRODUCTION

Malaria remains a significant public health challenge globally, particularly in endemic regions of sub-Saharan Africa and South Asia, where timely and accurate diagnosis is critical for effective management and control[1,2]. According to the World Health Organization's World Malaria Report 2023, there were an estimated 249 million malaria cases and 608,000 deaths worldwide in 2022, with the African region accounting for 94% of the global burden.[3] Within the Southeast Asian region, particularly, India

contributes substantially to the malaria burden, reporting approximately 4.7 million cases in 2022[3,4]. In India, Plasmodium falciparum accounts for nearly 50% of infections and is associated with severe outcomes, including cerebral malaria and death.[4] Although *P. vivax* infections are rarely fatal, they pose an equally important challenge due to relapses and persistent morbidity[5,6]. Both species *P. vivax* and *P.falciparum* require prompt and accurate diagnosis to ensure effective treatment and prevent complications.

Microscopic examination of stained peripheral blood smears is regarded as the gold standard for malaria diagnosis[7]. Microscopy provides key advantages, including parasite identification, species differentiation, and quantification of parasitaemia[8]. However, its successful implementation relies heavily on well-trained personnel and fully functioning laboratory infrastructure, both of which are often lacking in resource-constrained, rural healthcare settings[9]. Moreover, the method is time-intensive and difficult to scale in healthcare systems burdened by high patient loads. In such settings, where skilled microscopists are scarce and laboratory facilities limited, delays in diagnosis can adversely impact clinical management and disease outcomes.

To address these challenges, rapid diagnostic tests (RDTs) such as the MAL Card have been developed as a simplified and field-friendly alternative for malaria diagnosis[10]. RDTs do not require sophisticated equipment or technical expertise and produce results within 15–20 minutes, making them particularly advantageous in rural areas[11]. However, concerns remain regarding their diagnostic performance, particularly in detecting low parasitaemia, identifying mixed Plasmodium infections, and distinguishing specific parasite species[12]. These limitations could compromise treatment decisions and disease control efforts in endemic settings.

Given the above considerations, there is a critical need for evidence-based evaluation of the diagnostic tools employed in resource-limited environments. This study compares the diagnostic performance of the MAL Card test with the gold standard microscopic slide method in a rural hospital setting. Parameters such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) will be analysed to assess the reliability and accuracy of the MAL Card for routine malaria diagnosis. By identifying the strengths and limitations of both methods, this study aims to provide actionable insights for optimizing diagnostic strategies in high-burden rural settings. The findings will contribute to improved malaria detection, timely treatment initiation, and strengthened control measures, particularly in healthcare systems with limited infrastructure and workforce.

MATERIALS AND METHODS

Study Design

This retrospective study was conducted in a Government hospital in Kannauj, over a three-month period from July 2024 to September 2024 after obtaining permission from institutional ethical committee. Data were retrieved from hospital records, including diagnostic test results for both the MAL card and slide methods.

Inclusion and Exclusion Criteria

The inclusion criteria comprised patients who presented with febrile illness and a clinical suspicion of malaria. Patients were screened with both MAL card and slide test results available for comparison. Additionally, demographic information, including age and gender, was required for each patient to ensure comprehensive data analysis. The exclusion criteria eliminated cases in which patients had received

malaria treatment prior to hospital admission, as prior treatment could influence test outcomes and skew comparative results.

Diagnostic Methods

a) **Microscopic Diagnosis Using Stained Thin and Thick Peripheral Blood Smears (PBS):** For microscopic diagnosis, both thin and thick smears were prepared on clean, grease-free glass slides. These slides were stained using Leishman stain by covering the smears with the stain for 2 minutes, followed by dilution with double the volume of buffered water for 7–10 minutes. After staining, the slides were rinsed, air-dried, and examined under an oil immersion objective (1000×) by trained microscopists. Thick smear was utilized to detect the presence of the parasites and thin smear used to identify the species of Plasmodium parasites.

b) **Advantage Mal Card Test:** Mal Card by J.Mitra and Co. Pvt., Ltd., is a rapid immunochromatographic assay for the detection of Plasmodium lactate dehydrogenase (pLDH) antigen, was performed according to the manufacturer's instructions. A 5 µL drop of whole blood was applied to the sample well of the test device, followed by the addition of four drops of buffer solution provided in the kit. Results were visually interpreted after 15 minutes. A positive result was indicated by the appearance of both a control and a test line, while a single control line signified a negative result. Tests lacking a control line were considered invalid and excluded from further analysis.

Data Analysis

Statistical analyses were performed using R version 4.4.1 (R Core Team, 2023), an open-source software environment for statistical computing (available at: https://www.R-project.org/), and JASP (Version 0.19.2). To assess the diagnostic accuracy of the MAL Card rapid test, we conducted receiver operating characteristic (ROC) curve to compare between the two diagnostic methods. We calculated the following diagnostic metrics: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR), accuracy, and false discovery rate (FDR). Likelihood ratios for positive and negative test results were also calculated. Microscopic detection served as the reference standard for all test parameter evaluations.

RESULTS

Study population demographics

A total of 1,793 patients suspected of malaria were screened for malarial parasites using both thick and thin peripheral blood smears (PBS) and the Advantage Mal Card from July 2024 to September 2024. This study enrolled symptomatic individuals from a rural hospital, representing a demographically diverse cohort. The age distribution ranged predominantly from 20 to 40 years, with an approximately equal proportion of male and female participants. All participants presented with clinical symptoms suggestive of malaria, such as fever, chills and headaches.

Parasite prevalence by microscopy and RDT

Malaria prevalence was assessed using microscopy and the MAL Card rapid diagnostic test (RDT). The distribution of malaria cases identified by the MAL Card and peripheral blood smear microscopy is detailed in Table 1.

Table 1:Malaria Status by Mal Card and Peripheral Blood Smear's Microscopy.

Mal Card	**Microscopy**	**Result**	**Frequency(n)**	**(%)**
Negative	Negative	True negative	1751	97.7
Negative	Positive	False negative	2	0.1
Positive	Negative	False positive	8	0.4
Positive	Positive	True positive	32	1.8

Microscopy confirmed Plasmodium infection in 34 of the 1,793 participants (1.9%), while the MAL Card identified 40 positive cases (2.2%). The confirmed cohort of 34 patients consisted of 32 true positives and 2 false negatives. This cohort had a gender composition of 53% females and 48% males, with a mean age of 16.5 years (Table 2).

Table 2: Demographics of the positive patients

Age (Yrs)	***Gender***		***Total (%)***
	M (%)	**F (%)**	
>1	1 (2.9)	1 (2.9)	2 (5.8)
1 -10	1 (2.9)	4 (11.7)	5 (14.7)
10 - 20	8 (23.5)	8 (23.5)	16 (50)
20 - 30	5 (14.7)	1 (2.9)	6 (17.64)
30 - 40	2 (5.8)	1 (2.9)	3 (8.82)
>40	1 (2.9)	1 (2.9)	2 (5.8)
Total	**18 (52.9)**	**16 (47.1)**	**34 (100)**

Among these confirmed cases, 97.1% were positive for *P. vivax* only, while 2.9% were positive for *P. falciparum*. No cases of mixed infection were observed. The relationships between the malaria diagnostic methods among the two malaria species can be observed in Figure 1.

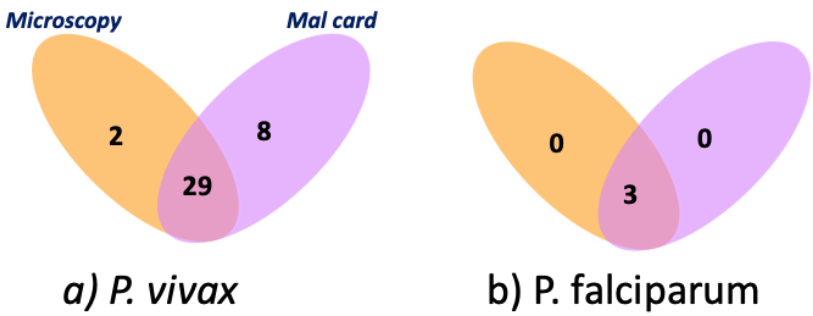

Figure 1: Venn diagrams show a summary of the relationships between the malaria diagnostic methods among the two malaria species

Performance Analysis

Table 3: Performance analysis of the Mal card

Metric	Value (%)
Sensitivity	94.12 %
Specificity	99.54 %
Positive Predictive Value (PPV)	80.00 %
Negative Predictive Value (NPV)	99.88 %
False Positive Rate (FPR)	0.46 %
Accuracy	99.44 %
False Discovery Rate (FDR)	20.00 %
Likelihood Ratio For Positive Test	204.58
Likelihood Ratio For Negative Test	0.0591
Youden's J-Index	93.66

The metrics for the performance of the Mal card is mentioned in Table 3. The sensitivity of the MAL Card test was found to be approximately 94.12%, indicating that the test correctly identified 94.12% of true malaria cases. The specificity was 99.54%, demonstrating that the test accurately identified 99.54% of individuals who did not have malaria. The positive predictive value (PPV) was calculated to be 80.00%, meaning that 80% of those who tested positive were true positives, while the negative predictive value (NPV) was very high at 99.88%, showing that nearly all negative test results were true negatives. The false positive rate (FPR) was low at 0.46%, indicating that false positives were rare. Overall, the accuracy of the MAL Card test was approximately 99.44%, reflecting its strong performance in the study. The false discovery rate (FDR) was 20.00%, showing that 20% of positive test results were false positives. The likelihood ratio for a positive test (LR+) was 204.58, indicating a strong increase in the likelihood of malaria when the test was positive, while the likelihood ratio for a negative test (LR-) was 0.0591, signifying a strong decrease in the likelihood of malaria when the test was negative. The Youden's J-index, calculated to be 93.66%, confirms the test's high diagnostic accuracy, balancing sensitivity and specificity effectively.

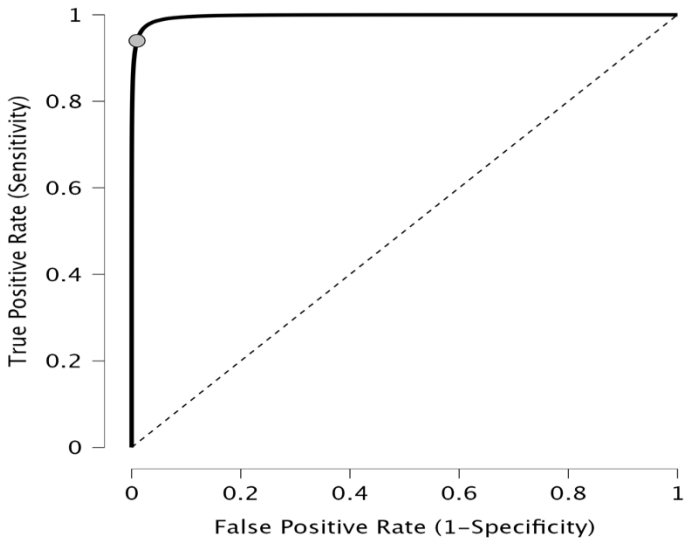

Figure 2: Receiver Operating Characteristic (ROC) curve for the MAL Card test. The curve demonstrates the relationship between sensitivity and false positive rate across various threshold

values. The Area Under the Curve (AUC) is >0.9, indicating excellent diagnostic performance of the MAL Card in distinguishing malaria-positive cases from malaria-negative cases.

The ROC curve for the MAL Card test (Figure 2) illustrates its diagnostic performance. The curve demonstrates a near-perfect shape, reflecting the high sensitivity and specificity metrics. The Area Under the Curve (AUC) was calculated > 0.9, indicating excellent overall diagnostic ability of the MAL Card in distinguishing between malaria-positive and malaria-negative cases. This high AUC highlights the utility of the MAL Card as a reliable diagnostic tool for malaria.

DISCUSSION

The MAL Card test is a rapid, qualitative, solid-phase immunochromatographic assay that detects *Plasmodium Lactate Dehydrogenase* (pLDH), an enzyme specific to *Plasmodium* species[13]. This test targets both *Plasmodium falciparum* and non-*falciparum* species such as *P. vivax, P. malariae,* and *P. ovale*, making it a versatile tool in malaria diagnostics[13]. The detection of pLDH, a stable and reliable biomarker across the parasite's lifecycle, underpins the working principle of the MAL Card test, allowing for accurate diagnosis in infectious cases.

The sensitivity of the MAL Card test, as observed in this study, was 94.12%, highlighting its ability to correctly identify the majority of malaria cases. Its high specificity (99.54%) ensures minimal false-positive results, providing strong diagnostic reliability. The test's negative predictive value (NPV) of 99.88% further emphasizes its utility in ruling out malaria with high confidence. While its positive predictive value (PPV) was 80.00%, indicating occasional false positives, this limitation is not uncommon for rapid diagnostic tests (RDTs) and is outweighed by the test's rapid and broad applicability. The high diagnostic accuracy and favourable likelihood ratios—204.58 for positive tests and 0.0591 for negative tests—further validate the MAL Card's effectiveness as a diagnostic tool.

The Receiver Operating Characteristic (ROC) curve was used to assess the diagnostic performance of our model. The ROC curve provides an effective evaluation of the trade-off between sensitivity (true positive rate) and false positive rate at various classification thresholds. Our analysis revealed an impressive Area Under the Curve (AUC) value of >0.9, indicating excellent discriminatory ability of the model in distinguishing between positive and negative cases. An AUC value greater than 0.9 suggests that the model exhibits high accuracy and reliability in its diagnostic predictions[14].

The MAL Card test is particularly helpful in resource-limited and rural settings where traditional microscopy may not be feasible. Its ease of use, requiring minimal training and no advanced laboratory infrastructure, makes it ideal for deployment in field conditions. The test provides results within minutes, allowing healthcare providers to initiate prompt and appropriate treatment, which is essential for controlling disease progression and reducing malaria morbidity.

Overall, the MAL Card test bridges critical gaps in malaria diagnostics by providing a sensitive, specific, and user-friendly tool that can be easily integrated into malaria control programs. By detecting infections promptly and reliably, particularly in endemic and resource-poor areas, the MAL Card test contributes significantly to the global fight against malaria, saving lives and improving health outcomes. Thus making it as valuable first-line diagnostic tool.

CONCLUSION

The findings of this study underscore the MAL Card test as an effective and promising diagnostic tool for

malaria, especially in remote and resource-limited regions. Its demonstrated high sensitivity, specificity, and overall accuracy make it a valuable resource for identifying malaria infections and ruling out non-malaria causes, making it well-suited to rural areas with limited access to traditional diagnostic methods. The rapid turnaround time and user-friendly design further strengthen the case for its adoption in low-resource settings, offering a notable advantage over conventional microscopy, which requires skilled personnel and infrastructure.

Nevertheless, the occurrence of false positives, as indicated by the false discovery rate, necessitates caution and highlights the importance of confirmatory testing in certain instances. This step is crucial for minimizing the risk of misdiagnosis and ensuring appropriate treatment. Despite these limitations, the MAL Card test offers substantial benefits as a first-line diagnostic tool. When integrated into malaria control initiatives, it has the potential to improve disease surveillance, enhance timely diagnosis, and support effective treatment strategies. In doing so, it plays a vital role in advancing efforts to reduce malaria's burden in endemic regions worldwide.

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