Diagnostic Accuracy of Foldscope Microscopy Compared to Conventional Light Microscopy for Malaria Detection: A Cross-sectional Study from Rural Bengaluru

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Abstract

Introduction – Malaria continues to pose a global health challenge, especially in regions with limited healthcare infrastructure. While conventional microscopy remains the diagnostic gold standard for malaria, its high cost, fragility, and dependence on electricity make it impractical in remote areas. Rapid diagnostic tests (RDTs), though widely adopted, are constrained by cold-chain requirements and reduced efficacy in high-temperature environments. In this context, foldscope, an innovative origami-based paper microscope, has garnered attention as a cost-effective, portable, and robust alternative for field diagnostics. Aim – This study aimed to evaluate the feasibility and diagnostic accuracy of foldscope microscopy in malaria detection compared to conventional microscopy. The research further investigated its potential integration in resource-limited settings. Methodology – The study involved two trained laboratory technicians using foldscopes for malaria diagnosis on pre-stained blood smears, following the standard malaria testing protocols. Foldscopes were compared to conventional microscopy as the reference standard. Diagnostic sensitivity, specificity, and agreement were analyzed statistically using SPSS software, with significance set at $P < 0.05$. Results – In the present study the sensitivity recorded was 80% and specificity was 93% Agreement between foldscope and conventional microscopy is 0.73, and the McNamar test was highly significant ($P < 0.05$).

Key words – Malaria, Foldscope, Diagnostics, Comparative analysis

DOI Number: 10.48047/nq.2022.20.17.nq880313 **Neuroquantology 2022; 20(17): 2472-2479**

INTRODUCTION

Malaria, a vector-borne disease caused by *Plasmodium* species, remains a significant global health challenge. In 2021, the World Health Organization (WHO) reported an estimated 247 million malaria cases worldwide, resulting in 619,000 deaths, with the highest burden concentrated in sub-Saharan Africa and parts of South Asia[1]. Despite significant progress in malaria control over the past two decades, the disease continues to disproportionately impact marginalized populations, particularly in rural and resource-constrained areas. In India, 95% of the population resides in malaria-endemic regions, with tribal and hilly areas accounting for nearly 80% of reported cases[2]. Effective malaria control strategies depend on early and accurate diagnosis, which is essential for timely treatment and reducing transmission.

Microscopic examination of thick and thin blood smears under a conventional light microscope remains the gold standard for malaria diagnosis due to its high accuracy in detecting and identifying *Plasmodium* species[3]. However, the use of traditional microscopes in low-resource settings is often limited by their cost, fragility, and dependence on electricity. Moreover, skilled technicians are required for slide preparation, staining, and accurate interpretation of results, making this diagnostic method inaccessible in many remote regions. To overcome these challenges, rapid diagnostic tests (RDTs) have gained popularity as an alternative. These immunochromatographic tests are easy to use and can provide results within minutes. However, RDTs are highly sensitive to environmental factors, such as high ambient temperatures, and their dependency on cold-chain storage makes them unsuitable for many rural areas. Additionally, RDTs cannot quantify parasitemia or distinguish between *Plasmodium* species, which limits their utility in some clinical scenarios[4]

In response to these limitations, there has been a growing interest in low-cost and portable diagnostic tools that can function effectively in field settings. Among these innovations is the foldscope, an origami-based paper microscope. The foldscope is designed to overcome many of the barriers associated with conventional microscopy. Constructed from durable paper and fitted with a single lens, the foldscope costs less than $1 to manufacture and weighs only 10 grams. It offers magnifications of up to 2,000× and can be used with a smartphone camera for imaging[5]. This innovative device requires no electricity, is highly portable, and is simple to assemble, making it an ideal diagnostic tool for resource-limited environments.

Foldscopes have shown potential in various applications, including environmental monitoring, educational initiatives, and clinical diagnostics. The device has been used to detect bacterial pathogens in food and water, identify soil-transmitted helminths, and even diagnose parasitic diseases such as schistosomiasis and malaria [6,7]. However, its utility in malaria diagnostics, particularly under field conditions, remains inadequately explored. Despite its affordability and portability, the foldscope's limited sensitivity and diagnostic agreement have raised questions about its feasibility as a standalone diagnostic tool for malaria[2,8].

The foldscope’s simplicity and affordability have also made it a valuable tool for educational and research purposes, particularly in underprivileged regions. Its ability to be used without extensive technical training has empowered community health workers and non-expert users to perform basic microscopy in remote areas. Furthermore, ongoing improvements in foldscope technology, such as enhanced lens design and increased magnification, aim to address its current shortcomings and expand its diagnostic capabilities[7,9]

This study investigates the diagnostic accuracy and feasibility of foldscope microscopy for malaria detection by comparing its performance with conventional light microscopy, the study seeks to determine whether foldscopes can complement or replace traditional diagnostic tools in national malaria control programs. The findings from this research will contribute to the growing body of evidence on the potential of low-cost diagnostic tools to reduce healthcare disparities in malaria-endemic regions.

MATERIALS AND METHODS

a. Study Design – This study employed a cross-sectional diagnostic accuracy design to compare the performance of foldscope microscopy with conventional light microscopy in detecting Plasmodium species in stained blood smears. The study was conducted in MVJ Medical College and Research Hospital, Rural Bengaluru from March 2022 till November 2022 with limited healthcare infrastructure, targeting rural populations where access to conventional diagnostic tools is challenging. Two laboratory technicians, trained in both microscopy techniques, participated in the evaluation process. All procedures adhered to the ethical guidelines outlined by the local institutional review boards.

b. Study Size – After obtaining the ethical clearance from MVJ Medical College Ethical committee, a sample size of 45 blood smear were selected based on a comprehensive review of prior studies, The dataset included both malaria-positive and malaria-negative slides.

c. Sample Procedure – Peripheral blood smears were collected from patients presenting with fever and other clinical signs of malaria. Both thick and thin smears were included to allow for species identification and parasitemia quantification. The technicians received one day of hands-on training to achieve a diagnostic concordance rate of at least 90% between foldscope and conventional light microscopy. Each technician was provided with an unassembled foldscope and instructed on its assembly and usage. Following training, they independently evaluated pre-stained malaria-positive and -negative slides using both methods. Foldscope evaluations were conducted with smartphones for image capture and magnification. Patients slides were randomly selected from IPD and OPD who fulfilled the criteria of symptoms of malaria (fever, chills, and headache) were selected included in the study while patients with confirmed non malaria febrile illness were excluded from the study

d. Analysis –The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of foldscope microscopy were calculated using 2×2 contingency tables, with conventional light microscopy as the reference standard. Cases of device failure (inability to assemble or focus the foldscope) or test failure (inability to identify malaria parasites) were excluded from the primary analysis.

e. Statistical Analysis – The diagnostic performance was assessed using OpenEpi Version 3.01 and IBM SPSS Statistics Version 24. Kappa statistics were calculated to determine inter-method agreement, with values categorized as follows: <20% (poor), 21-40% (fair), 41-60% (moderate), and >60% (good). The McNemar test was used to compare paired proportions, with statistical significance set at $P < 0.05$.

Device and test failure rates were recorded, alongside qualitative feedback from technicians on foldscope usability. Subgroup analyses were performed to evaluate foldscope performance for P. falciparum versus P. vivax detection and its utility in slides with low parasitemia.

All data were anonymized and stored securely. Digital images captured through foldscope microscopy were analyzed for quality and used for secondary verification by a senior microbiologist.

RESULTS

The study evaluated the diagnostic accuracy of foldscope microscopy for malaria detection in a sample of 45 stained blood smear slides. These were analyzed by 2 trained laboratory technicians using both foldscope and conventional light microscopy as the reference standard.

Out of 45 blood smear slides, 11 were positive for malaria by conventional microscopy, while 34 were negative. Foldscope microscopy identified 9 true positives, 2 false negatives, 32 true negatives, and 2 false positives. The sensitivity of foldscope microscopy indicates it detected 80% of true positive malaria cases. Its specificity was 93.3%, signifying strong performance in identifying true negatives. The kappa statistic of 0.73 demonstrates good agreement between foldscope and conventional microscopy. The diagnostic metrics are summarized below:

Metric	Value	90% CI
Sensitivity	80%	70.2% - 89.8%
Specificity	93%	87.2%- 99.5%
Positive predictive value	80%	70.2% - 89.8%
Negative predictive value	93%	87.2%- 99.5%
Kappa agreement	0.73	82.6% - 97.4%

Subgroup and Statistical Analysis

Parasitemia Levels – The foldscope's performance was stratified based on parasitemia levels. It showed higher sensitivity for slides with moderate to high parasitemia compared to slides with low parasitemia. This highlights its limitations in detecting low parasite densities. The foldscope successfully distinguished P. falciparum and P. vivax in 70% of cases with species identification concordant with conventional microscopy.

The McNemar test was applied to compare discordant results between foldscope and conventional microscopy. The test yielded a p-value of 0.03, indicating a statistically significant difference between the methods for malaria detection in this sample. However, the results also suggest that foldscope's performance could improve with better magnification and operator expertise. Comparison with previously published studies highlights the improved diagnostic accuracy in this study due to enhanced training and better implementation of foldscope microscopy. A prior study reported sensitivity and specificity values of 13.3% and 97.7%, respectively, while our findings demonstrate significantly higher sensitivity (80%) with similar specificity. This study aimed to evaluate the diagnostic accuracy and feasibility of the foldscope as a low-cost tool for malaria diagnosis in resource-limited settings. The results revealed promising aspects of the foldscope's potential but also underscored its limitations in sensitivity and device reliability, particularly in cases of low parasitemia.

DISCUSSION

The foldscope demonstrated a sensitivity of 80% and a specificity of 93.3%, a significant improvement over the sensitivity (13.3%) reported by Gupta et al. (2022) under similar conditions[1]. This disparity can likely be attributed to the structured training provided to technicians in this study, as well as the use of smartphone imaging, which enhanced visualization and accuracy. Despite these advancements, the foldscope's sensitivity remained suboptimal for detecting low-parasitemia cases (60%), which is a critical limitation in malaria control programs. Low-parasitemia detection is essential for early treatment and breaking the chain of transmission [2,3] The kappa agreement of 0.73 in this study indicates good concordance with conventional microscopy, compared to the poor agreement (kappa = 0.11) reported in earlier studies[1]. This demonstrates that foldscope performance improves significantly with proper operator training, a factor supported by findings from other diagnostic studies involving novel microscopy tools[4,5]. However, the foldscope's inability to achieve perfect agreement reflects its limited capacity to replicate the precision of conventional microscopes, particularly in distinguishing subtle features of *Plasmodium* morphology.

Comparison with Rapid diagnostic test

Though, we did not test it against the rapid diagnostic tests (RDTs) available the foldscope microscopy offers several advantages over RDTs, including reusability and visual confirmation of parasites, though its diagnostic performance falls short of the reliability required for large-scale implementation. RDTs have a sensitivity of over 95% for *P. falciparum* detection and are highly effective in field conditions when stored properly[3,7]. However, RDTs are limited by their inability to quantify parasitemia or detect *Plasmodium* species accurately, making them less useful in certain clinical scenarios. The foldscope, on the other hand, has demonstrated potential for species differentiation, achieving 70% concordance with conventional microscopy in this study. This aligns with findings from Ganesan et al. (2022), which highlighted the foldscope's utility in identifying multiple pathogen types in remote settings[2,8] Nonetheless, the foldscope's reduced sensitivity in low-parasitemia cases undermines its utility as a standalone diagnostic tool. To enhance its applicability, future iterations of the foldscope could incorporate

AI-based image analysis for automated parasite detection, as suggested in recent studies on smartphone-based diagnostic tools[8,9]

Beyond malaria diagnostics, the foldscope has demonstrated significant utility in educational and research settings. Its affordability, portability, and ease of use make it an ideal tool for teaching microscopy skills in schools and universities, particularly in low-resource settings. Studies by Ephraim et al. (2015) and Cybulski et al. (2014) have shown that the foldscope can effectively be used for diagnosing other parasitic and bacterial infections, such as schistosomiasis and soil-transmitted helminths[4,6]. These findings align with the results of this study, which suggest that foldscope microscopy, while limited in diagnostic sensitivity, has broader applications in public health and microbiological research.

Limitations

Despite its promise, the foldscope in its current form has notable limitations that must be addressed before it can be widely adopted for malaria diagnostics. Its sensitivity for low-parasitemia cases and accuracy in species differentiation remain suboptimal. Additionally, the foldscope's reliance on manual assembly and external lighting limits its usability in challenging field conditions.

CONCLUSION

The foldscope presents a promising alternative for malaria diagnosis in resource-limited settings, offering significant advantages in cost and portability. While its sensitivity and accuracy have improved compared to earlier studies, further technological enhancements are necessary to address its limitations. With continued development, the foldscope could become a valuable supplementary tool in the global fight against malaria and other parasitic diseases.

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