

Comparative Performance of Microscopy, Loop-mediated Isothermal Amplification (LAMP) and Quantitative PCR for Malaria Detection: A Narrative Review

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ABSTRACT

Malaria still remains a global health concern and accurate and timely diagnosis was an important management. This narrative review compared the three methods typically used in detection of malaria, specifically, microscopy, loop-mediated isothermal amplification, and real-time PCR. With the aid of PubMed and Google Scholar, comprehensive literature was done with the studies within 2015 to 2026 focusing on comparing at least two methods of detection using whole blood sample. Performance of each method must be included, specifically, sensitivity, specificity, limit of detection, cost, turnaround time and equipment needed. Moreover, microscopy has been a consistent and important screening method with symptomatic cases. But it failed to detect asymptomatic with low parasitic count. LAMP provided molecular-like sensitivity with increased field applicability and logistics, and consistently achieves sensitivities of 83.3% to 100% against PCR. It is less tedious than qPCR and personnel was not required to be exceptionally trained. Real-time PCR remained the most sensitive and specific and detected lowest parasitic densities. No single method was best for all scenarios, but there was an applicable method if categorizing the specific need of the facility or the patients for the specific situation was done.

Keywords: LAMP, Loop-mediated Isothermal Amplification, Malaria, Microscopy, *Plasmodium*, qPCR, Sensitivity, Specificity.

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INTRODUCTION

Malaria was a vector-borne disease caused by genus *Plasmodium*. The parasite was transmitted by Anopheles mosquito through biting or taking a blood from its another host, humans (Fikadu and Ashenafi, 2023). The parasite is composed of five known species infecting the intermediate host, humans, which are *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium knowlesi* (Fikadu and Ashenafi, 2023; Li *et al.*, 2025). They are endemic in places around tropics and sub-tropics.

Malaria spreads when Anopheles mosquito injected saliva with the malaria sporozoite, which is the infective form to humans. This will then infect liver cells and mature into schizonts. The development to schizont will end through its rupture which causes the release merozoites into the bloodstream leading to the infection of other red blood cells. Erythrocyte involvement initiates

the erythrocytic stage and ends the exo-erythrocytic or liver stage of multiplication. Merozoites develop to immature trophozoites and will then mature to mature trophozoites eventually develop to schizont or diagnostic gametocytes. The gametocytes are also the infective stage for the uninfected Anopheles mosquito when it takes a blood meal which initiates the sporogonic cycle wherein the parasite in the mosquito further develops (CDC - DPDx - Malaria, n.d.; Fikadu and Ashenafi, 2023).

The rupture of the schizont in the erythrocytic stage triggers the appearance of fever and chills symptoms and worse are severe complications like capillary obstruction, cerebral malaria, severe malarial anemia, coma and death (Crutcher and Hoffman, 1996; Buck and Finnigan, 2023). There are 610,000 deaths in total among 282 million cases this 2024 according to World Health Organization World Malaria Report (2025) making malaria still one of the global burdens that we face in today's world. Amidst this, there is an observable decreasing trend of incidence case and mortality rate of malaria from 2000-2015 by 26% which happened due to massive prevention methods and treatment tools (*World Malaria Report*, 2025). But the progress has stalled from 2025-2024, 22.6% increase, due to various reasons and one is drug and insecticide resistance. Thus, accurate and early diagnosis remain important aspect in malaria management since treatment will not commence without reliable results and early



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detection (Amir *et al.*, 2018; Jain *et al.*, 2014; Mediavilla *et al.*, 2024). Also, severe symptoms development might be a product of delaying detection and treatment. This may lead to formation of complications and early death (Gupta and Wassmer, 2021) as early diagnosis makes malaria more curable (Singh and Sharma, 2014). Thus, availability of rapid detection tests, microscopy using the thick and thin smear, and other detection methods helps with management, surveillance and elimination efforts.

Overview of Diagnostic Methods

Microscopy of malarial smears remains the gold standard for detection and diagnosis of malaria parasite as per WHO and CDC (2024). Blood is extracted from a suspected patient and two sets of thick and thin smears are created which are stained and dried before viewing under the microscope. The parasite is present inside the erythrocyte in either immature trophozoite or ring, mature trophozoite, schizont, and gametocyte stage (Siahaan, 2018). Microscopy requires training and good microscope to accurately view and detect *Plasmodium spp.* by magnifying cells specifically erythrocytes making detection depends entirely upon the microscopists' or examiners' competence and training (Maturana *et al.*, 2022; Siahaan, 2018). However, it is still the cheapest to perform among the diagnostic methods discussed in this study due to limited and low-cost equipment needed (Maturana *et al.*, 2022).

Loop-mediated Isothermal Amplification (LAMP) is a molecular detection method used to amplify *Plasmodium* species nucleic acid without thermocycling equipment by using strand-displacing *Bst* DNA polymerase. It utilizes specifically designed primers of two or three sets (four or six primers) binding to six target sites of *Plasmodium* DNA sequence, which is the important aspect of the method, to displace loop structures. In 1 hr, LAMP could generate nucleic acid copies of up to 10. Moreover, it is inherently cost-effective and suitable for areas of limited equipment because it mainly requires centrifuge and heat block to provide constant temperature and no thermal cycler is needed (Yang *et al.*, 2026).

Moreover, real-time Polymerase Chain Reaction (qPCR) is a molecular technique that quantifies *Plasmodium* DNA by amplifying the target sequence in a thermal cycler. Quantification is done by Cycle threshold (Ct) value measurement, which correlates with the initial amount of target nucleic acid. Quantification is one of its best features and qPCR is currently the most sensitive method, capable of identifying as few as 1-10 parasites/ μ L. However, it is expensive since materials and equipment used are costly. These requirements are not typically available in a low-resource setting and utilizing those requires trained personnel (Siahaan, 2018; Slater *et al.*, 2022). It is also labor-intensive and time-consuming to perform (Slater *et al.*, 2022), making it a less utilized and less practical method in routine practice.

Rationale for a Narrative Review

Malaria is still one of the diseases that can be treated but end lives due to lack of preventing, misdiagnosis and late treatment although different diagnostic methods are available. Most of the studies available were focused on utilizing each method, microscopy, LAMP and qPCR, for detection. Also, there are limited narrative reviews about these techniques and usually they are focused on review of each method, and not comparing these three and lack of narrative synthesis based on performance like result accuracy, cost, availability and much more. This narrative review collects studies and evidences to compare each method to become a helpful tool for choosing technique for future researches and showcase strengths and weaknesses for future development of the methods for malaria detection.

Objectives

This study aims to determine the diagnostic performance of LAMP, qPCR and microscopy methods; highlight strengths and limitations of each method; identify clinical scenarios where one method may best be applicable; and propose future research priorities.

METHODOLOGY

A comprehensive search of the PubMed and Google scholar online databases was performed with search terms 'malaria or *Plasmodium*' AND 'Loop-mediated isothermal amplification' OR 'LAMP' AND 'qPCR' OR 'quantitative PCR' OR 'real-time PCR or microscopy or blood smear' AND 'diagnosis' OR 'detection' OR 'sensitivity' OR 'specificity'. The search is limited only for studies within 2015 to present which are English peer-reviewed primary paper or comparative studies that includes performance metrics such as sensitivity, specificity and limit of detection, at least one of the three on human blood samples. Case reports, editorials, conference abstracts without full data, animal studies or *in vitro* only DNA samples and used conventional PCR without quantification were excluded from this review. Moreover, selection was done through title or abstract screening followed by full-text review and snowball citation searching for additional relevant studies.

Thematic Sections

Analytic or Diagnostic Performance: Sensitivity, Specificity, and Limit of Detection

LAMP was compared with real-time PCR testing for detecting *Plasmodium* and the research of Cook *et al.* in 2015, showed 83.3% sensitivity and 99.7% specificity. Meanwhile, Tegegne *et al.* in 2017 results with 100% and 93.5% for LAMP and 90% and 98.7% for microscopy sensitivity and specificity, respectively, in comparison with nPCR. Moreover, Charpentier *et al.* in 2020 reported 99.6% specificity and 100% sensitivity for LAMP and 100% for both sensitivity and specificity for microscopy in

comparison with qPCR. However, Tambo *et al.* in 2018 used LAMP as reference method and PCR had 72.73% and 100% sensitivity and specificity, respectively. On the other hand, Benié *et al.* in 2022 compared LAMP and microscopy against nested PCR method which resulted to sensitivity of 93.3% and 20%, and specificity of 95.4% and 100%, respectively. Lastly, Ocker *et al.* in 2016 resulted with sensitivity of 100% and 99%, specificity of 93% and 100% microscopy and LAMP, respectively, compared with nPCR.

Detection of Low Density and Asymptomatic Infections

The data from these studies shows that conventional diagnostics must be of high sensitivity to accurately detect low parasitic density and in asymptomatic patients. Benié *et al.* in 2022 revealed that in mass screening that microscopy method detected asymptomatic prevalence at only 5.2% and LAMP identified 18.8%, which is a four-fold increase in detecting low parasitic count. Moreover, this is supported by another study of Cook *et al.* in 2015 wherein both LAMP and PCR can detect 1.8% of below 5 parasites per μL . These may be low percentages but, these two methods are better in detecting low amount of parasitic load. However, real-time PCR can detect ultra-low-density infections, less than 1-2 parasites/ μL (Tambo *et al.*, 2018; Charpentier *et al.*, 2020). But LAMP has been a practical method in the field with limited equipment and facilities (Cook *et al.*, 2015; Tegegne *et al.*, 2017) which can detect 83% to 100% of asymptomatic carriers (Cook *et al.*, 2015; Tegegne *et al.*, 2017; Charpentier *et al.*, 2020; Tambo *et al.*, 2018; Benié *et al.*, 2022; Ocker *et al.*, 2016).

Operational and Logistical Comparisons

LAMP used a method of boil-and-spin for DNA extraction that used 60 μL of whole blood and mixed it with an equal amount of extraction buffer. It was subjected at 95°C for 5 min after and proceeded with centrifugation. Therefore, with these steps, no commercial kits are required and template DNA could be produced in less than 10 min (Cook *et al.*, 2015). This characteristic was one of the important features of LAMP method compared with qPCR which required a tedious and time-consuming purifications or Chelex extractions that could be finished after an overnight incubation (Tambo *et al.*, 2018; Charpentier *et al.*, 2020). However, among all the methods, microscopy is the simplest to do since it only needs a microscope, but highly trained personnel must be available to accurately read the slides or samples.

Comparing time of testing, LAMP was accomplished and resulted to a readout in 60 to 90 min (Tambo *et al.*, 2018; Tegegne *et al.*, 2017) microscopy required approximately 60 min of staining and reading of the slide, but qPCR was extended up to 310 min (Tegegne *et al.*, 2017). Moreover, LAMP required only a simple heat-block or a water bath, centrifuge and UV lamp for fluorescence reading which could be put on a small facility

making it good for field applications (Cook *et al.*, 2015; Benié *et al.*, 2022). The studies also mentioned that even with less to no prior molecular experience, LAMP can be used and be proficient just after three days of theory and practice sessions. However, microscopy demands training to maintain the accuracy of reading the samples (Charpentier *et al.*, 2020). This was challenging since patients to be tested for malaria will not be everyday especially in non-endemic places which may cause less practice of the skill that may lead to inaccuracy through time (Table 1).

Cost Effectiveness and Health System Integration

Based on the study of Tambo *et al.* in 2018 stated that LAMP costs around \$0.70 per sample and qPCR costs \$5-\$7 per sample. This difference with the price per test is driven by the equipment required with the two methods. The LAMP requires heating block and a centrifuge while the qPCR needs thermocyclers alongside the trained personnel. This showed that LAMP equipment is a fraction of the price of the requirement materials for qPCR. Moreover, another study by Tambo *et al.* in 2018 which performed the LAMP method DNA extraction successfully by directly using a cut from nitrocellulose membrane which resulted to be comparable with the DBS-extracted DNA.

DISCUSSION

Synthesis of Findings

These different studies conducted in different places with diverse epidemiological settings gave overview of the performance of the three methods for malaria detection. The main finding was microscopy has been a consistent and important screening method especially with symptomatic cases. However, it failed to detect asymptomatic with low parasitic count. LAMP provided molecular-like sensitivity with increased field applicability and logistics. Real-time PCR remained the most sensitive and specific and detected lowest parasitic densities. This made the qPCR as a good reference method.

Specifically, the studies showed that microscopy has 9.3% 5 and 17.8% 6 sensitivity when they were compared to qPCR. Its performance is good but failed to detect low parasitemia which was vital especially for testing in non-endemic areas. LAMP consistently achieves sensitivities of 83.3% to 100% against PCR across multiple studies, effectively capturing four-fold more asymptomatic carriers than microscopy. Moreover, the method done for LAMP method is less tedious than qPCR and personnel was not required to be exceptionally trained but only required theoretical and practical exposure that would be accomplished within three days. It is more economic which could be used in low-resource area especially the equipment used were low-cost and easier to transport. However, microscopy remained the important method in identifying specific *Plasmodium spp.* identifier. Also, qPCR remained essential as confirmatory test which was essential with detection of ultra-low parasitemia

Table 1: Summary of the comparison of operation and logistics of three methods.

Feature	Microscopy	qPCR	LAMP
Equipment cost	Low	High	Moderate
Per-test cost	Low	High	Low-moderate
Time to result	30-60 min	2-4 hr	60-90 min
Cold chain needed	No	Yes (reagents)	Minimal
Skilled personnel	High	High	Low-moderate
Field deployable	Yes	No	Yes

or infection of less than 5 parasites/ μ L that could be missed by LAMP.

Strengths of the Existing Literatures

Majority of the studies compare the methods against the reference method, real-time PCR. The testing of the methods were done in different settings and scenarios, endemic area, non-endemic and samples with low-parasitic load.

Limitations of the Existing Literatures

The comparison among the three methods were rarely available in the studies. Also, the reference methods were varying depending on the study as some are based on qPCR, nPCR or even LAMP. This heterogeneity in reference method does not standardize the sensitivity and specificity results calculated. Moreover, results of microscopy were heavily reliant on the personnel and his or her training, length of exposure or practice and frequency of encountering the test. This means that it is reliant on the technician's expertise and may cause variation with the results of diagnostic performance. Also, sample size of the studies was limited especially with studies that focus on asymptomatic cases testing.

FUTURE RESEARCH DIRECTIONS

First was the comprehensive cost-effectiveness and budget impact analyses of the different methods including not just the facility and equipment requirement but also, training of personnel, equipment maintenance, and quality assurance. These were important to inform the policymakers and laboratory management to carefully choose the method of testing fit for their situation. Second, researches should be focused on creating point-of-care testing kits based on LAMP principle to be available in low-resource and far-flung areas that physical healthcare or laboratory facilities could not reach. Simple kits based on LAMP could be used as a screening or general detection of malaria, aiding early detection and management. Finally, stability of reagents especially, LAMP reagents when being exposed in varying and extreme field conditions to know the effects and alteration with testing accuracy.

CONCLUSION

Microscopy, among all the methods discussed, provide rapid results and suitable for symptomatic cases. It exhibited low sensitivity to low-density and asymptomatic parasitic infection. However, the method remained an important method for specifically identifying the species of malaria and quantification purposes. Also, the accuracy depends greatly from the trained personnel.

LAMP was optimal for molecular-based method for field use and monitoring patients. It gave high sensitivity, 83.3%-100%, against PCR methods, nPCR and qPCR. It could provide result with 60 to 90 min with the use of simple equipment that could be transported to areas with limited facilities. Moreover, reliability of results does not fully rely on the personnel expertise and training. Cost of testing a sample is relatively cheaper compared to the molecular test method used, PCR.

Real-time PCR remained the reference method and offered highest sensitivity but required demanding equipment and facility, and trained personnel that all contribute to its higher cost per testing. Also, turnaround time was longer compared with the mentioned methods above.

No single method was best for all scenarios but there was an applicable method if categorizing the specific need of the facility or the patients for the specific situation was done. Example was microscopy for facilities that offers malarial identification, qPCR for reference and quality assurance testing, and LAMP for mass screening and check asymptomatic cases. Moreover, combination of methods of testing.

ABBREVIATIONS

CDC: Centers for Disease Control and Prevention; **Ct:** Cycle threshold; **DBS:** Dried blood spot; **DNA:** Deoxyribonucleic acid; **LAMP:** Loop-mediated isothermal amplification; **LOD:** limit of detection; **nPCR:** Nested polymerase chain reaction; **PCR:** Polymerase chain reaction; **qPCR:** Quantitative polymerase chain reaction; **RDT:** Rapid diagnostic test; **UV:** Ultraviolet; **WHO:** World Health Organization.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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