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Comparison of Microscopic Accuracy, Rapid Diagnostic Test (RDT), Polymerase Chain Reaction (PCR), and Loop-Mediated Isothermal Amplification (LAMP) in Malaria Diagnosis: A Literature Review

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ABSTRACT: Malaria is an infectious disease that remains a global public health problem, especially in tropical and subtropical countries such as Indonesia. This disease is caused by the Plasmodium parasite, which is transmitted through the bite of infected female Anopheles mosquitoes. According to the 2024 World Malaria Report, there were approximately 249 million cases of malaria and 597,000 deaths worldwide, with Indonesia accounting for approximately 1.8 million cases or 46% of the total cases in Southeast Asia. This condition shows that malaria is still a major challenge in the national health system, especially in endemic areas such as Papua, Nusa Tenggara, and parts of Kalimantan. Rapid and accurate diagnosis of malaria is crucial in reducing morbidity and mortality rates. Peripheral blood microscopy is still considered the gold standard because it can identify Plasmodium species and assess the degree of parasitemia, but its sensitivity decreases in infections with low parasite density. Advances in diagnostic methods have led to the development of Rapid Diagnostic Tests (RDTs), which detect specific parasite antigens and provide rapid results, although the results can be affected by HRP2 gene mutations and reagent storage conditions. Furthermore, molecular methods such as Polymerase Chain Reaction (PCR) offer the highest sensitivity with the ability to detect up to 0.25–5 parasites/µL, but require advanced laboratory facilities. The latest innovation, Loop-Mediated Isothermal Amplification (LAMP), can amplify parasite DNA at a constant temperature of 60–65°C without a thermal cycler, with sensitivity and specificity reaching 95–99%. Therefore, this literature review highlights that a combination of conventional and molecular methods is essential to improve diagnostic accuracy and support malaria elimination efforts in Indonesia.

KEYWORDS: Malaria, Diagnosis, Microscopic, Rapid Diagnostic Test (RDT), Polymerase Chain Reaction (PCR), Loop-Mediated Isothermal Amplification (LAMP)

1. INTRODUCTION

Malaria is an infectious disease that remains a public health problem worldwide, especially in tropical and subtropical countries. The parasite that causes malaria belongs to the Plasmodium genus and is transmitted through the bite of an infected female Anopheles mosquito. Currently, there are five known species of Plasmodium that can infect humans, namely *P. falciparum, P. vivax, P. malariae, P. ovale, and P. knowlesi* (Kementerian Kesehatan, 2023). Based on World Malaria Report 2024, in 2023 there were an estimated 249 million cases of malaria and 597,000 deaths worldwide, with 94% of cases occurring in Africa. In Southeast Asia, Indonesia has the highest malaria burden, accounting for approximately 46% of total cases in the region, or around 1.8 million cases, most of which are caused by *P. falciparum and P. vivax*. This situation shows that malaria remains a major challenge for the national health system, especially in endemic areas such as Papua, Nusa Tenggara, and parts of Kalimantan.

Rapid and accurate diagnosis of malaria is a step toward reducing morbidity and mortality from this disease. With the development of science and technology, malaria diagnosis methods continue to undergo innovation from conventional techniques to modern approaches based on molecular biology (WHO, 2016). Peripheral blood microscopy is still the gold standard in malaria diagnosis because it can identify Plasmodium species and assess the degree of parasitemia. However, this method requires trained analysts and is highly dependent on the quality of the blood sample and the examiner's precision (CDC, 2024).

Efforts to improve efficiency and expand access to malaria diagnosis have led to the development of Rapid Diagnostic Tests

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(RDTs), which work by detecting parasite antigens such as Histidine-Rich Protein 2 (HRP2) and Plasmodium lactate dehydrogenase (pLDH). RDTs have the advantages of being easy to use, providing rapid results, and not requiring complex laboratory facilities. However, variations in sensitivity and specificity remain a challenge, mainly due to genetic differences between parasites and reagent storage conditions. In addition, the deletion of the pfhrp2 and pfhrp3 genes in Plasmodium falciparum has been reported to be one of the causes of an increase in false negative results in several endemic areas (Ngasala et al., 2024).

Advances in molecular technology have led to the development of diagnostic methods with higher sensitivity and specificity, such as Polymerase Chain Reaction (PCR) and Loop Mediated Isothermal Amplification (LAMP). Both methods are capable of detecting the presence of parasite DNA even at very low levels of parasitemia, which make them highly valuable for identifying submicroscopic infections. PCR is known to have high accuracy, however, its use remains limited to laboratories equipped with sufficient tools and resources. In contrast, the LAMP method offers a more practical alternative because DNA amplification is carried out at a constant temperature without the need for thermal cycling, so it can be applied in areas with limited facilities (Selvarajah et al., 2020).

The development of various malaria diagnostic methods requires a comprehensive review to assess the advantages, limitations, and potential applications of each technique in supporting early detection and efforts to eliminate this disease. Therefore, the purpose of this literature review is to thoroughly review the development and main characteristics of various malaria diagnostic methods, ranging from microscopic examination to molecular technologies such as PCR and LAMP.

2. METHODS

This literature review employed a narrative and descriptive-analytical methodology to synthesize current evidence regarding malaria diagnostic approaches. A systematic search of relevant literature was conducted using several electronic databases, including PubMed, Google Scholar, Science Direct, and Research Gate. The search utilized specific keywords such as *"malaria diagnosis," "rapid diagnostic test (RDT)," "microscopic examination," "polymerase chain reaction (PCR),"* and *"loop-mediated isothermal amplification (LAMP)."* The inclusion criteria encompassed original research articles published within the last ten years, written in English or Indonesian, and addressing the diagnostic performance or comparative evaluation of malaria detection methods. Articles were excluded if they were non-empirical, such as reviews, editorials, commentaries, or abstracts lacking full-text availability. Eligible studies were critically appraised, and relevant data were extracted concerning methodological principles, diagnostic accuracy, sensitivity, specificity, and operational feasibility. The extracted information was subsequently analyzed and synthesized qualitatively to provide a comprehensive and evidence-based overview of advancements and challenges in malaria diagnostic methodologies.

3. RESULT AND DISCUSSION

3.1 Result

Following an extensive literature search and screening process, the author examined a number of journals considered most relevant to the chosen research topic. The reviewed articles include scientific studies that applied quantitative, qualitative, or mixed-method designs and were published in recognized national and international journals.

Table 1: Summary of studies comparing diagnostic methods for malaria detection

No	Title	Author and Year	Country	Method	Result
1	Performance of Rapid Diagnostic Tests, Microscopy, Loop-Mediated Isothermal Amplification (LAMP) and PCR for Malaria Diagnosis in Ethiopia: A Systematic Review and Meta-Analysis	Feleke et al. (2021)	Ethiopia	Systematic review and meta-analysis	RDT vs microscopy showed sensitivity ranging 79 - 100% and specificity 80 - 100%. LAMP demonstrated 100% sensitivity and 85 - 99% specificity, indicating excellent diagnostic performance and practicality in low-resource settings.

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2	Accuracy of Diagnosis among Clinical Malaria Patients: Comparing Microscopy, RDT, and a Highly Sensitive Quantitative PCR	Opoku Afriyie et al. (2023)	Ghana	Cross-sectional	Parasite prevalence was 17.5% (microscopy), 24.5% (RDT), and 42.1% (qPCR). Using qPCR as the gold standard, RDT was more sensitive (55.7%) than microscopy (39.3%) and had similar specificity (98%). Both tests missed >40% of submicroscopic infections
3	Comparison of Plasmodium spp Detection Results Between Thick Blood Smear Microscopic Examination and Polymerase Chain Reaction Technique	Sandra et al. (2015)	Indonesia	Experimental	PCR demonstrated better performance than Thick Blood Smear Microscopy in detecting Plasmodia8888. The diagnostic test results for PCR (when compared to TBSM) were Sensitivity 100%, Specificity: 60% Positive Predictive Value 83.33% ;Negative Predictive Value 100%
4	Sensitivity and Specificity of Nested PCR for Diagnosing Malaria: Cases in Several Areas of Indonesia	Arifin et al. (2018)	Indonesia	Cross-sectional	Nested PCR showed higher sensitivity (97.5%) than microscopy (88.2%) but lower specificity (40.7%). Nested PCR was more effective in detecting mixed infections compared to microscopy.
5	Quantification of the Misidentification of Plasmodium knowlesi as Plasmodium malariae by Microscopy: An Analysis of 1569 P. knowlesi Cases	Mahittikorn et al. (2021)	Southeast Asia (Malaysia, Thailand, Indonesia)	Systematic review	The pooled prevalence of P. knowlesi misidentified as P. malariae was 57% (95% CI: 37–77%). The highest misidentification occurred in Sarawak (87%) and Sabah (85%). Molecular methods are essential for accurate species differentiation.
6	Comparison of the Effectiveness of Rapid Diagnostic Tests (RDT) with Microscopic Examination in Clinical Malaria Patients at Mubune Public Health Center, West Likupang District	Ritung et al. (2018)	Indonesia	Cross-sectional	RDT sensitivity was 67%, specificity 97%, positive predictive value 67%, and negative predictive value 97%. RDT examination was nearly as effective and accurate as microscopic examination for malaria diagnosis.
7	Performance of four HRP-2/pLDH combination rapid diagnostic tests and field microscopy as screening tests for malaria in pregnancy in Indonesia: a cross-sectional study	Ahmed et al. (2015)	Indonesia	Cross-sectional	Compared to PCR, the overall sensitivity of RDTs and field microscopy ranged from 24.6– 31.1%, with specificity >98.4%. First-Response had the best diagnostic accuracy (sensitivity 31.1%, specificity 98.9%). Combination RDTs are a suitable alternative for malaria screening in pregnant women in rural Indonesia

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8	Laboratory challenges of Plasmodium species identification in Aceh Province, Indonesia, a malaria elimination setting with newly discovered P. knowlesi	Coutrier et al. (2018)	Indonesia	Laboratory Investigation (Comparative Diagnostic Accuracy)	Microscopy misclassified or missed up to 56% of confirmed cases, including all P. knowlesi cases. The standard 18S rRNA nPCR missed P. knowlesi infections. Genus-specific LAMP reliably detected all infections, highlighting its potential for rapid and accurate detection in elimination settings
9	Molecular detection of human Plasmodium species using a multiplex real time PCR	Lazrek et al. (2023)	France	Validation of a multiplex real-time PCR assay	The qPCR method showed 100% sensitivity and specificity for all five species in whole blood samples. It ha s low Limits of Detection (LoD) which is around 0.25 parasites/µL for P. vivax, 0.5 parasites/µL for P. falciparum and P. knowlesi, 1 parasite/µL for P. ovale, and 5 parasites/µL for P. malariae
10	Evaluation of Multiplex/Nested Polymerase Chain Reaction and Loop-Mediated Isothermal Amplification for Malaria Diagnosis in Southeastern Iran	Mirahmadi et al. (2022)	Iran	Cross-sectional	mn-PCR and LAMP showed high sensitivity and specificity, indicating they are good alternatives to nPCR for malaria diagnosis. mn-PCR achieved 100% sensitivity and 100% specificity. LAMP achieved 92.1% sensitivity and 100% specificity
11	Head-to-head comparison of two loop-mediated isothermal amplification (LAMP) kits for diagnosis of malaria in anon-endemic setting	Ivarsson et al. (2023)	Sweden	Retrospective Validation Study	The HumaTurb Loopamp™ Malaria PDT kit showed superior performance, achieving 100% sensitivity and 100% specificity compared to qPCR. The Alethia® Illumigene Malaria kit had lower performance, with 90.24% sensitivity and 95.65% specificity compared to qPCR
12	Loop-Mediated Isothermal Amplification (LAMP) in the Diagnosis of Molecular-Based Malaria P arasite Infection	Suwandi, J.F (2021)	Indonesia	Literature Review	LAMP is a molecular-based method with high accuracy. Its sensitivity and specificity are noted to be above 90% in almost all previous studies. The method is also capable of detecting Plasmodium species in various conditions, such as in endemic or non-endemic (imported) areas, during pregnancy, in conditions of low parasitemia density, and in low transmission areas
13	Detection of Plasmodium knowlesi, Plasmodium falciparum and Plasmodium vivax using loop-mediated isothermal	Piera et al. (2017)	Malaysia	Evaluation/Diagnostic Accuracy Study	The Pan LAMP assay showed 100% sensitivity for P. knowlesi, P. falciparum, and P. vivax. The Pf LAMP was 100% sensitive and specific for P. falciparum and did not react with P.

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amplification (LAMP) in a co-endemicarea in Malaysia	knowlesi samples. The Pan LAMP's limit of detection for P. knowlesi was 2 parasites/µL, which was comparable to PCR. LAMP sensitivity was far superior to the RDTs, which only detected 10% and 28% of P. knowlesi cases, respectively. the LAMP assay presents a highly sensitive and specific molecular alternative for malaria diagnosis, particularly in areas co-endemic for P. knowlesi.

3.2 Discussion Microscopic Examination

Thick blood smear microscopy is the gold standard in malaria diagnosis and is widely used throughout the world. This examination is performed by observing blood preparations that have been stained using Giemsa stain to detect asexual forms of Plasmodium in erythrocytes. This method has fairly good sensitivity and specificity in detecting malaria parasites, especially in areas with limited laboratory facilities. In addition, microscopy also allows for the determination of Plasmodium species and the calculation of the degree of parasitemia, thereby assisting in determining the severity of infection and monitoring therapy (Sandra et al., 2014).

The advantages of microscopic examination include relatively low cost, simple procedures, and the ability to detect various Plasmodium species. However, this method has a number of limitations. The accuracy of the examination results is highly dependent on the skill and accuracy of the laboratory analyst and the quality of the blood sample being examined. In cases with low parasite density or in patients who have received antimalarial treatment, the sensitivity of this method may decrease, increasing the possibility of false negative results. In addition, microscopic examination takes a long time and is prone to variations in results between examiners (Sandra et al., 2014).

World Health Organization reports that the sensitivity of the microscopic method ranges from 75- 90%, while its specificity reaches 95 - 98%, with a parasite detection limit of approximately 50 100 parasites/µL of blood (WHO, 2016). Several studies show variations in results, such as in Ethiopia (sensitivity 75.2%; specificity 97.1%) and Ghana (sensitivity 39.3%; specificity 98.3%) compared to PCR (Feleke et al., 2021). In Indonesia, study by Arifin et al. (2018) reported a sensitivity of 88.2% and specificity of 78.5% compared to nested PCR. Meanwhile, Opoku et al (2023)found a drastic decrease in sensitivity to 6.6% at parasite densities <200 parasites/µL.

Microscopic examination has limitations in accurately identifying *Plasmodium knowlesi*. This is due to the morphological similarity of *P. knowlesi* with other species at various stages of its intraerythrocytic development. *Plasmodium knowlesi* has a very short erythrocytic cycle, approximately 24 hours, causing rapid and periodic fluctuations in the number of parasites in the blood. This condition results in the possibility of false negative results if blood sampling does not coincide with the peak of parasitemia. In addition, the morphology of *P. knowlesi* changes according to its cycle; the young trophozoite phase often resembles P. falciparum while the advanced and schizont phases resemble *P. malariae*. This morphological similarity between species increases the risk of misidentification in conventional microscopic examination, particularly in endemic areas with diverse Plasmodium species (Mahittikorn et al., 2021).

Research by Nainggolan et al (2022) in Langkat Regency, North Sumatra, showed that of the 342 blood samples examined in six villages in Langkat Regency, North Sumatra, only one sample (0.3%) tested positive for malaria through microscopic examination and was identified as *Plasmodium malariae*. However, when confirmed using the Polymerase Chain Reaction (PCR) method, three additional positive cases were found that were not previously detected microscopically, consisting of one case of *P. malariae* and two cases of *P. knowlesi*. A meta-analysis by Mahittikorn et al (2021) also reported that more than 50% of *P. knowlesi* cases were misidentified as *P. malariae* through microscopy. These findings indicate that although microscopy remains the primary method for diagnosing malaria in endemic areas, molecular-based confirmatory testing is still necessary to ensure accurate species identification, particularly in *P. knowlesi* infections, which have a morphology similar to other species.

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Rapid Diagnostic Test (RDT)

The development of Rapid Diagnostic Tests (RDTs) represents a significant step forward in expanding access to malaria diagnosis, particularly in resource-limited areas. RDTs work by detecting specific parasite antigens such as Histidine-Rich Protein 2 (HRP2) for Plasmodium falciparum and Plasmodium lactate dehydrogenase (pLDH) or aldolase for other species (P. vivax and mixed infections) (Yerlikaya et al., 2018).

In a meta-analysis in Ethiopia, RDT sensitivity reached 95.05% and specificity 96.47% compared to microscopy (Feleke et al., 2021). Research in Indonesia also showed significant variation. A study in North Sumatra showed RDT sensitivity of 63.8% and specificity of 100% compared to microscopy (Siahaan, 2011). Meanwhile, a study at the Mubune Community Health Center in North Sulawesi found sensitivity of 67% and specificity of 97% (Ritung et al., 2018). In endemic areas such as Sumba, the sensitivity of four types of HRP2/pLDH RDTs was only 24.6–31.1%, while their specificity remained high (>98%) compared to nested PCR. These data confirm that although RDTs are practical, their sensitivity decreases in infections with low parasitemia or HRP2 gene mutations (Ahmed et al., 2015).

RDT is considered highly useful for field testing because it is easy to perform and does not require advanced laboratory equipment, thereby expanding diagnostic access in endemic areas. However, reduced sensitivity may occur in cases with low parasite loads or HRP2 gene deletions, leading to false-negative results. Therefore, although RDT is effective in detecting malaria, particularly P. falciparum, confirmation with microscopic or PCR methods remains necessary for accurate diagnosis in malaria elimination efforts (Ahmed et al., 2015)

Polymerase Chain Reaction (PCR)

PCR is a molecular method with the highest sensitivity in detecting Plasmodium DNA. This technique works by amplifying parasite DNA fragments so that it can detect infection even when the number of parasites in the blood is very low (Lazrek et al., 2023). In a study by Lazrek et al (2023), it was reported that the real-time multiplex PCR method has a very low limit of detection (LOD), which is around 0.25 parasites/µL for P. vivax, 0.5 parasites/µL for P. falciparum and P. knowlesi, 1 parasite/µL for P. ovale, and 5 parasites/µL for P. malariae from whole blood samples.

PCR has the advantage of detecting Plasmodium species that are difficult to distinguish microscopically, especially *Plasmodium knowlesi*. Research in Aceh by Coutrier et al (2018) reported that nested PCR was able to identify *P. knowlesi* infections that were previously undetectable by microscopic examination due to morphological similarities with *P. malariae.* With its ability to amplify parasite-specific DNA, PCR enables more accurate detection of malaria infections, including mixed infections and asymptomatic cases that are often missed by conventional methods. Therefore, PCR is considered the reference standard in molecular malaria diagnosis and is highly recommended to support surveillance and malaria elimination efforts in low-endemic areas (Mirahmadi et al., 2022).

Loop-Mediated Isothermal Amplification (LAMP)

LAMP is a molecular diagnostic innovation developed to provide a fast, sensitive alternative to PCR that does not require a thermal cycler. The amplification process is carried out at a constant temperature (60–65 °C) with six specific primers that accelerate the DNA amplification reaction (Ivarsson et al., 2023).

A global meta-analysis shows that the Pan-LAMP method has a sensitivity of 95% and a specificity of 98%. For the detection of P. falciparum (Pf-LAMP), the sensitivity reaches 96% and the specificity 99% (Selvarajah et al., 2020). LAMP has high accuracy (>90%) and is capable of detecting all Plasmodium species, including infections with low parasitemia, non-endemic (imported) cases, and malaria in pregnancy (Suwandi, 2021).

A recent study by Martín-Ramírez et al (2022) evaluated the use of the commercial Alethia Malaria LAMP kit and reported a sensitivity of 98.8% and specificity of 94.7% compared to PCR, with a test time of approximately 40 minutes and a very low detection limit (~0.075 parasites/µL). Research by Piera et al (2017) in Malaysia supports these findings, where the Pan-LAMP method showed 100% sensitivity with a detection limit of 2 parasites/µL for P. knowlesi, and still produced positive detections at levels of 0.2 parasites/µL.

Overall, LAMP has detection capabilities comparable to PCR but with advantages in terms of speed and ease of procedure. In addition to being fast and accurate, LAMP is also practical for use in endemic areas with limited laboratory facilities because it only requires a simple heating device and can provide results in less than an hour. However, the risk of DNA contamination and the cost of commercial kits remain challenges in the widespread application of this method (Martín-Ramírez et al., 2022).

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4. CONCLUSION

The development of malaria diagnostic methods shows significant progress from conventional techniques to more sensitive and specific molecular approaches. Microscopic examination remains the gold standard in diagnosis because it can identify Plasmodium species and assess the degree of parasitemia, although its accuracy is highly dependent on the skill of the examiner and decreases in infections with low parasitemia.

Rapid Diagnostic Test (RDT) methods provide a quick and easy alternative for application in primary health care facilities, but variations in sensitivity and specificity remain a challenge due to parasite genetic factors and reagent quality. Meanwhile, the Polymerase Chain Reaction (PCR) method has the highest sensitivity because it can detect parasite DNA at very low levels of parasitemia, but its application is limited by the need for laboratory equipment and high costs.

As the latest molecular innovation, Loop-Mediated Isothermal Amplification (LAMP) has the advantage of amplifying DNA at a constant temperature without requiring a thermal cycler, with sensitivity and specificity almost equivalent to PCR. Thus, each diagnostic method has its own advantages and limitations. The appropriate method must be tailored to facility conditions, examination objectives, and regional endemicity levels to support early diagnosis and malaria elimination efforts in Indonesia.

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