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The Challenge of Diagnosing Asymptomatic Malaria During the Elimination Phase: An Implications for the Sensitivity of Laboratory Methods and Surveillance Strategies in Indonesia

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Abstract

Malaria remains a significant global health challenge, particularly during the elimination phase, where asymptomatic and sub microscopic infections become major obstacles to interrupting transmission. Individuals with asymptomatic malaria often do not seek treatment but may serve as hidden reservoirs that sustain parasite circulation in endemic communities. Conventional diagnostic methods, including microscopy and rapid diagnostic tests (RDTs), have limited sensitivity in detecting infections with low parasite density, creating challenges for accurate surveillance and case management. This study aims to analyze the challenges of diagnosing asymptomatic malaria, compare the sensitivity of available laboratory methods, and evaluate their implications for malaria elimination strategies in Indonesia. This study employed a literature review with an analytical-critical approach by reviewing scientific articles published between 2015 and 2025 from PubMed, Scopus, and Google Scholar. The reviewed studies focused on diagnostic performance comparisons among microscopy, RDT, polymerase chain reaction (PCR), quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP). The findings indicate that microscopy and RDT have limited sensitivity in detecting sub microscopic infections, whereas molecular methods demonstrate superior diagnostic accuracy, with PCR and qPCR capable of identifying parasite densities below the detection limits of conventional approaches. However, the implementation of molecular diagnostics remains constrained by infrastructure, cost, and human resource limitations. In conclusion, malaria elimination in Indonesia requires an integrated diagnostic strategy combining conventional methods with selective molecular testing, strengthened laboratory capacity, and improved surveillance systems to detect hidden infections and prevent residual transmission.

INTRODUCTION

Malaria is still one of the world's major parasitic infectious diseases with a significant burden of morbidity and mortality, especially in tropical and subtropical regions.(World Health Organization, 2023) According to a report by the World Health Organization (WHO), despite the decline in incidence in the last two decades, malaria remains a public health challenge in various endemic countries, including Indonesia.(World Health Organization, 2023)· The Government of Indonesia has a strong commitment to a nationwide malaria elimination program, with most districts/cities having achieved elimination status.(Peraturan Menteri Kesehatan Republik Indonesia Nomor 22 Tahun 2022 Tentang Penanggulangan Malaria, 2022)·(Sitohang et al., 2018) However, the challenges in the pre-elimination and elimination

phases are different from the control phases, especially related to infections with low parasitemia that are difficult to identify with conventional examination.(Oyegoke et al., 2022)

Asymptomatic malaria infection is a condition when an individual is infected *Plasmodium spp.* in the absence of typical clinical symptoms.(Chen et al., 2016) Individuals with asymptomatic infections often do not seek therapy, but these individuals can be a source of transmission because there are parasites that are still circulating in the peripheral blood and potentially infecting mosquitoes *Anopheles*(vector).(Kotepui et al., 2023) In areas with low transmission, the proportion of asymptomatic and submicroscopic infections tends to increase due to the formation of partial immunity in populations that are repeatedly exposed to this parasite.(Chen et al., 2016),(Kotepui et al., 2023) Submicroscopic malaria infection refers to the density of parasites that are at the lower limit of parasite identification by conventional methods but can still be identified using molecular methods. (Policy & Committee, 2017)

In Indonesia, microscopic examination of thick and thin blood preparations is still *Gold standard* in the diagnosis of malaria because it can identify parasites and calculate the density of parasitemia.(UNDP, 2023) However, the microscopy method has limitations in identifying malaria parasites, it is estimated that only about 50–100 parasites/μL of blood can be identified, and it can also only detect about 55-60% of asymptomatic malaria cases, in addition to the effectiveness of this method depends on the quality of the preparation, the competence of the laboratory analyst, and the level of parasitemia in the blood sample.(Oyegoke et al., 2022),(Deo et al., 2022) In addition to microscopy, rapid diagnostic tests (RDTs) are used as an alternative examination, especially in laboratory facilities with limited trained human resources, but the sensitivity of this method varies depending on the type of antigen detected and the density of the parasite.(Oyegoke et al., 2022),(Berzosa et al., 2018) In infections with low parasitemia, both microscopy and RDT are at risk of producing false-negative results because asymptomatic infections cannot be detected.(Whittaker et al., 2021),(Ilyas & Muh, 2025) ,(Ende et al., 2026)

The development of molecular diagnostic technology has provided new opportunities in detecting asymptomatic malaria. The polymerase chain reaction (PCR), quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP) methods are reported to have much higher sensitivity than microscopy and RDT.(Ilyas & Muh, 2025),(Feleke et al., 2021),(Calderaro et al., 2024),(Fajri et al., 2026) ,(Zainabadi, 2021) PCR is able to detect infections with a parasite density of less than 5 parasites/μL of blood, while qPCR can identify lower parasitemia while providing quantitative information about the number of parasites.(Ilyas & Muh, 2025) Meanwhile, the LAMP method offers a simpler alternative with diagnostic performance that is close to PCR so that it has the potential to be applied to areas with laboratory facilities.(Ilyas & Muh, 2025),(Feleke et al., 2021) Various studies have shown that the use of molecular methods is able to identify a much higher proportion of asymptomatic malaria infections than conventional methods.(Ilyas & Muh, 2025),(Ahmad et al., 2022),(Oyegoke et al., 2022) However, the widespread implementation of molecular methods in routine surveillance systems still faces obstacles in the form of limited laboratory infrastructure, operational costs, the need for special equipment, and the technical competence of human resources.(Berzosa et al., 2018)

In the context of malaria elimination, the sensitivity of diagnostic tools is a crucial factor because if there is a failure to detect asymptomatic malaria infection, it can increase residual transmission in the community.(Surendra et al., 2020),(Sitohang et al., 2018) Although various

studies have stated the advantages of molecular methods in detecting asymptomatic malaria, their implementation in elimination programs still faces various obstacles, including: high costs, the need for special equipment, limited trained personnel, and uneven laboratory capacity in endemic areas.(Calderaro et al., 2024)·(Ahmad et al., 2022) Therefore, a comprehensive study is needed on the challenges of asymptomatic malaria diagnosis, the sensitivity of various diagnostic methods, and their implications for malaria surveillance strategies in Indonesia. This literature review aims to analyze the current scientific evidence on the performance of asymptomatic malaria diagnostic methods and their role in supporting sustainable malaria elimination efforts in Indonesia.

METHOD

This study was a literature review with an analytical-critical approach that aims to synthesize scientific evidence regarding the challenges of asymptomatic malaria diagnosis in the perspective of diagnostic methods and their implications for national elimination strategies. This approach was chosen to integrate epidemiological findings, diagnostic aspects, and policy relevance in the malaria surveillance system, especially in the pre-elimination and elimination phases. This review not only assesses the technical performance of diagnostic methods, but also evaluates their suitability in supporting population-based public health interventions.

Article searches are conducted electronically through three main databases, namely PubMed, Scopus, and Google Scholar. The search strategy used a combination of keywords and Boolean operators as follows: "asymptomatic malaria", "submicroscopic infection", "malaria diagnosis", "microscopy", "rapid diagnostic test", "PCR", "molecular detection", and "malaria elimination". The search is limited to articles published in the range of 2015–2025 to ensure the up-to-date scientific evidence, and is limited to articles in English and Bahasa Indonesia.

Inclusion criteria in this review include: (1) original research articles, (2) observational studies, community surveys, or evaluation of malaria diagnostic methods, (3) studies that report detection of asymptomatic or submicroscopic malaria infection, and (4) articles that present comparative data on the sensitivity of diagnostic methods such as microscopy, rapid diagnostic test (RDT), polymerase chain reaction (PCR)/quantitative PCR (qPCR) and Loop-mediated isothermal amplification (LAMP). Exclusion criteria include: non-systematic review articles, editorials, letters to editors, single case reports, experimental animal research, as well as articles without full-text access.

The literature selection process was carried out in two stages. The first stage is the screening of titles and abstracts to identify suitability with the research topic. The second stage is the evaluation of the full text to ensure the fulfillment of the inclusion and exclusion criteria. Articles that met the criteria were then extracted using a structured extraction sheet that included information about the study location, study design, number of samples, diagnostic methods used, limits of detection, as well as key findings related to the proportion of submicroscopic infections and examination sensitivity.

Data synthesis was carried out narratively and thematically by grouping the results of the study based on the type of diagnostic method and detection performance in asymptomatic populations. The analysis focused on the comparison of sensitivity of microscopy, RDT, and molecular methods, as well as their implications for laboratory capacity in supporting malaria

elimination surveillance. To improve the validity of the results, only studies with clear and standardized diagnostic methods are included in the final analysis.

RESULTS AND DISCUSSION

Selection and Study Characteristics

Based on the results of literature searches from PubMed, Scopus, and Google Scholar, a number of articles were obtained which were then selected according to inclusion and exclusion criteria. After the screening process of titles, abstracts, and full text review, a number of studies that met the criteria were further analyzed. The included studies consisted of cross-sectional observational studies, community surveys, and comparative evaluation of diagnostic methods in asymptomatic populations in low- to moderate-transmission areas.

Most of the research was conducted in endemic countries of Southeast Asia and Africa, with some studies coming from Indonesia, particularly the Papua and Nusa Tenggara regions. The number of samples in the study varied, ranging from hundreds to thousands of respondents, with a primary focus on the detection of malaria infection without clinical (asymptomatic) symptoms. The diagnostic methods evaluated included microscopy, rapid diagnostic test (RDT), polymerase chain reaction (PCR), quantitative PCR (qPCR), loop-mediated isothermal amplification (LAMP), and serological surveillance. (Table 1)

Table 1. Study characteristics

Yes	Researcher (Year)	Country	Study Design	Number of Samples (n)	Research Population	Diagnostic Methods	Rated Externalities	Key Findings
1	Chen I (2016)	Multinational	Narrative review	Literature	Asymptomatic malaria infection	Microscopy, RDT, PCR	The role of asymptomatic malaria	Asymptomatic malaria is an important reservoir that needs to be detected in the elimination phase.
2	Cody M (2023)	Asia	Systematic review & meta-analysis	106 studies	Asian Population	Microscopy, PCR	Asymptomatic prevalence of malaria	Asymptomatic infections are still high and many cases are only detected by PCR.
3	Berzosa P (2018)	Guinea Ecuador	Cross-sectional	1.724	Endemic population	Microscopy, RDT, PCR	Diagnostic accuracy	PCR shows the highest sensitivity compared to microscopy and RDT.
4	Whittaker C (2021)	Global	Systematic review & meta-analysis	387 surveys	Endemic population	Microscopy, PCR	Submicroscopic infections	The proportion of submicroscopic infections increases in

Yes	Researcher (Year)	Country	Study Design	Number of Samples (n)	Research Population	Diagnostic Methods	Rated Externalities	Key Findings
								areas of low transmission.
5	Frederick DG (2021)	Ethiopia	Systematic review & meta analysis	24 studies	Malaria diagnostic studies	Microscopy, RDT, LAMP, PCR	Sensitivity and specificity	LAMP and PCR have higher sensitivity than microscopy and RDT.
6	Calderaro A (2024)	Italy	Narrative review	Literature	Laboratory diagnosis	Microscopy, RDT, PCR, qPCR	Development of diagnostic methods	Molecular examination is the most sensitive method for low parasitemia.
7	Zainabadi K (2021)	Global	Review	Literature	Low-density infections	Ultrasensitive PCR	Molecular diagnosis	Ultrasensitive PCR is able to detect <1 parasite/μL of blood.
8	Deo DA (2022)	Indonesia	Cross-sectional	409	Malaria patients	Microscopy, PCR	Evaluation of the diagnosis	PCR found more cases than microscopy.
9	Van der Ende J (2026)	Ecuador	Cross-sectional	1.022	Pre-elimination population	PCR	Asymptomatic parasitemia	PCR detects reservoirs of infection that are not microscopically identified.
10	Afriyie SO (2023)	Ghana	Cross-sectional	1.040	Malaria patients	Microscopy, RDT, qPCR	Accuracy of diagnosis	qPCR has the highest sensitivity to submicroscopic infections.
11	Zaw MT (2017)	Myanmar	Cross-sectional	485	Endemic population	Microscopy, PCR	Submicroscopic infections	The majority of asymptomatic infections are detected only using PCR.
12	Mandefro A (2025)	Ethiopia	Multicenter diagnostic study	1.874	Plasmodium falciparum *patients*	HRP2-RDT	RDT Performance	Detection **of hrp2/hrp3** decreases the sensitivity of RDT.
13	Fajri M (2026)	Indonesia	Comparative cross-sectional	1.200	Keerom Society	Microscopy, RDT, PCR	Comparison of diagnostic performance	PCR has the highest sensitivity in areas of high transmission.
14	Surendra H (2020)	Indonesia	Cross-sectional	4.095	Elimination region	Serology	Malaria surveillance	Serology effectively identifies

Yes	Researcher (Year)	Country	Study Design	Number of Samples (n)	Research Population	Diagnostic Methods	Rated Externalities	Key Findings
								receptive areas.
15	Ahmad RA (2022)	Indonesia	Health system evaluation	National data	Surveillance system	Surveillance sensitivity analysis	Laboratory capacity affects the sensitivity of malaria surveillance.	
16	Theresia A (2024)	Indonesia	Cross-sectional	328	Elimination region	Epidemiological analysis	Clinical factors of malaria	Epidemiological factors affect the clinical degree of malaria in the elimination area.
17	Fauzia h N (2025)	Indonesia	Systematic review	18 studies	*Plasmodium knowlesi*	Microscopy vs PCR	Accuracy of diagnosis	PCR is superior to microscopy in detecting *P. knowlesi*.
18	Wardhani P (2020)	Indonesia	Comparative diagnostic study	276	Malaria patients	Two RDTs, microscopy, real-time PCR	RDT Performance	Real-time PCR provides the highest sensitivity, while RDT is suitable for rapid screening.

Diagnostic Method Sensitivity Comparison

The results of the study showed that there was a significant difference in the sensitivity of each method between microscopy, rapid diagnostic test (RDT), polymerase chain reaction (PCR)/quantitative PCR (qPCR) and LAMP (loop-mediated isothermal amplification) methods. (Table 2)

Microscopy

Microscopic examination of thick and thin blood preparations remains the *gold standard* method in diagnosing malaria in many health facilities. However, in asymptomatic malaria populations with low parasite density, the sensitivity of the microscope decreased significantly. Some studies report that microscopes are only able to detect a small percentage of infections that are later confirmed positive through molecular methods. The detection limit of microscope which ranges from 50–100 parasites/μL is a major factor in the limitation in identifying submicroscopic infections. In addition to the parasite density factor, the quality of the preparation, staining techniques, and the competence of laboratory analysts also affect the accuracy of the results. Inter-observer variability was found in several studies, especially in examinations with low parasitemia.

Rapid Diagnostic Test (RDT)

RDT is widely used as an alternative to microscopes due to its ease of use and short examination time. However, the results of the study show that the sensitivity of RDT to asymptomatic infections is also limited. Some studies reported negative results on RDT which were later proven positive through PCR. Variations in RDT sensitivity are influenced by the type of antigen detected (e.g. HRP2 or pLDH), the stability of the reagent, and the likelihood of deletion of the target gene in a particular parasite. In populations with low parasitemia, RDT performance tends to decrease, potentially producing false negative results. This suggests that RDT has limitations in elimination surveillance that demands the detection of low-density infections.

Molecular Method (PCR/qPCR)

The molecular method shows the highest sensitivity in detecting asymptomatic malaria infection. Some studies report that the proportion of infections detected only by PCR can be two to five times greater than microscopic. The detection limit of molecular methods is reported <5 parasites/μL, even in ultra-sensitive techniques that can detect lower densities. The qPCR method also allows the quantification of parasite density, providing a more accurate picture of the level of parasitemia in asymptomatic populations. However, the routine implementation of molecular methods is still limited to reference laboratories or research because it requires special equipment, strict quality control, and trained personnel.

Molecular Method (LAMP)

The LAMP (loop-mediated isothermal amplification) method has high sensitivity in detecting asymptomatic malaria. Although slightly inferior to PCR and qPCR, this method offers the advantages of shorter examination times, simpler procedures, and does not require special equipment (*thermal cycler*), so it has the potential to be applied to regional laboratories with limited resources.

Table 2. Performance Comparison of Asymptomatic Malaria Diagnostic Methods

Diagnostic Methods	Sensitivity (%)	Specificity (%)	Limit of Detection (LoD)	Pros	Limitations	References
Microscopy [2, 8, 10, 15, 20]	45–75	90–98	±50–100 parasites/μL	Gold standard in primary service, able to determine the species and density of parasites, relatively low cost	Decreased sensitivity in low parasitemia, depending on the competence of the analyst and the quality of the preparation	
Rapid Diagnostic Test (RDT) [4, 10, 14, 15, 20]	60–85	90–98	±100–200 parasites/μL	Fast (15–20 minutes), easy to use in the field, no microscope required	Low sensitivity to submicroscopic infections, affected by deletion **of the hrp2/hrp3** gene, unable to account for parasitemia	

Diagnostic Methods	Sensitivity (%)	Specificity (%)	Limit of Detection (LoD)	Pros	Limitations	References
Loop-mediated Isothermal Amplification (LAMP) [5,14,15]	90–98	95–99	±1–5 parasites/µL	High sensitivity, shorter examination time than PCR, no need for conventional thermal cycler	The cost is higher than microscopy/RDT, still limited to certain laboratories	
Conventional PCR [8,10,14–17]	92–99	95–100	±1–5 parasites/µL	Highly sensitive to detect submicroscopic infections, able to identify species	Requires molecular facility, longer examination time, relatively high cost	
Quantitative PCR (qPCR) [6, 7, 10, 14, 15]	95–99	98–100	<1 parasite/µL	Highest sensitivity, able to measure parasite density, very suitable for elimination surveillance	Equipment is expensive, requires trained human resources and strict quality control	
Ultrasensitive PCR (uPCR) [7, 14]	>98	>99	<0.1 parasites/µL	Detects infections with very low parasitemia, suitable for research and surveillance of elimination	Not yet widely available, high cost, not yet a routine inspection	

Findings of the study in Indonesia

Several studies in Indonesia show that asymptomatic malaria is still found in areas that are on the verge of elimination, especially in Papua, East Nusa Tenggara, and some areas with a history of malaria endemic. Most studies report that the PCR method is able to detect more cases than microscopy and RDT. In addition, serological surveillance also shows potential in identifying areas that still have residual malaria transmission even though the number of clinical cases has decreased. (Table 3)

Table 3. Findings of the Indonesian study

Yes	Researcher (Year)	Research Location	Study Design	Method	Key Findings	Implications for Malaria Elimination
1	Sitohang V	Indonesia (national)	Commentary	Analysis of elimination programs	Indonesia has made progress towards malaria elimination, but transmission is still concentrated in Papua and some eastern regions, so there is a need to	Strengthening early detection and laboratory surveillance is a priority in high endemic areas.

Yes	Researcher (Year)	Research Location	Study Design	Method	Key Findings	Implications for Malaria Elimination
					strengthen diagnosis and surveillance.	
2	Deo DA	Southwest Sumba, East Nusa Tenggara	Cross-sectional	Microscopy and nested PCR	Out of 409 samples, PCR detected more cases than microscopy, especially in infections with low parasitemia and mixed infections.	PCR is suitable for use as a confirmatory check in endemic areas with many submicroscopic infections.
3	Surendra H	Sabang, Aceh	Cross-sectional	Healthcare facility-based serology surveillance	Serological examinations are able to identify receptive areas that are at risk of retransmission despite the low number of clinical cases.	Serology can complement routine surveillance in the malaria elimination phase.
4	Ahmad RA	Indonesia (national)	Evaluation of the surveillance system	Sensitivity analysis of surveillance systems	The sensitivity of surveillance is influenced by access to services, quality of laboratory diagnosis, case reporting, and epidemiological investigations.	Strengthening laboratory capacity and reporting systems increases the effectiveness of malaria elimination programs.
5	Fajri M	Keerom Regency, Papua	Comparative cross-sectional	Microscopy, RDT, PCR	PCR showed the highest sensitivity compared to microscopy and RDT in areas with high transmission.	Supports the use of molecular methods as reference testing in high-risk elimination regions.
6	Theresia A	Indonesia (elimination area)	Cross-sectional	SISMAL data analysis	Epidemiological factors and case characteristics are related to the clinical degree of malaria in the elimination area.	Risk factor-based surveillance can improve the effectiveness of case detection.
7	Wardhani P	Indonesia	Comparative diagnostic study	Two RDTs, microscopy, real-time PCR	Real-time PCR has the highest sensitivity, while RDT is suitable for rapid screening in the field.	PCR is recommended as a confirmation method to improve the accuracy of the diagnosis.
8	Fauziah N	Indonesia	Systematic review	Microscopy and PCR	PCR is superior to microscopy in detecting *Plasmodium knowlesi*, especially in low parasitemia.	Strengthen recommendations for the use of molecular methods in reference laboratories.

Proportion of Submicroscopic Infections

Most of the studies in this review reported the presence of submicroscopic infections that were not identified by microscopes or RDTs but were identified by PCR. In areas with low transmission or in the pre-elimination phase, the proportion of submicroscopic infections reported to be quite significant compared to infections with clinical symptoms.

These submicroscopic infections are generally found in individuals without fever complaints, so they are not caught in the *passive case detection system*. The existence of this reservoir has the potential to maintain latent malaria transmission in the community, especially if active screening is not carried out with sensitive diagnostic methods.

Implementation Challenges in the Laboratory

The results of the study also show that the main challenge in the detection of asymptomatic malaria is not only in the technological aspect, but also in the laboratory capacity. Some of the obstacles identified include:

1. Limited PCR facilities at district/city level laboratories.
2. Relatively high operational costs and molecular reagents.
3. The need for technical training for laboratory analysts in molecular engineering.
4. Uneven external standardization and quality control.

On the other hand, microscopy remains the most operationally realistic method in primary health facilities, but its sensitivity is limited to low parasitemia.

The results of this review show that the main challenge in the detection of asymptomatic malaria in the elimination phase lies in the limited sensitivity of conventional diagnostic methods, especially microscopy and rapid diagnostic tests (RDT), in identifying infections with low parasite density. Consistent sensitivity differences between conventional and molecular methods show significant diagnostic gaps in asymptomatic (asymptomatic) malaria populations.(Oyegoke et al., 2022),(Chen et al., 2016),(Kotepui et al., 2023),(Zainabadi, 2021)

Limitations of microscopic examination in Low Parasitemia

Microscopy examination of thick and thin blood preparations is still the standard in the diagnosis of malaria in most health facilities. However, based on the results of the synthesis of several quantitative studies obtained, the sensitivity of microscopes in asymptomatic populations ranges from 45–70%. While the detection limit of microscope is generally around 50–100 parasites/μL of blood,(Zaw et al., 2017) So this is the main limiting factor in detecting submicroscopic malaria infection.(UNDP, 2023),(Calderaro et al., 2024),(Zaw et al., 2017)

The successful identification of malaria in low parasitemia is highly dependent on the quality of the preparation, the Giemsa staining technique, the thickness of the blood film, and the time of observation under the microscope. Inter-observer variability is also a challenge, especially at parasite densities close to the detection limit.(Calderaro et al., 2024) This suggests that although microscopy has a high specificity (>95%), its sensitivity in the elimination phase is still less than optimal.(Calderaro et al., 2024),(Fajri et al., 2026)

It is necessary to improve competence through regular training, comparative testing, and external quality assurance (EQA) programs which are very important to minimize misinterpretations, especially in conditions of low parasitemia.(UNDP, 2023),(Calderaro et al., 2024),(Ahmad et al., 2022)

Performance of Rapid Diagnostic Test (RDT) in the Context of Elimination

RDT has the advantages of easy use and fast examination time, simple procedures so that this test is widely used in primary care facilities.(Oyegoke et al., 2022)·(Berzosa et al., 2018)·(Calderaro et al., 2024) However, the results of the synthesis of several studies quantitatively show that the sensitivity of RDT in asymptomatic infections ranges from 60–85%, and is considered lower than the molecular method and is influenced by the type of antigen used, especially HRP2 and pLDH.(Berzosa et al., 2018)·(Feleke et al., 2021)·(Calderaro et al., 2024)In addition, the presence of hrp2/hrp3 gene deletion in some populations of Plasmodium falciparum can reduce the sensitivity of RDT resulting in false negatives.(Oyegoke et al., 2022)·(Calderaro et al., 2024)·(Mandefro et al., 2025)The stability of the kit, storage conditions, and ambient temperature also contribute to the variation in diagnostic results.(Ahmad et al., 2022)Although RDT has a relatively high specificity (90–98%), there can be a risk of false negatives in asymptomatic populations, making RDT less than ideal as the only screening method in the elimination phase.(Oyegoke et al., 2022)·(Afriyie et al., 2023) Therefore, the selection of RDT types and storage quality control are important aspects in laboratory practice.

Advantages of Molecular Methods and Their Implications

The polymerase chain reaction (PCR) and quantitative PCR (qPCR) methods showed the highest sensitivity (>90%) with a limit of detection <5 parasites/μL, even <1 parasite/μL in ultra-sensitive techniques.(Feleke et al., 2021)·(Afriyie et al., 2023) This difference explains why the proportion of infections detected with PCR can be two to four times higher than microscopic examination. The ability of qPCR to quantify parasitemia also provides added value in understanding the dynamics of submicroscopic infections.(Afriyie et al., 2023) In the context of elimination, the identification of hidden reservoirs can be key to preventing residual transmission.(Ilyas & Muh, 2025)·(Theresia & Helda, 2024)However, the implementation of molecular malaria testing in routine laboratories still faces a number of obstacles, including: the need for special equipment (thermal cycler), the high price of reagents, contamination must be strictly controlled, and the limited number of trained personnel who understand DNA amplification techniques.(Berzosa et al., 2018) In addition, there is a risk of cross-contamination and technical errors that can affect the validity of the results if the procedure is not executed in accordance with the standards.(Berzosa et al., 2018)

Implications for Laboratory and Surveillance Practices

In this review it was found that no one diagnostic method is completely ideal in all contexts. Microscopic examination remains relevant as a basic method due to its ability to determine parasite species and calculate parasite density directly, as well as its relatively low cost.(Berzosa et al., 2018)·(Fauziah et al., 2025)·(Wardhani et al., 2020) RDT has an important role in rapid detection in the field.(Wardhani et al., 2020) Meanwhile, PCR is more suitable to be used as a confirmation or surveillance method in areas with low transmission.(Berzosa et al., 2018)·(Afriyie et al., 2023)·(Wardhani et al., 2020)

In malaria elimination programs, integrated diagnostic strategies are the most rational approach. Regional reference laboratories can be strengthened to conduct selective molecular examinations on individuals with high epidemiological risk, especially if the results of microscopy or RDT are negative but there is still a suspicion of malaria infection.(Fajri et al., 2026)·(Ahmad et al., 2022)·(Surendra et al., 2020)

Currently, it is necessary to improve the competence of health analysts in microscopic examination and molecular engineering, the application of internal quality control and external quality assurance, as well as understanding the limits of detection of each method are important components in maintaining the quality of malaria diagnosis. Periodic evaluation of the performance of the equipment, the quality of the reagent, and the competence of laboratory officers is also needed to improve the accuracy of the examination results.(Peraturan Menteri Kesehatan Republik Indonesia Nomor 22 Tahun 2022 Tentang Penanggulangan Malaria, 2022),(UNDP, 2023),(Calderaro et al., 2024),(Ahmad et al., 2022)

Limitations of the Review

This review has several limitations, including the heterogeneity of the study design, the variation of the molecular methods used, and the differences in population characteristics in each study so that the synthesis is carried out narratively. In addition, not all studies report complete sensitivity and specificity data, so quantitative comparisons between methods cannot be carried out thoroughly. However, the consistency of results from various studies shows that molecular methods have a higher sensitivity than conventional methods in detecting asymptomatic malaria.

CONCLUSION

Asymptomatic malaria is a major challenge in the malaria elimination phase because infected individuals are generally asymptomatic but have the potential to become a reservoir of transmission, especially in infections with low parasitemia or submicroscopic. The results of the literature review show that conventional diagnostic methods, namely microscopy and *rapid diagnostic test* (RDT), still have limitations in detecting asymptomatic malaria infection due to decreased sensitivity at low parasite density. In contrast, molecular methods such as *polymerase chain reaction* (PCR), *quantitative PCR* (qPCR), and *loop-mediated isothermal amplification* (LAMP) show higher sensitivity and are more effective in identifying malaria parasites that have the potential to cause transmission in the elimination region. Findings from various studies, including studies in Indonesia, show that strengthening laboratory capacity, improving the competence of health analysts/laboratories, implementing an examination quality assurance system, and selective integration of molecular methods in reference laboratories are important strategies to increase the sensitivity of asymptomatic malaria case detection. In addition, strengthening the surveillance system that combines laboratory examinations with epidemiological approaches is needed to support early identification of cases and prevent retransmission in areas that have reached elimination status. Thus, malaria diagnosis strategies in the elimination phase cannot rely only on conventional methods, but require an adaptive, risk-based, and supported diagnostic approach with high sensitivity according to the capacity of the health system. Therefore, further research is needed in Indonesia to evaluate the effectiveness of the implementation of molecular methods at various levels of health services and also analyze the costs and benefits, so that it can be the basis for the formulation of sustainable malaria surveillance and elimination policies.

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