



Characteristics and performance of participants in the malaria microscopic proficiency testing program over the years 2022-2024 in Vietnam

Características y desempeño de los participantes en el programa de pruebas de competencia microscópicas de malaria durante los años 2022-2024 en Vietnam

Phuc Huynh Thi Diem¹ <https://orcid.org/0000-0002-8173-4115>

Phuong Nguyen Thi Be¹ <https://orcid.org/0009-0002-7455-8886>

Huynh Nguyen Tien¹ <https://orcid.org/0000-0003-3017-6149>

Xuan Bui Thi Le¹ <https://orcid.org/0009-0007-1315-055X>

Van Tran Thi Hue^{1*} <https://orcid.org/0009-0007-0677-2553>

¹University of Medicine and Pharmacy at Ho Chi Minh City. Quality Control Center for Medical Laboratory. Ho Chi Minh City, Vietnam.

*Author for correspondence. Email: huevan@ump.edu.vn

ABSTRACT

Introduction: Malaria is a serious infectious disease with significant public health impact. Microscopic examination of blood smears remains a key diagnostic method, and external quality assessment through proficiency testing helps ensure diagnostic accuracy.

Objective: To describe the characteristics and evaluate the quality of participants in the malaria microscopic proficiency testing program over the years 2022-2024 in Vietnam.

Methods: Retrospective research. The subjects were 32 participants in the blood smear program for malaria parasites from 2022-2024. STATA 14.0. was used for data analysis.

Results: Participants were predominantly public units (71.9%), Grade I hospitals (50%), provincial and equivalent levels (62.5%), and located in the Southeast region (68.8%). Over three years,

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approximately 95% of units achieved a total score over 50%, with only 5% scoring less than 50%. By component, the proportion of units achieving $\geq 50\%$ for identification steadily increased (from 96.4% in 2022 to 97.8% in 2024), reaching 100% for individual parasite analysis in both 2023 and 2024. However, the density counting estimation component consistently had a lower proportion of units achieving $\geq 50\%$, remaining around 88% throughout the three years.

Conclusion: The main characteristics are: belonging to units of the public type, ranked I, to the provincial level and equivalent, in the Southeast region. The quality of participation is increasingly improved when evaluating the overall and individual components, but the component always accounts for a lower proportion than the density counting.

Keywords: external quality assessment; malaria; malaria parasite counting; parasite density.

RESUMEN

Introducción: La malaria es una enfermedad infecciosa que impacta la salud pública. La microscopía sanguínea es una herramienta diagnóstica esencial y la evaluación externa de la calidad es importante para garantizar la precisión diagnóstica.

Objetivo: Describir las características y evaluar la calidad del desempeño de los participantes en el programa de pruebas de aptitud para la microscopía de malaria en Vietnam, durante el periodo 2022-2024.

Métodos: Se realizó una investigación retrospectiva con datos de 32 participantes del programa de frotis sanguíneo de malaria, de 2022 a 2024. El análisis estadístico se realizó con STATA 14.0.

Resultados: Participaron predominantemente unidades públicas (71,9 %), hospitales de Grado I (50 %), de nivel provincial o equivalente (62,5 %) ubicados en la región Sudeste (68,8 %). En tres años, aproximadamente 95 % de las unidades obtuvieron una puntuación total superior al 50 %, con solo el 5 % por debajo. Específicamente, la proporción de unidades que lograron $\geq 50\%$ en la identificación aumentó de manera sostenida (96,4 % en 2022 a 97,8 % en 2024); alcanzó el 100 % para el análisis individual de parásitos en 2023 y 2024. No obstante, el componente de estimación del recuento de densidad, consistentemente mostró menor proporción de unidades con $\geq 50\%$ y se mantuvo alrededor del 88 % durante todo el periodo.



Conclusión: La calidad de la participación mejora progresivamente en los componentes generales e individuales, pero el componente de recuento de densidad representa, sistemáticamente, una proporción de éxito inferior entre los participantes.

Palabras clave: evaluación externa de la calidad; identificación; parásitos de la malaria; recuento de densidad.

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INTRODUCTION

Malaria is a serious infectious disease that has a significant impact on public health, especially in tropical and subtropical countries with difficult economic conditions. Malaria remains a public health challenge. In 2023 alone, there were an estimated 597 000 malaria deaths and 263 million new cases – an increase of 11 million cases over the previous year.⁽¹⁾ This disease not only poses a clinical challenge but also causes enormous socio-economic burdens due to its prevalence in underdeveloped areas, thereby strongly hindering the development process. Fever cold is an infectious disease caused by the parasite *Plasmodium*. Vector transmission is mosquitoes *Anopheles*.⁽²⁾ There are 5 species of malaria parasites that cause diseases in humans, namely: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*.⁽³⁾ In Central Vietnam, in the period 2018–2022, the infection rate of *P. falciparum* has increased from 67% (2018) to 82% (2022) of the total number of malaria cases.⁽⁴⁾ Notably, the trend of resistance to artemisinin and piperazine has been detected and spread in provinces such as Binh Phuoc, Gia Lai, and Kon Tum in the period 2018–2020.⁽⁵⁾

According to the guidelines for malaria surveillance and prevention of the Ministry of Health, currently localities in Vietnam are in the malaria elimination stage. The diagnosis and identification of patients infected with malaria parasites is carried out by the method of Giemsa staining and

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microscopy.⁽⁶⁾ Although there are more modern diagnostic techniques, the Giemsa staining technique for malaria parasites is still considered by the World Health Organization (WHO) to be the “gold standard” in the diagnosis of malaria parasites.⁽⁷⁾ However, due to this being a manual method, there is a potential risk of errors or confusion during manipulation. Therefore, quality assurance is extremely important. Each laboratory needs to carry out both internal and external quality inspections by reputable and competent organizations.

In recent years, External Quality Assessment (EQA) programs in the field of malaria parasite diagnosis have been widely implemented in many countries to improve the accuracy and reliability of testing. Around the world, WHO and the Centre for International Cooperation on Malaria (WHO-FIND, UK NEQAS) have developed EQA programs for malaria laboratories at the national and regional levels, with a particular focus on microscopy, rapid diagnostic (RDT), and PCR techniques. Recent studies have shown that EQA plays an important role in detecting technical errors, assessing the capacity of testers, and verifying the suitability of internal testing procedures in laboratories.⁽⁸⁾

In Vietnam, the national malaria parasite external inspection program has been implemented since 2019; the percentage of units participating in external inspection has increased steadily, especially in malaria-endemic provinces. A study in central Vietnam noted that although the accuracy of detection *P. falciparum* and *P. vivax* relatively high (> 90%), but the rate of accurate identification of rare species such as *P. malariae* or coordinated infection is still limited. Research of Tam LT et al.⁽⁴⁾ shows that the parallel implementation of external inspection and epidemiological surveillance helps detect asymptomatic cases, improve microbiological diagnostic capacity, and support Viet Nam's goal of malaria elimination by 2030.

External examination of blood smears for malaria parasites helps ensure accurate diagnosis and avoid missing cases. Microscopy techniques depend on the skills of technicians, so external inspection helps standardize tests, detect errors and improve professional capacity, supporting effective malaria control.

This study aims to describe the characteristics and evaluate the quality of participants in the malaria microscopic proficiency testing program in Vietnam over the period from 2022 to 2024.



METHODS

Study design

A retrospective descriptive study was conducted among units participating in the external inspection program from 2022 to 2024.

Subjects

The study population included all healthcare units that participated in the External Quality Assessment (EQA) for malaria parasite detection during the period from 2022 to 2024. In total, 32 hospitals participated. The participants were laboratories that qualified and agreed to participate with varying levels of experience.

Data Collection

Data were collected from records of EQA activities, which included the results of identification, density estimation, and individual analysis of malaria parasites in blood smear samples. From 2022 to 2024, there were 9 EQA rounds (3 rounds per year). The results of the performance assessment of participants in the malaria microscopic were aggregated from the results returned by the units (fluctuating below 32). The aggregated data recorded 88 results returned for 2022, 90 results returned for 2023 and 2024.

Variables

The key variables included:

Identification score are determined when comparing the reported results from a participating unit with the assigned value. At sample P01, the assigned value indicates no malaria parasite, and the assigned value of sample P02 is the presence of one specific species of malaria parasite. The unit returns results for each sample. If the unit reports accurately is 3 points. If the unit reports not applicable or P+ other species is 1 point. If the unit reports a different result is 0 points. Identification points range from 0 - 6 points.



Density counting score are scored by comparing the unit's result with an assigned value for each sample. If the reported density is within $\pm 25\%$ of the assigned value is 3 points. The reported density deviates between $\pm 25\%$ and $\pm 50\%$ is 1 point. A greater 50% deviation is 0 points. Density points range from 0 - 6 points.

Individual parasite analysis score assess the correct identification of individual parasite forms. Each sample contains 03 morphological stages/forms of the malaria parasite. For each correctly identified form, 1 point is awarded. Maximum of 3 points per sample. Individual parasite analysis points range from 0 - 6 points.

Total score is the total score of three indicators identification, density counting, and individual parasite analysis points. Range from 0 - 18 points.

Identification, density counting, and individual parasite analysis variables are binary variables that take the value of 1 when the variables' identification, density counting, and individual parasite analysis score are greater than or equal to 3 points (I.e., Reaching 50% or more). Otherwise, identification, density counting, and individual parasite analysis categorical take the value of 0 (Less than 50%).

Overall performance are binary variables that take the value of 1 when total score are greater than or equal to 9 points (I.e., Reaching 50% or more). Otherwise, Overall performance categorical take the value of 0 (Less than 50%).

Other variables about the characteristics of participating units were surveyed in region, grade hospitals, type of hospitals, and level hospitals. Specifically, regarding region, the units were distributed across the Southeast, Central–Central Highlands, and other areas, reflecting the geographical diversity of the sample. In terms of grade hospitals, the institutions were classified according to the Ministry of Health's regulations, including Grade I, Grade II, Grade III, and Special hospitals, allowing for comparisons based on scale and technical capacity. With respect to type of hospitals, both public and private hospitals were included, highlighting the variety of operational models represented. Finally, according to management level, hospitals were categorized as central, provincial, district, and others, illustrating the hierarchical structure of the healthcare system and ensuring representative coverage across different levels of service delivery.



Data analysis

Data were analyzed using STATA 14.0. Descriptive statistics were applied to summarize the performance scores and unit characteristics. Qualitative variables were presented as frequencies and percentages, while quantitative variables were expressed as means and standard deviations. Comparisons of mean outcome scores across the years 2022, 2023, and 2024 were conducted using one-way analysis of variance (ANOVA).

Ethical considerations

The study used secondary data from a national quality assurance program. No personal or identifiable patient data were used. Institutional permission was obtained for the use of program data in this analysis. All information was handled confidentially and used solely for research purposes.

RESULTS

Quality of units participating in the program of extracurricular blood smear for malaria parasites

Each sample set designed according to WHO was sent to units participating in the blood smear program for malaria parasites including 2 blood smears (Fig. 1 A), including thin droplet area (Fig. 1 B) and thick droplet area (Fig. 1 C).

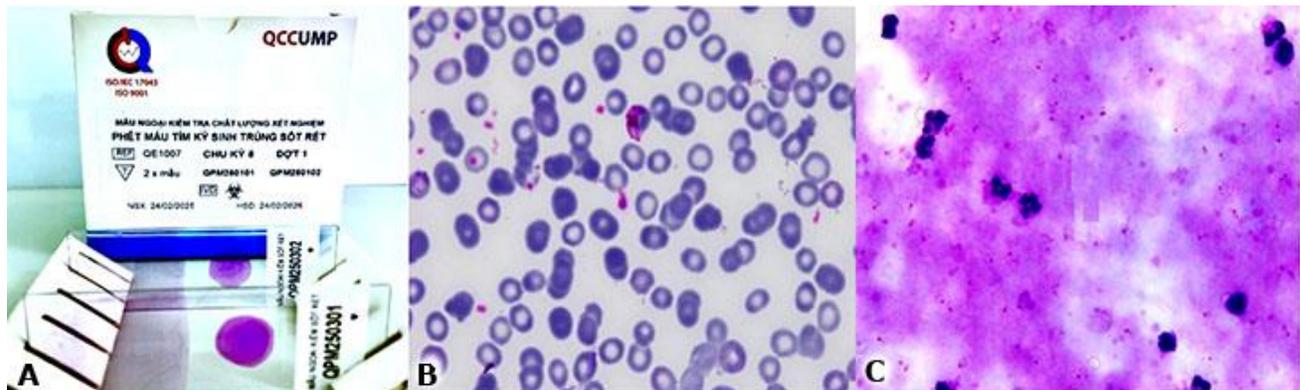


Fig. 1 - Morphology observed in thin droplet (left) and thick droplet (right) samples.

Characteristics of units participating in the proficiency testing program

The distribution rate by region is markedly different when considering units participating in the program of blood smear for malaria parasites across the country. The Southeast region accounts for the majority of participating units, with nearly 68.8%. The Central – Central Highlands region follows with a significant percentage of participation, close to 18.8%. Meanwhile, the remaining regions (categorized as "other") have a very low rate of participation in the external quality assessment program (table 1).

Within the scope of assessing the distribution of participation in external inspection by hospital grade, Grade I hospitals show the highest participation rate, accounting for 50%. This is followed by Grade II and Grade III hospitals, with participation rates ranging from 21.9% to 25%. In contrast, the special hospital sector has a very low participation rate, at only 3.1%. Regarding hospital ownership type, the results reveal a clear distinction: public hospitals make up more than 70% of participants, while non-public hospitals account for less than 30%. As for the hospital level of care, the provincial and equivalent levels represent 62.5% of participation, which is significantly higher than the combined participation of the central and district-equivalent levels (table 1).



Table 1 – Characteristics of participating units in the malaria microscopic proficiency testing program over the years 2022-2024

Characteristics	n	%
Regions		
Others	4	12.4
Southeast	22	68.8
The Central - Central Highlands	6	18.8
Grade hospitals		
Grade I	16	50.0
Grade II	7	21.9
Grade III	8	25.0
Special hospital	1	3.1
Types hospitals		
Public	9	28.1
Private	23	71.9
Level hospitals		
Central	4	12.5
Provincial	20	62.5
District	7	21.9
Others	1	3.1

Performance of participants in the malaria microscopic proficiency testing program over the years 2022-2024

The results show the highest performance total score in 2023, with a slight but consistent decline observed in 2024 across all components. Specifically, the total score in 2023 was 14.6 ± 3.1 , followed by 14.3 ± 2.8 in 2022. In 2024, there was a notable drop to 13.0 ± 3.0 . The change in performance total score over the years was statistically significant with $p < 0.001$ (table 2).

The mean identification score decreased over time, from 5.5 ± 1.3 in 2022 to 5.0 ± 1.5 in 2023 and 4.8 ± 1.6 in 2024, statistically significant with $p = 0.004$. The average scores for parasite density estimation remained relatively stable, ranging from 3.0 ± 1.7 in 2023 to 3.5 ± 1.5 in both 2022 and the overall average. However, a slight decrease to 3.3 ± 1.5 was observed in 2024. The best performance was observed in 2023 with a mean score of 5.7 ± 0.7 , followed by 5.4 ± 1.0 in 2022.



A decline was seen in 2024 with an average of 4.9 ± 1.0 , suggesting a reduction in the ability to accurately recognize parasite forms, statistically significant with $p < 0.001$ (table 2).

Table 2 – Mean scores performance of participating units in the malaria microscopic proficiency testing program, Vietnam, 2022–2024

Mean Score	Total	2022	2023	2024	P value
Identification score	5.2 ± 1.5	5.5 ± 1.3	5.0 ± 1.5	4.8 ± 1.6	0.004
Density score	3.5 ± 1.5	3.5 ± 1.5	3.0 ± 1.7	3.3 ± 1.5	0.275
Individual analysis score	5.3 ± 1.0	5.4 ± 1.0	5.7 ± 0.7	4.9 ± 1.0	< 0.001
Total score	14.0 ± 3.0	14.3 ± 2.8	14.6 ± 3.1	13.0 ± 3.0	< 0.001

One-way ANOVA.

In general, the overall performance of units in the malaria microscopic proficiency testing program over the years 2022-2024 achieved performance scores above 50% or more consistently account for a high proportion, ranging from 94.4% to 95.6% (Fig. 2).

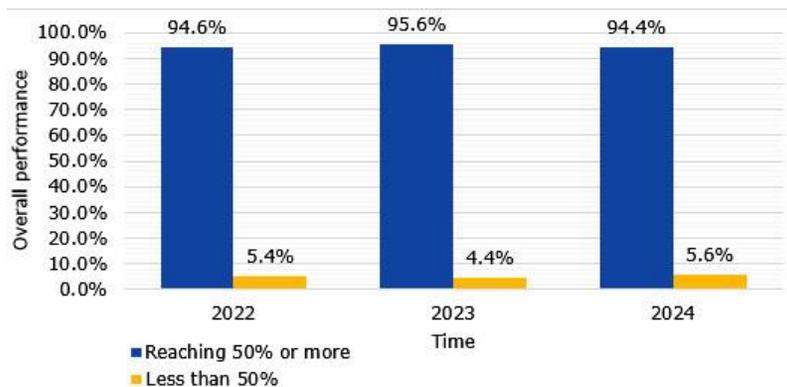


Fig. 2 - Proportion overall performance of units participating in external inspections from 2022 to 2024.

When analyzing the proportion of participating units achieving scores of 50% or higher for each indicator from 2022 to 2024, the identification shows a steady improvement: from 96.4% in 2022, to 96.7% in 2023, and 97.8% in 2024. The density counting displayed slight fluctuations, starting at 88.2% in 2022, dropping marginally to 87.8% in 2023, and then increasing to 88.9% in 2024.



Meanwhile, the individual parasite analysis showed excellent performance, increasing from 97.3% in 2022 to a perfect 100% in 2023, and consistently maintained at 100% in 2024 (Fig. 3).

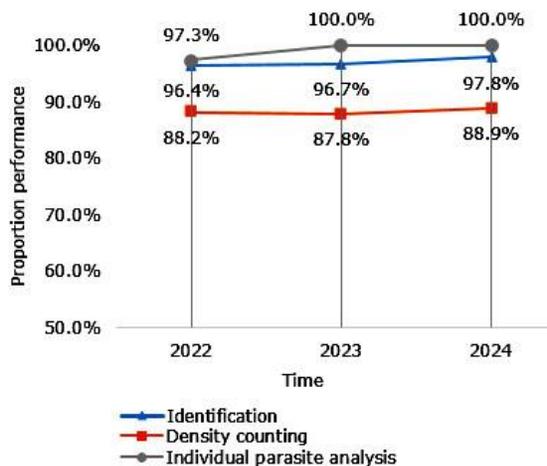


Fig. 3 - Proportion performance of individual indicators in external inspections from 2022 to 2024.

DISCUSSION

The high participation rate in the Southeast region may be attributed to the fact that it is an area with a large concentration of malaria in Vietnam, highlighting a greater awareness of the importance of testing quality. Similarly, the Central – Central Highlands region shows a relatively good level of participation. However, the low rate of participation in other regions especially border and remote areas where malaria infections are still common, suggests a lack of awareness or prioritization regarding the role of external quality assessment in ensuring accurate blood smear testing for malaria parasites.

The high participation rate of Grade I hospitals likely reflects their stronger commitment and capacity to ensure diagnostic quality, especially for malaria, which requires skilled microscopy. The modest participation of Grade II and III hospitals may indicate limited resources or varying levels of prioritization. The extremely low rate among special hospitals could be due to the nature of their patient population, where malaria testing is less frequently performed. The dominance of



public hospitals in the external inspection program suggests that government policies and mandates may be more strongly enforced in the public sector, whereas non-public facilities might have less oversight or incentive to participate. Finally, the fact that provincial-level hospitals contribute the majority of participants underscores their central role in regional disease control. In contrast, the lower participation from central and district levels highlights gaps in engagement that may affect the overall consistency of malaria diagnostic quality across the health system.

Yan H et al.⁽⁹⁾ showed overall scoring rates of 96.6% for microscopy and 85.0% for nucleic acid amplification tests (NAAT). In the national NAAT quality assessment, 124 samples were analyzed, showing an accuracy of 87.9%, with no significant difference between reference and non-reference laboratories. These findings indicate generally high diagnostic performance, though NAAT results suggest room for improvement in molecular testing accuracy across laboratory levels.

The upward trend in the identification score reflects a gradual yet consistent improvement in the ability of units to identify accurately malaria parasites in blood smear testing. In contrast, the density counting score remains notably lower and more variable than the other indicators, suggesting that this step is technically more challenging or lacks consistency in practice, and therefore requires targeted improvement. The perfect performance in individual analysis over the last two years demonstrates that this aspect of the testing procedure has been well-standardized and effectively maintained, likely due to improved training and adherence to protocols.

Maintaining a 100% score for two consecutive years also suggests a mature quality assurance system, which can serve as a model for enhancing other testing components, particularly density counting.

According to the results of the study evaluating the performance of the laboratory by confirming the reliability and accuracy of the results obtained in accordance with the requirements of the ISO 15189 standard in order to verify the method of diagnosing malaria by microscopy at LPM, belonging to the Aristide Le Dantec hospital (HALD) in Dakar, Senegal. The results showed that a minimum threshold of 50% consistency was used for comparison, (4) of the six (6) parasite density assessment slides achieved less than 50% consistency, thirteen (13) of the fourteen (14) identification slides achieved greater than 50% consistency. For species identification, consistency



greater than 80% was noted and the reading technician achieved a score of 0.20 to 0.4 on a scale of 0 to 1 for the parasite density reading. The microscope reader achieves 100% accuracy, sensitivity, specificity, and both negative and positive predictive values.⁽¹⁰⁾

In Uganda, a cross-sectional study found that less-experienced technicians and those without refresher training have significantly lower diagnostic accuracy, underscoring the need for ongoing capacity building and quality monitoring.⁽¹¹⁾ Similarly, a 2023 study from Ethiopia reported frequent misdiagnosis and species misidentification when comparing routine microscopy results with PCR findings, emphasizing the necessity of regular performance assessments and corrective actions to strengthen malaria diagnostic quality.⁽¹²⁾

The small proportion of units scoring below 50% represents facilities that may face significant challenges in maintaining the accuracy and reliability of malaria microscopy. These underperforming units are often located in remote areas, where access to training, supervision, and quality laboratory materials is limited. Many of these laboratories are classified as Grade II or III and operate with fewer qualified staff and less consistent participation in quality improvement activities. The low performance likely reflects gaps in technical skills—particularly in parasite density counting, slide preparation, and species identification—as well as weaknesses in internal quality control systems. Strengthening communication between regional reference centers and lower-level facilities can also help improve consistency and reliability in malaria diagnosis across all regions. This finding is consistent with the WHO Malaria Microscopy External Quality Assessment Progress Report (2022),⁽⁸⁾ which states that laboratories located in remote and hard-to-reach areas often achieve lower performance scores due to limited access to training and supervision.

The units participating in the external blood smear for malaria parasites are mainly of the public, provincial level and equivalent, grade I, mainly distributed in the Southeast region. The percentage of participating units with results of 50% or more over the years accounts for a high rate and is always stable. The quality change in each indicator tends to get better over the years (except for the density counting indicator).



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Conflicts of interest

None of conflicts of interest in relation to the work.

Authorship contribution

Conceptualization: *Phuc Huynh Thi Diem.*

Data curation: *Phuc Huynh Thi Diem, Xuan Bui Thi Le, Huynh Nguyen Tien.*

Formal analysis: *Phuc Huynh Thi Diem, Phuong Nguyen Thi Be.*

Research: *Tran Thi Hue Van, Phuc Huynh Thi Diem, Phuong Nguyen Thi Be, Xuan Bui Thi Le, Huynh Nguyen Tien.*

Methodology: *Phuc Huynh Thi Diem, Xuan Bui Thi Le, Huynh Nguyen Tien.*

Supervision: *Tran Thi Hue Van.*



Writing – original draft: *Phuc Huynh Thi Diem, Phuong Nguyen Thi Be, Xuan Bui Thi Le, Huynh Nguyen Tien.*

Writing – review and editing: *Phuc Huynh Thi Diem, Phuong Nguyen Thi Be.*

Data availability

Raw data is stored in excel files and is ready to be provided upon request. Email:

huevan@ump.edu.vn