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Diagnostic Performance of Rapid Diagnostic Tests and Microscopy for Malaria Detection in Jayapura, Indonesia: A Pilot Study

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Abstract: Malaria remains a major public health concern in Jayapura, Indonesia. Limited microscopy availability in primary healthcare facilities has increased reliance on rapid diagnostic tests (RDTs) for early case detection. This study aimed to evaluate the diagnostic performance of two malaria RDTs, AllCheck® and Orient Gene®, using microscopy as the reference standard. A cross-sectional study was conducted at a primary healthcare center in Jayapura in December 2025, involving 49 patients with clinical symptoms suggestive of malaria. Capillary blood samples were collected for RDT testing and preparation of thick and thin blood smears. Diagnostic accuracy was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), as well as agreement analysis using Cohen's Kappa and McNemar test. Both RDTs demonstrated comparable diagnostic performance, with sensitivities of 93.75% (95% CI: 69.77–99.84) and specificities of 100% (95% CI: 89.42–100.00). The PPV was 100% (95% CI: 78.20–100.00), and the NPV was 97.06% (95% CI: 84.67–99.93). Agreement analysis showed almost perfect concordance between each RDT and microscopy ($\kappa = 0.953$; $p < 0.001$). No significant difference was observed between AllCheck® and Orient Gene® results based on the McNemar test ($p = 1.000$). No significant association was found between diagnostic outcomes and patient sex or age group ($p > 0.05$). The RDTs showed good performance in detecting *Plasmodium falciparum* and *Plasmodium vivax*, but reduced sensitivity for *Plasmodium malariae*. In conclusion, AllCheck® and Orient Gene® RDTs demonstrate strong diagnostic performance and almost perfect agreement with microscopy, supporting their suitability for malaria screening and initial diagnosis in primary healthcare settings in Jayapura. Nevertheless, microscopy remains essential for confirming non-falciparum infections and ensuring comprehensive case detection.
Keywords: Microscopic; *Plasmodium falciparum*; *Plasmodium malariae*; *Plasmodium vivax*; rapid diagnostic tests.

INTRODUCTION

Malaria remains a significant public health problem in Papua. The disease burden has increased, with reported cases rising from 28,075 in 2020 to 30,235 in 2021, and the Annual Parasite Incidence (API) in Jayapura reached 99.49 per 1,000 population[1]. This high incidence is closely associated with local environmental conditions, including housing quality, the presence of mosquito breeding sites around residential areas, and

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climatic factors such as temperature, rainfall, and humidity that directly support the survival of malaria vectors[1, 2].

Beyond environmental determinants, Papua also faces challenges related to case management and community behavior. Resistance to sulfadoxine–pyrimethamine has long been reported and may reduce the effectiveness of commonly used therapies[3]. At the same time, unsafe self-medication practices remain prevalent, often leading to delayed diagnosis and inappropriate treatment[4]. Under these circumstances, rapid and accurate diagnostic methods are essential to ensure appropriate therapy and reduce transmission. Consequently, rapid diagnostic tests (RDTs) are widely relied upon as diagnostic tools in primary health care settings.

Several studies have demonstrated that malaria RDTs generally perform well, although sensitivity and specificity may vary across regions and parasite species. In Indonesia, for example, the Advantage Malaria Card Pf/Pv Ag demonstrated 100% sensitivity for *Plasmodium vivax* and 89.5% for *Plasmodiun falciparum*[5]. More recent findings from Sorong City, West Papua, also reported promising results, with a sensitivity of 81.8% and specificity of 100%[6]. Similar performance variations have been documented in other countries, including India, Ethiopia, Nigeria, and Côte d'Ivoire, where sensitivity is generally high, and specificity exceeds 95%, particularly for the detection of *Plasmodium falciparum*[7-9]. These findings are supported by a systematic review involving 14 studies from five countries evaluating 14 malaria RDTs. Overall, RDTs demonstrated good diagnostic accuracy, especially for *Plasmodium falciparum*, with a sensitivity of 91.8% and specificity of 97.7%. For *Plasmodium vivax*, sensitivity tended to be lower, although specificity remained very high at 99.2%. The review also highlighted that RDT performance improves with increasing parasite density in the blood[10].

Although several studies have evaluated malaria rapid diagnostic tests (RDTs) in Indonesia, data from Papua remain limited, despite its distinct transmission characteristics and species distribution. In Jayapura, particularly at the primary healthcare level, the diagnostic performance of the AllCheck® and Orient Gene® RDTs has not been specifically assessed. Local evidence is important because variations in parasite density, species composition, and the possible presence of pfhrp2/3 gene deletions may affect the accuracy of HRP2-based RDTs.

A study conducted in Ethiopia reported that 63.6% of *Plasmodium falciparum* isolates lacked the Pfhrp2 gene, and a substantial proportion also showed deletions of the Pfhrp3 gene. The authors concluded that these deletions may lead to false-negative results in HRP2-based RDTs and could compromise malaria diagnostic strategies in endemic settings[11].

Malaria RDTs detect specific parasite antigens, primarily histidine-rich protein 2 (HRP2) in *Plasmodium falciparum* and parasite lactate dehydrogenase (pLDH) expressed by different Plasmodium species. The selected antigen target influences diagnostic sensitivity, particularly in non-falciparum infections. Therefore, evaluating RDT performance within the local epidemiological context is essential to ensure accurate diagnosis. Accordingly, this study aimed to evaluate the diagnostic performance of the AllCheck® and Orient Gene® malaria rapid diagnostic tests compared with microscopy in symptomatic patients at primary healthcare facilities in Jayapura, Papua, Indonesia.

MATERIALS AND METHODS

The study subjects were outpatients presenting to primary health care facilities with clinical complaints suggestive of uncomplicated malaria. Clinical criteria included fever with a temperature ≥37.5°C or a history of fever within the previous 24 hours. This study was designed as a pilot study; therefore, the sample size was not determined using a formal diagnostic sample size calculation. A total of 50 participants were recruited consecutively to obtain preliminary data on RDT performance and to assess the feasibility of examination procedures prior to a larger-scale study.

Eligible participants were individuals aged ≥1 year who had not received antimalarial therapy within the previous seven days and did not exhibit signs or symptoms of severe malaria according to WHO criteria. Individuals with severe comorbid conditions, such as acute malnutrition, and those who declined to provide written informed consent were excluded from the study. A total of 50 individuals were screened for eligibility. One participant was excluded because they were younger than 1 year of age. The remaining 49 participants met the inclusion criteria and were included in the final analysis.

Specimen collection was performed using capillary blood obtained through the fingerprick method. Thick and thin blood smears were prepared on a single glass slide. The thin smear was prepared by placing approximately 2 µL of blood on a spreader slide at an angle of about 45°. The thick smear was prepared by placing approximately 6 µL of blood and spreading it to form a 10 mm diameter area. After air-drying, the smears were processed according to standard procedures and stained using Giemsa.

Microscopic examination of thick and thin blood smears was performed by certified and experienced laboratory personnel in accordance with WHO guidelines. Slides were examined systematically under oil immersion, and parasite identification was based on standard morphological criteria. For negative smears, microscopic examination was conducted according to WHO recommendations before declaring the sample negative. Two malaria rapid diagnostic tests were used in parallel for each blood sample: the AllCheck® Malaria Pf/PAN Rapid Test, which detects *Plasmodium falciparum* histidine-rich protein 2 (HRP2) and pan-Plasmodium lactate dehydrogenase (pLDH), and the Orient Gene® Malaria Pf/Pv Rapid Test, which detects Pf-specific and Pv-specific antigens. Both tests were performed according to the manufacturer's instructions. RDT results were read within 15–20 minutes by laboratory staff and interpreted as positive or negative based on the presence of valid control and test lines.

Diagnostic performance was assessed by comparing RDT results with microscopy findings. Demographic data and examination results were recorded using standardized forms, entered, and cleaned in Microsoft Excel. Statistical analyses were performed using IBM SPSS Statistics version 29.0. The association between categorical variables (sex and age group) and malaria positivity based on microscopy was evaluated using the Chi-square test. When the assumption of minimum expected cell counts was not met, the Fisher–Freeman–Halton exact test was applied. A p-value <0.05 was considered statistically significant.

Diagnostic accuracy parameters were calculated using microscopy as the reference standard. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were derived from 2×2 contingency tables, and exact binomial (Clopper–Pearson) 95% confidence intervals were computed for each estimate. Agreement

between each RDT and microscopy was assessed using Cohen's kappa coefficient. To compare paired diagnostic outcomes between AllCheck® and Orient Gene®, the McNemar test was applied.

This study received ethical approval from the Health Research Ethics Committee of Jayapura Health Polytechnic, Ministry of Health, under approval number 139/KEPK-J/XI/2025.

RESULTS AND DISCUSSION

The results of malaria examinations using AllCheck®, Orient Gene®, and microscopic analysis among malaria patients at the laboratory unit of Koya Barat Primary Health Center are presented in Table 1.

Table 1. Overall Prevalence of Malaria Parasite Infection

Variable	AllCheck®			Orient Gene®			Microscopy		
	Positive n (%)	Negative n (%)	p-value	Positive n (%)	Negative n (%)	p-value	Positive n (%)	Negative n (%)	p-value
Sex									
Male	8 (27.4)	21 (72.4)	0.580	8 (27.4)	21 (72.4)	0.580	9 (31.0)	20 (69.0)	0.771
Female	7 (35.0)	13 (65.0)		7 (35.0)	13 (65.0)		7 (35.0)	13 (65.0)	
Age (yr)									
0–5	2 (28.6)	5 (71.4)	0.862	2 (28.6)	5 (71.4)	0.862	2 (28.6)	5 (71.4)	0.872
6–15	2 (20.0)	8 (80.0)		2 (20.0)	8 (80.0)		2 (20.0)	8 (80.0)	
16–30	4 (28.6)	10 (71.4)		4 (28.6)	10 (71.4)		5 (35.7)	9 (64.3)	
31–45	5 (35.7)	9 (64.3)		5 (35.7)	9 (64.3)		5 (35.7)	9 (64.3)	
>45	2 (50.0)	2 (50.0)		2 (50.0)	2 (50.0)		2 (50.0)	2 (50.0)	

A total of 49 participants were included in the final analysis. Based on microscopic examination as the reference standard, 16 participants (32.7%) were positive for malaria infection and 33 (67.3%) were negative. The distribution of malaria positivity according to sex and age group is presented in Table 1. No statistically significant association was observed between malaria positivity and sex ($p = 0.771$) or age group ($p = 0.872$).

The cross-tabulation between both RDT brands and microscopy is presented in Table 2. Each RDT correctly identified 15 of 16 microscopy-confirmed malaria cases and produced no false-positive results. One false-negative case was observed for each RDT.

Table 2. Cross-Tabulation of AllCheck® and Orient Gene® Compared with Microscopy

Result Category	AllCheck® Microscopy +	AllCheck® Microscopy −	Orient Gene® Microscopy +	Orient Gene® Microscopy −
RDT Positive	15	0	15	0
RDT Negative	1	33	1	33
Total	16	33	16	33

Diagnostic performance parameters are summarized in Table 3. Both RDT brands demonstrated identical performance. Sensitivity was 93.75%, while specificity reached 100.00%. The PPV was 100.00%, and the NPV was 97.06%. The 95% confidence intervals reflect the precision of these estimates.

Table 3. Diagnostic Performance of RDT Brands Compared with Microscopy (Exact 95% Confidence Intervals)

Parameter	AllCheck® % (95% CI)	Orient Gene® % (95% CI)
Sensitivity	93.75 (69.77–99.84)	93.75 (69.77–99.84)
Specificity	100.00 (89.42–100.00)	100.00 (89.42–100.00)
PPV	100.00 (78.20–100.00)	100.00 (78.20–100.00)
NPV	97.06 (84.67–99.93)	97.06 (84.67–99.93)

Both RDT brands demonstrated high diagnostic accuracy when compared with microscopy. Sensitivity reached 93.75% (95% CI: 69.77–99.84), indicating that 15 of 16 microscopy-confirmed cases were correctly identified. Specificity was 100.00% (95% CI: 89.42–100.00), with no false-positive results observed.

The relatively wide confidence interval for sensitivity reflects the limited number of positive cases included in this pilot study. The confidence interval for specificity, although reaching 100% at the upper bound, showed a lower limit of 89.42%, indicating statistical uncertainty inherent to the sample size.

Table 4. Agreement and Paired Comparison Analysis

Analysis	Comparison	Statistic	p-value	Interpretation
Cohen's Kappa	AllCheck® vs Microscopy	$\kappa = 0.953$	<0.001	Almost perfect
Cohen's Kappa	Orient Gene® vs Microscopy	$\kappa = 0.953$	<0.001	Almost perfect
McNemar Test	AllCheck® vs Orient Gene®	Discordant pairs: 0 vs 0	1.000	No difference

Agreement analysis showed almost perfect concordance between each RDT and microscopy (Cohen's $\kappa = 0.953$, $p < 0.001$), Table 4. Paired comparison using the McNemar test identified no discordant pairs and no statistically significant difference between AllCheck® and Orient Gene® ($p = 1.000$), Table 4.

The findings of this study indicate that age and sex were not significantly differentiating factors in malaria diagnostic outcomes, whether assessed using RDTs or microscopy. This observation aligns with evidence from Cameroon, where relatively balanced proportions of positive results were reported between males and females, with no significant differences across age groups in an asymptomatic population[12]. A retrospective pediatric study in Pakistan similarly found that age and sex did not significantly influence diagnostic outcomes, whereas parasite density was the primary determinant of test accuracy[13]. Collectively, these findings support the notion that malaria detection is more strongly influenced by infection-related characteristics, particularly parasite density, rather than by demographic factors.

In terms of diagnostic performance, the AllCheck® and Orient Gene® RDTs demonstrated equivalent accuracy in this study. Sensitivity reached 93.75%, with specificity and PPV both at 100%, and an NPV of 97.06%. These results indicate strong capability in correctly identifying infected individuals while minimizing false-positive diagnoses. However, a sensitivity of 93.75% implies that approximately 6.25% of

microscopically confirmed malaria cases may remain undetected if RDT is used as the sole diagnostic method. This finding indicates that both RDTs demonstrate a very high level of agreement with microscopy and show no statistically significant difference in diagnostic performance.

False-negative results are well documented in real-world settings. A large-scale analysis involving more than 85,000 children across 19 African countries reported that approximately 20% of microscopy-positive cases were RDT-negative, particularly in low-transmission areas, among younger children, and in urban environments[14]. Likewise, a clinical study using PCR as the reference standard demonstrated that both RDT and microscopy failed to detect more than 40% of infections identified by quantitative PCR[15]. These findings highlight how low parasitemia and inherent methodological limitations may allow a substantial proportion of infections to remain undiagnosed and continue contributing to malaria transmission. In addition, deletions of the histidine-rich protein 2 (HRP2) gene in *Plasmodium falciparum* may further compromise the performance of HRP2-based RDTs, although genotyping was not conducted in the present study.

The diagnostic performance observed here falls within ranges reported in previous Research. Meta-analyses have described pooled sensitivity and specificity values of approximately 93%, demonstrating relatively stable performance across diverse health care settings[16]. A study in the United Kingdom among pediatric populations reported comparable findings, particularly for *Plasmodium falciparum* detection, with sensitivity and NPV reaching 100%[17]. Evidence from studies conducted in India and Indonesia further supports the reliability of RDTs when compared with microscopy[5, 18].

Nevertheless, microscopy was used as the reference standard in this study, and it is important to acknowledge that microscopy is not an absolute gold standard. Evidence indicates that microscopy may miss 20–30% of infections detected by PCR, especially in submicroscopic cases[19]. Comparative evaluations of RDT, microscopy, and PCR have shown that PCR can identify an additional 12–23% of cases beyond those detected by conventional methods[20]. Consequently, some cases categorized as negative by both RDT and microscopy may represent true infections detectable only by more sensitive molecular techniques, potentially introducing verification bias when microscopy is assumed to be definitive.

The predictive values reported in this study should also be interpreted within the context of local malaria prevalence. Although PPV reached 100% and NPV 97.06%, predictive values are highly dependent on disease prevalence. Modeling analyses in African countries have demonstrated that in low-prevalence settings, PPV of RDTs may decline to below 20%, while NPV remains above 90%, despite unchanged intrinsic sensitivity and specificity[21]. Conversely, in higher-prevalence clinical settings, PPV may remain high at approximately 95–100%, whereas NPV may decrease due to a higher proportion of false-negative results[22]. Therefore, the predictive performance observed in this study reflects the epidemiological characteristics of the study area and may not be directly generalizable to regions with differing transmission intensities.

From a clinical and public health perspective, the 6.25% proportion of missed cases warrants careful consideration. In practical terms, this suggests that approximately 6 out of every 100 symptomatic malaria patients could be overlooked if diagnosis relies solely on RDT. Community-level analyses estimate that nearly 20% of infections in African pediatric surveys were not detected by RDT and consequently did not receive

treatment, thereby sustaining transmission[14]. Furthermore, molecular-based studies have shown that submicroscopic infections undetected by RDT or microscopy constitute a substantial portion of the infectious reservoir, particularly in pre-elimination contexts, where even a small number of missed cases may perpetuate local transmission[19].

Several limitations should be acknowledged. The relatively small sample size, consistent with the pilot study design, limits the generalizability of these findings. The low proportion of non-falciparum infections restricted a comprehensive evaluation of RDT performance for these species. Additionally, the study was conducted at a single health care facility over a limited period, limiting the full assessment of potential seasonal variations in malaria transmission.

Despite these limitations, the findings have important implications for malaria elimination efforts in Papua. In resource-limited settings where access to microscopy and laboratory infrastructure is constrained, RDTs with strong diagnostic performance provide valuable tools for screening and early case detection. Appropriate integration of RDTs into diagnostic algorithms can facilitate timely treatment initiation, reduce diagnostic delays, and support sustainable malaria control and elimination strategies in endemic regions.

CONCLUSION

Overall, the findings indicate that the RDTs used in this study provided consistent and reliable diagnostic results within primary health care practice, achieving sensitivity and specificity comparable to those reported in previous studies. However, given the exploratory nature of this pilot study and the limited sample size, these findings should be interpreted as preliminary evidence that requires further confirmation through larger-scale studies before broader implementation.

CONFLICT OF INTEREST

In this study there is no conflict of interest.

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