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Comparative Evaluation of Light Microscopy, Rapid Diagnostic Tests and the Sysmex Xn-31 Analyzer for Malaria Diagnosis in a Hospital Setting in the Ashanti Region of Ghana

Francis Agyei Amponsah

amponsahfa@yahoo.com

Kwame Nkrumah University of Science and Technology

Joseph Acquah

Kwame Nkrumah University of Science and Technology

Isaac Acheampong

Kwame Nkrumah University of Science and Technology

Prince Adoba

Kwame Nkrumah University of Science and Technology

Edward Yaw Afriyie

Komfo Anokye Teaching Hospital

Bright Antwi

Kwame Nkrumah University of Science and Technology

John Kwaku Onipayede

Kwame Nkrumah University of Science and Technology

Samuel Kwasi Appiah

University for Development Studies

Nathan Okai Odame

St. John of God Catholic Hospital

Samuel Wiafe Mintah

Seventh Day Adventist Hospital, Kwadaso

Benedict Sackey

Kwame Nkrumah University of Science and Technology

Lilian Antwi Boateng

Kwame Nkrumah University of Science and Technology

Otchere Addai-Mensah

Kwame Nkrumah University of Science and Technology

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COMPARATIVE EVALUATION OF LIGHT MICROSCOPY, RAPID DIAGNOSTIC TESTS AND THE SYSMEX XN-31 ANALYZER FOR MALARIA DIAGNOSIS IN A HOSPITAL SETTING IN THE ASHANTI REGION OF GHANA

Francis Agyei Amponsah[1], Joseph Acquah[1], Isaac Acheampong[1], Prince Adoba[1], Edward Yaw Afriyie[2], Bright Antwi[1], John Kwaku Onipayede[1], Samuel Kwasi Appiah[3], Nathan Okai Odame[4], Samuel Wiafe Mintah[5], Benedict Sackey[1], Lilian Antwi Boateng[1], Otchere Addai-Mensah[1].

1. **Department of Medical Diagnostics, Faculty of Allied Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana**
2. **Department of Haematology, Komfo Anokye Teaching Hospital, Kumasi, Ghana**
3. **Department of Haematology, School of Allied Health Sciences, University for Development Studies, Tamale, Ghana**
4. **Laboratory Department, St. John of God Hospital, Duayaw Nkwanta, Ghana**
5. **Laboratory Department, Seventh Day Adventist Hospital, Kwadaso-Kumasi, Ghana**

Corresponding author: Francis Agyei Amponsah; amponsahfa@yahoo.com, fa.amponsah@knust.edu.gh

ABSTRACT

Background: *Plasmodium falciparum* accounts for approximately 98.1% of malaria infections in Ghana, and the Ashanti Region experiences year-round transmission. Because malaria symptoms overlap with other febrile illnesses, laboratory confirmation is essential before treatment. Light microscopy, rapid diagnostic tests (RDTs) and the Sysmex XN-31 automated hematology analyzer are all used for malaria diagnosis, but each has recognized limitations, and no study in Ghana has compared all three methods simultaneously on the same patient samples. This study evaluated the diagnostic performance of RDTs, light microscopy and the Sysmex XN-31 for malaria detection in a Ghanaian hospital setting.

Methods: This cross-sectional study recruited 234 patients with suspected malaria at Kwadaso SDA Hospital, Ashanti Region. Participants' venous blood samples were simultaneously tested by light microscopy (reference standard), RDT, and the Sysmex XN-31. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Cohen's kappa were calculated for each pairwise comparison. Spearman's correlation and Bland-Altman analysis assessed agreement in parasite density between the Sysmex XN-31 and microscopy, and Cochran's Q test assessed overall agreement among all three methods.

Results: Of 234 participants, 26 (11.1%) tested positive by both microscopy and the Sysmex XN-31, while the RDT detected 23 (9.8%). *P. falciparum* accounted for 23 (88.5%) of positive cases. Against microscopy, the RDT showed 88.5% sensitivity, 100% specificity, 100% PPV, 98.6% NPV and kappa = 0.932; identical figures were obtained against the Sysmex XN-31. Parasite density measurements from the Sysmex XN-31 correlated strongly with microscopy ($r_s = 0.948$, $p < 0.001$), with Bland-Altman analysis showing a mean bias of −1,063 parasites/μL. Cochran's Q test found no statistically significant difference in overall accuracy among the three methods ($Q(2) = 6.000$, $p = 0.05$).

Conclusion: Light microscopy, RDTs and the Sysmex XN-31 all showed reliable diagnostic performance for malaria detection, with almost perfect to perfect agreement across all pairwise comparisons. The RDT's narrower species coverage was the only meaningful point of difference among the three methods. These findings support the parallel deployment of all three methods

across Ghana's tiered healthcare system, rather than treating any single method as a universal replacement.

Keywords: Malaria; Rapid diagnostic test; Light microscopy; Sysmex XN-31; Diagnostic accuracy; Ghana

Introduction

Malaria remains a global health concern, particularly in nations with low and intermediate incomes. According to the World Health Organization, there were approximately 249 million malaria cases and 608,000 deaths worldwide in 2022, with over 94% of these deaths occurring in the WHO African Region [1]. In Ghana, human malaria continues to account for a significant burden of disease, with *Plasmodium falciparum* being responsible for approximately 98.1% of cases. The middle belt of Ghana, including the Ashanti Region, experiences year-round transmission, with malaria parasitemia prevalence around 22.8% [2]. Malaria remains a primary contributor to morbidity and mortality across all age groups in the region, despite ongoing interventions and treatment programs [3].

Accurate and timely diagnosis remains essential for human malaria control, enabling effective case management, reducing unnecessary antimalarial use and preventing drug resistance [4]. Light microscopy has traditionally served as the gold standard for quantifying parasitemia and distinguishing between *Plasmodium* species. Under optimal conditions, it can detect parasite densities as low as 50 to 100 parasites/μL [5]. However, its performance is highly dependent on technical factors such as slide preparation, staining quality, and operator skill [6]. In resource-limited settings, sensitivity declines significantly, particularly for low-density infections.

To overcome these constraints, malaria rapid diagnostic tests (RDTs) were developed. These immunochromatographic tools detect malaria-specific antigens such as HRP2 (Histidine-rich protein-2) or pLDH (*Plasmodium* lactate dehydrogenase), and are praised for their speed, simplicity, and overcoming the need for expensive equipment [2,7]. However, RDTs are affected

by factors such as *hrp2* gene deletions, prozone effects and antigen persistence, which can lead to false negatives and false positives especially in low parasitemia cases [8].

More recently, automated hematology analyzers such as the Sysmex XN-31 have been introduced. These analyzers utilize fluorescence flow cytometry to detect infected red blood cells and provide parasitemia quantification alongside a full blood count. While offering high-throughput and operator-independent results, they may struggle with species differentiation, particularly for non-*falciparum* infections [4], [9]. Although Opoku Afriyie *et al*. evaluated the diagnostic accuracy of microscopy and RDTs against PCR among clinical malaria cases in Ghana [8], there is no published study in Ghana which comprehensively compares microscopy, RDTs, and the Sysmex XN-31 analyzer for malaria diagnosis. This evidence gap highlights the need for a comparative evaluation of all three diagnostic methods in actual clinical settings, especially in countries like Ghana, where malaria is endemic. This study, therefore, compared the diagnostic performance of light microscopy, RDTs, and the Sysmex XN-31 analyzer for malaria detection among patients in a hospital setting in the Ashanti Region of Ghana.

Materials and Methods

Study Design/ Setting

This study utilized a cross-sectional design to compare the diagnostic performance of light microscopy, rapid diagnostic tests (RDTs), and the Sysmex XN-31 analyzer for malaria detection. The study was carried out at Kwadaso SDA Hospital in the Ashanti Region of Ghana, between October and November 2025. Kwadaso SDA Hospital is one of the major hospitals that serves as a referral hospital in the Kwadaso municipality, and malaria is one of the top ten outpatient cases in the hospital.

Participants Recruitment

The study population consisted of patients presenting to Kwadaso SDA Hospital with clinical symptoms suggestive of malaria, including fever, chills, headache, fatigue, nausea, and other features commonly associated with acute malaria infection. Participants of all ages and genders,

including both adults and children, were eligible for inclusion provided they met the study criteria. Study participants were chosen by a convenience sampling technique; patients who exhibited clinical signs of malaria during the study period were contacted one after the other and enrollment continued until the desired sample size of 234 was attained. The sample size was determined using Cochran's formula for estimating proportions in cross-sectional studies. A 95% confidence level ($Z = 1.96$), a margin of error of 5% ($e = 0.05$), and an estimated malaria prevalence of 17.5% among clinical malaria cases in Ghana were applied, based on previous findings [8], yielding a minimum requirement of 221.8, adjusted to 234 to account for a possible 5% non-response rate.

Inclusion and exclusion criteria

Participants were included in the study if they presented with one or more symptoms suggestive of malaria and consented to blood sample collection for malaria testing using all three diagnostic methods. Written informed consent was obtained from adult participants, while assent and parental or guardian consent were obtained for minors. Participants who had not received antimalarial treatment within seven days prior to testing were eligible. Participants were excluded if they declined or were unable to provide informed consent. Individuals who had taken antimalarial medication within seven days prior to testing were excluded to avoid false-negative or altered diagnostic results. Patients requiring immediate emergency medical intervention and those whose blood samples were hemolyzed, clotted, contaminated, or insufficient for all diagnostic tests were also excluded.

Ethical consideration

The study's protocol was approved by the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medicine and Dentistry, KNUST (CHRPE/AP/931/25), and the management of Kwadaso SDA Hospital before commencing the study. The style and purpose of the study were explained to the participants before the interview. Informed consents were sought from adult participants or the legal guardians of children participants prior to sample collection. All methods were performed in accordance with the relevant guidelines and regulations.

Sample Collection and Laboratory Investigations

Blood samples were taken from each individual who exhibited symptoms suggestive of malaria after informed consent. 5ml of whole blood samples were drawn into 3.2% EDTA tubes using a 5ml syringe and needle. To guarantee uniformity across diagnostic techniques, the same blood sample was used for all testing and every test was performed on the same day.

For light microscopy, thick and thin blood smears were prepared on the same slide. While the thin smear aided in identifying the *Plasmodium* species, the thick smear was used to find parasites. The thin blood film was fixed with methanol before staining both thick and thin smears with 10% Giemsa working solution for 10 minutes. Blood films were observed under 100X objectives for detection of malaria parasites and the result was reported as positive if malaria parasites were seen or negative if malaria parasites were not seen after observing 100 fields of the thick smear. Parasite count was determined by counting the number of asexual forms of parasites against 200 leucocytes, this number was then multiplied by the participant's leucocyte count which is then divided by the 200 leucocytes and the result expressed as parasite per microliter of blood.

Malaria Rapid Diagnostic Tests (RDTs) operate based on the principle of lateral flow immunochromatography, detecting specific parasite antigens such as Histidine-Rich Protein 2 (HRP2) and/or *Plasmodium* Lactate Dehydrogenase (pLDH) in human blood. In this study, the RDT used was the HIGHTOP Malaria Pf Ag Test Cassette (Qingdao Hightop Biotech Co., Ltd., Qingdao, Shandong, China), a single-line cassette specific to the detection of *Plasmodium falciparum* Histidine-Rich Protein 2 (HRP2). When present, these antigens bind to monoclonal antibodies embedded in the test strip, producing a visible line at the test region indicates a positive result, with results typically available within 15–20 minutes. The appearance of a visible line at the control region of the kit validates the test.

The Sysmex XN-31 analyzer (Sysmex Corporation, Kobe, Japan) was operated by adhering to the manufacturer's protocol and safety procedures. The analyzer uses a combination of optical light scatter, electrical impedance, and fluorescence flow cytometry methods to evaluate complete blood counts, and utilizes fluorescence flow cytometry to detect malaria-infected red blood cells (MiRBCs) in peripheral blood by staining intracellular parasite nucleic acids with a proprietary fluorescent dye. When excited by a violet semiconductor laser (405 nm), the dye fluoresces, allowing the analyzer to identify and enumerate cells containing parasitic DNA distinct from

uninfected erythrocytes. The system provides a quantitative parasitemia output, expressed as the percentage of MiRBCs (% MiRBCs), with a lower detection limit of approximately 20 parasites/μL.

Statistical Analysis

Data were entered, cleaned and analyzed using SPSS version 27. Descriptive statistics including means, standard deviations, frequencies, and percentages were used to summarize participant characteristics. Light microscopy was used as the reference standard for diagnostic performance evaluation. The diagnostic performance of RDTs and the Sysmex XN-31 analyzer were assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) using 2 × 2 contingency tables. Ninety-five percent confidence intervals (95% CI) were calculated for all diagnostic performance measures. Spearman's rank correlation analysis and Bland–Altman analysis were additionally performed to evaluate the relationship and agreement between parasite density measurements obtained by light microscopy and the Sysmex XN-31 analyzer. Agreement between diagnostic methods was evaluated using Cohen's kappa (κ) statistic, and Cochran's Q test was used to assess overall agreement in diagnostic accuracy among all three methods. A p-value less than 0.05 was considered statistically significant.

RESULTS

Participant Characteristics and Malaria Positivity

A total of 234 participants were included in this study. The median age of the participants was 26 years (IQR 14–44.25), with ages ranging from 1 to 90 years. Using light microscopy and the Sysmex XN-31 analyzer, 26 individuals (11.1%) tested positive for malaria, while 208 individuals (88.9%) tested negative. In contrast, the RDT identified 23 positive cases (9.8%) and 211 negative cases (90.2%). Among the 26 positive cases, *P. falciparum* accounted for 23 (88.5%), *P. malariae* for 2 (7.7%), and *P. ovale* for 1 (3.8%).

Table 1: General Descriptive of Study Participants (N = 234)

Variable	Frequency (n)	Percentages (%)
Age (years) M(IQR)	26(14-44)	
Range	1-90	
Sex		
Male	92	39.3
Female	142	60.7
Microscopy Results		
Positive Cases	26	11.1
Negatives	208	88.9
RDT Reactivity		
Positive	23	9.8
Negative	211	90.2
Sysmex XN-31 Results		
Positive	26	11.1
Negative	208	88.9
Species Distribution (among positive cases, N=26)		
P. f	23	88.5
P. m	2	7.7
P. o	1	3.8

The results are presented in frequencies and percentages unless otherwise stated. M(IQR): Median (Inter-quartile Range), RDT: Rapid Diagnostic Test, p.f: Plasmodium falciparum, p.m: Plasmodium malariae, p.o: Plasmodium ovale. Species distribution percentages are calculated among malaria-positive cases only (N=26).

Comparison of Sociodemographic and Hematological Parameters between Malaria-Positive and Malaria-Negative Participants

Sociodemographic and hematological parameters of the study participants were compared based on malaria status and are presented in Table 2. Median age did not differ significantly between malaria-positive participants (19.50 years, IQR 16.00–27.25) and malaria-negative participants (27.00 years, IQR 13.00–45.00; $p = 0.677$). Sex distribution also did not differ significantly between the two groups ($p = 0.288$), with the malaria-positive group comprising 13 males (50.0%) and 13 females (50.0%), compared with 79 males (38.0%) and 129 females (62.0%) among malaria-negative participants.

Malaria-positive participants had significantly higher hemoglobin concentration than malaria-negative participants (13.40 g/dL versus 12.20 g/dL; $p = 0.001$) and significantly higher hematocrit (40.15% versus 35.75%; $p < 0.001$). Red blood cell count and white blood cell count did not differ significantly between the two groups ($p = 0.263$ and $p = 0.206$, respectively). Platelet count was significantly lower among malaria-positive participants than malaria-negative participants (143×10^9/L versus 242×10^9/L; $p < 0.001$).

Table 2: Comparison of Hematological Parameters between Malaria-Positive and Malaria-Negative Participants.

Parameter	Malaria positive (N=26)	Malaria negative (N=208)	p-value
Age(years) [Median (IQR)]	19.50 (16.00–27.25)	19.50 (16.00–27.25)	0.677
Sex [n (%)]			
Male	13 (50.0%)	79 (38.0%)	0.288
Female	13 (50.0%)	129 (62.0%)	
Hb (g/dL) [Median (IQR)]	13.40 (12.4-14.2)	12.20 (11.3-13.2)	**0.001**
RBC count ($x10^{12}$) [Mean ± SD]	4.89 ± 0.73	4.42 ± 0.59	0.263

WBC count [Median (IQR)]	6.34 (5.1-7.6)	6.78 (5.3-8.7)	0.206
PLT count [Median (IQR)]	143 (85.5-197.3)	242 (198.8-310.5)	**<0.001**
HCT [Median (IQR)]	40.15 (36.8 -42.8)	35.75 (32.9-38.8)	**<0.001**

Age and other non-normally distributed continuous parameters are presented as median (interquartile range) and compared using the Mann-Whitney U test. RBC values are expressed as mean ± standard deviation and compared using the independent samples t-test. Sex is presented as frequency (%) and compared using Fisher's Exact Test. Significant value: P<0.05. N=frequency, Hb= Haemoglobin Concentration, RBC= Red Blood Cells, WBC= White Blood Cells, PLT= Platelets, HCT= Haematocrit, IQR= Interquartile Range, SD= Standard Deviation.

Diagnostic Accuracy of the Three Methods

The diagnostic performance of the RDT and the Sysmex XN-31 relative to light microscopy, and of the RDT relative to the Sysmex XN-31, is summarized in Table 3. Against light microscopy, the RDT correctly identified 23 of 26 positive cases and returned three false-negative results, with no false-positive results among the 208 negative cases, giving a sensitivity of 88.5%, specificity of 100%, PPV of 100%, NPV of 98.6%, and kappa = 0.932 ($p < 0.001$). Against the Sysmex XN-31, the RDT produced identical performance figures. The Sysmex XN-31 identified all 26 microscopy-positive cases and returned no false positives among the 208 negative cases, giving 100% sensitivity, specificity, PPV, and NPV against microscopy, with kappa = 1.00 ($p < 0.001$). In both RDT comparisons, all three false negatives were the non-falciparum cases (2 *P. malariae*, 1 *P. ovale*).

Table 3: Diagnostic accuracy of RDT and Sysmex XN-31 relative to light microscopy, and RDT relative to Sysmex XN-31

Comparison	Sensitivity	Specificity	PPV	NPV	Kappa (κ)	p-value
RDT vs Microscopy	88.5%	100%	100%	98.6%	0.932	<0.001

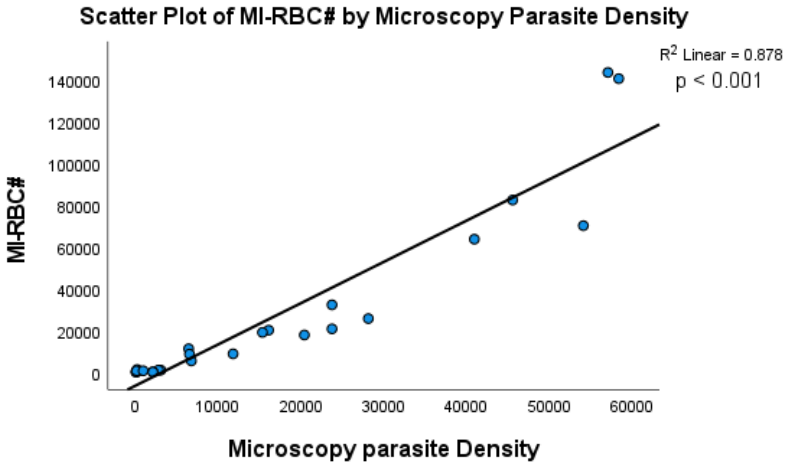
Comparison	Sensitivity	Specificity	PPV	NPV	Kappa (κ)	p-value
RDT vs Sysmex XN-31	88.5%	100%	100%	98.6%	0.932	<0.001
Sysmex XN-31 vs Microscopy	100%	100%	100%	100%	1.000	<0.001

PPV: positive predictive value; NPV: negative predictive value; κ: Cohen's kappa coefficient; kappa values were interpreted according to the classification of Landis and Koch (1977), where 0.81–1.00 indicates almost perfect to perfect agreement; light microscopy served as the reference standard for both the RDT and Sysmex XN-31 comparisons. RDT: Rapid Diagnostic Test.

Agreement in Parasite Density between the Sysmex XN-31 and Light Microscopy

Spearman's rank correlation revealed a very strong positive correlation between parasite density measurements obtained by light microscopy and the Sysmex XN-31 ($r_s = 0.948$, $p < 0.001$) (Figure 1A). Bland–Altman analysis showed a mean difference (bias) of −1,063 parasites/µL, with 95% limits of agreement of −17,428 to 15,302 parasites/µL; most observed differences fell within these limits, indicating acceptable agreement between the two methods (Figure 1B).

A



B

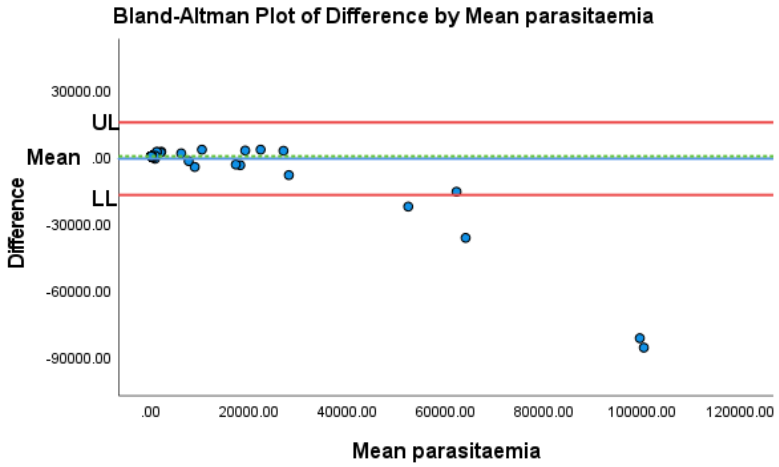


Figure 1: Agreement between light microscopy and the Sysmex XN-31 for parasite density. *(A) Correlation between microscopy parasite density and Sysmex XN-31 MI-RBC#. (B) Bland–Altman plot of the difference between the two methods against their mean.*

Overall Comparison of Diagnostic Accuracy among the Three Methods

Cochran's Q test was performed to compare the overall diagnostic accuracy of the three malaria diagnostic methods simultaneously. The results are presented in Table 4. The test revealed no statistically significant difference in diagnostic performance among the RDT, light microscopy, and the Sysmex XN-31 analyzer.

Table 4: Overall Comparison of Diagnostic Accuracy Among the Three Methods Using Cochran's Q Test

Test	N	Q Statistic	df	P-value
Cochran's Q	**234**	**6.000**	**2**	**0.05**

Significant value: P<0.05, df: degrees of freedom, RDT: Rapid Diagnostic Test

Discussion

This study compared the diagnostic performance of rapid diagnostic tests (RDTs), light microscopy, and the Sysmex XN-31 automated hematology analyzer for malaria detection among patients with suspected malaria attending Kwadaso SDA Hospital in the Ashanti Region of Ghana.

All three methods demonstrated strong and comparable overall diagnostic performance, with almost perfect to perfect agreement observed across all method pairs. One point of difference recurs throughout these findings. Because the RDT used in this study targets the *Plasmodium falciparum*-specific HRP2 antigen by design, it was, as expected, unable to detect the non-falciparum species that both microscopy and the Sysmex XN-31 identified.

The RDT showed almost perfect agreement with light microscopy, with a sensitivity of 88.5%, specificity of 100%, positive predictive value of 100%, negative predictive value of 98.6%, and a Cohen's kappa of 0.932. The specificity result is particularly notable since no malaria-negative participants were misclassified as positive. This finding is consistent with the high specificity reported for HRP2-based RDTs elsewhere in sub-Saharan Africa and carries direct clinical relevance, since false positives in an endemic setting such as Ghana translate into unnecessary antimalarial treatment [10], [11]. The RDT's sensitivity of 88.5% which is marginally lower than that of microscopy and the Sysmex XN-31, reflects the species composition of this sample. Because *Plasmodium falciparum* accounted for 88.5% of the 26 positive cases, an HRP2-based test was expected to perform near its ceiling; the same result also indicates precisely where its species-specific design constrains its detection range.

The three false-negative RDT results in this study were traced to non-*falciparum* species, specifically two cases of *P. malariae* and one case of *P. ovale*, each confirmed independently by microscopy. Because these species do not express the HRP2 antigen that the RDT is designed to detect, a negative result was the expected outcome rather than a test failure [12]. This distinction could only be established with confidence because every specimen in this study was tested simultaneously by all three methods; a two-method comparison could not have separated a species-specific detection boundary this cleanly from a reference-method artifact. It is also worth noting that *hrp2* gene deletions can lead to a second possible route to false-negative RDT results, even in true *P. falciparum* infections, and have been increasingly reported elsewhere in sub-Saharan Africa [13]. This study did not directly test for gene deletions, and therefore cannot confirm whether that mechanism contributed to any of the observed results, but it remains a relevant caveat for interpreting RDT-negative results more broadly. A negative RDT result in this study population should not, on its own, be interpreted as ruling out malaria, particularly where non-falciparum infection is clinically suspected.

Against the Sysmex XN-31, the RDT produced results almost identical to those obtained by microscopy, with a sensitivity of 88.5%, a specificity of 100%, a positive predictive value of 100%, a negative predictive value of 98.6%, and a kappa of 0.932. That these figures barely changed between the two reference methods is informative in itself; it indicates that the RDT's performance in this study was stable and not an artifact of which reference standard was used for comparison. The same three non-falciparum cases missed against microscopy were also missed here, which rules out the possibility that those misses were specific to microscopy as a comparator. This is consistent with a comparable evaluation of automated analyzers against RDTs [7]. For this study population, the practical implication is that the RDT remains a dependable frontline tool wherever *P. falciparum* predominates, as is the case for the great majority of cases observed here.

Against light microscopy, the Sysmex XN-31 matched every result exactly, with 100% sensitivity, specificity, positive predictive value, and negative predictive value, and a kappa of 1.00, the strongest agreement recorded anywhere in this study. This figure should be read carefully rather than taken at face value. With only 26 positive cases in the sample, there were few opportunities for the two methods to disagree, so a kappa of 1.00 reflects the dataset's small size and low error rate as much as it reflects the analyzer's true ceiling of accuracy. Similarly, strong agreement between the XN-31 and microscopy has been reported in comparable African settings, supporting the plausibility of this result, although confirming it with the same degree of certainty would require a larger sample size containing substantially more positive cases [4], [14]. Perfect detection agreement, however, did not mean equivalent diagnostic depth. The single *P. ovale* case in this dataset illustrates the gap directly: microscopy identified the parasite as *P. ovale*, while the Sysmex XN-31 recorded it only as an infection of an unspecified non-falciparum species. This distinction is not academic, since *P. ovale* carries dormant liver-stage hypnozoites and requires radical cure with primaquine in addition to standard treatment [15], a decision the XN-31 output alone could not have prompted.

Beyond detection accuracy, the Sysmex XN-31's simultaneous production of a full blood count added clinically useful information. Malaria-positive participants had significantly lower platelet counts than malaria-negative participants (143×10^9/L versus 242×10^9/L; $p < 0.001$), consistent with the thrombocytopenia widely documented in malaria infection [16]. The analyzer's standardized, operator-independent output also removes the inter-observer variability inherent to

manual microscopy [9], though its cost and infrastructure requirements currently restrict its practical use in Ghana to tertiary and reference laboratory settings.

Parasite density measurements from the Sysmex XN-31 correlated strongly with microscopy (r_s = 0.948, $p < 0.001$), consistent with comparable evaluations of Sysmex-based parasite quantification [9,14]. Bland–Altman analysis showed a mean bias of −1,063 parasites/μL, with the few outliers all occurring at high parasite densities, where the XN-31's fluorescence signal approaches background noise and manual microscopy counting becomes more sensitive to field-to-field sampling variation. Outside this low-density range, the quantitative agreement between the two methods was sufficiently close to support their reasonable interchangeability across this study population.

Across all three pairwise comparisons, kappa values ranged from 0.932 to 1.00, representing almost perfect to perfect agreement according to the classification proposed by Landis and Koch (1977) [17]. Cochran's Q test, however, returned $Q(2) = 6.000$, $p = 0.05$, sitting exactly on the pre-defined significance threshold. Read strictly, this supports accepting the null hypothesis of no significant difference in accuracy among the three methods. Read practically, a p-value landing precisely at the cutoff signals a real, if modest, divergence, traceable entirely to the same three non-falciparum cases discussed throughout. The high overall agreement observed is best explained by the composition of this sample: *P. falciparum*, reliably detected by all three methods, accounted for 88.5% of positive cases [18], and identical same-day sample handling across all specimens removed pre-analytical variability that might otherwise have introduced additional discordance.

Together, these findings describe three methods that are each reliable within a defined role, rather than a single method that supersedes the others. The RDT, with almost perfect agreement with both other methods and zero false positives across the entire study, remains justified as the primary diagnostic tool at the community and primary care level, where speed and independence from laboratory infrastructure matter most [1,19]. As expected, the RDT's inability to detect the three non-falciparum cases which fall outside the detection scope of HRP2-based testing, is precisely the gap which is filled by both microscopy and the Sysmex XN-31, supporting the combination of methods with different detection principles rather than reliance on any single one [20]. The Sysmex XN-31 occupies a position between the two, matching microscopy's detection accuracy

while adding a full blood count within the same workflow, but remaining feasible only where the supporting infrastructure already exists.

This study has several limitations. The relatively small number of malaria-positive cases (n = 26) limits statistical power, particularly for the Sysmex XN-31 versus microscopy comparison, and may have contributed to the very high concordance observed. The study was conducted at a single site over a short period from October to November 2025, which may limit generalizability to other healthcare settings, regions and seasonal transmission patterns in Ghana. Convenience sampling may have introduced selection bias since only symptomatic patients were enrolled and asymptomatic carriers were not captured. The absence of PCR as a molecular reference standard means submicroscopic infections may have been missed by all three methods, potentially underestimating true malaria prevalence. Finally, the Sysmex XN-31's limited capacity for definitive species identification, not able to classify non-falciparum infections limits its utility for detailed epidemiological surveillance and species-specific clinical management.

Conclusion

This study compared the diagnostic performance of light microscopy, rapid diagnostic tests, and the Sysmex XN-31 automated hematology analyzer for malaria detection among patients attending Kwadaso SDA Hospital in the Ashanti Region of Ghana. All three methods showed strong and comparable diagnostic performance, with almost perfect to perfect agreement across all pairwise comparisons and no statistically significant difference in overall accuracy. The RDT's narrower species coverage, an expected consequence of its HRP2-specific design, was the only meaningful point of difference among the three methods. These findings support the parallel deployment of light microscopy, RDTs, and the Sysmex XN-31 across Ghana's tiered healthcare system, with each method positioned at the level of care its strengths best serve, rather than treating any single method as a universal replacement.

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Author's contributions

Conceptualization: Francis Agyei Amponsah, Joseph Acquah, Otchere Addai-Mensah, Lilian Antwi-Boateng, Benedict Sackey, Edward Yaw Afriyie, Isaac Acheampong, Prince Adoba, Nathan Okai Odame

Data Curation: Joseph Acquah, Prince Adoba, Nathan Okai Odame, Samuel Kwasi Appiah, Samuel Wiafe Mintah

Formal analysis: Francis Agyei Amponsah, Prince Adoba, Joseph Acquah, Samuel Kwasi Appiah, Isaac Acheampong.

Investigation: Francis Agyei Amponsah, Joseph Acquah, Bright Antwi, Nathan Okai Odame, John Kwaku Onipayede, Samuel Wiafe Mintah.

Methodology: Francis Agyei Amponsah, Otchere Addai-Mensah, Edward Yaw Afriyie, Prince Adoba, Benedict Sackey, Samuel Wiafe Mintah.

Project Administration: Francis Agyei Amponsah, Lilian Antwi-Boateng, John Kwaku Onipayede.

Resources: Francis Agyei Amponsah, Joseph Acquah, Samuel Kwasi Appiah, Isaac Acheampong, Edward Yaw Afriyie, Otchere Addai-Mensah, Lilian Antwi-Boateng, Benedict Sackey, Samuel Wiafe Mintah.

Validation: Francis Agyei Amponsah, Otchere Addai-Mensah, Benedict Sackey, Samuel Kwasi Appiah.

Writing- original draft: Francis Agyei Amponsah, Joseph Acquah, Otchere Addai-Mensah, Lilian Antwi-Boateng, Benedict Sackey, Edward Yaw Afriyie, Isaac Acheampong, Prince Adoba, Bright Antwi, Nathan Okai Odame, John Kwaku Onipayede, Samuel Kwasi Appiah, Anthony Dwamena.

Writing- review and editing: Francis Agyei Amponsah, Joseph Acquah, Otchere Addai-Mensah, Lilian Antwi-Boateng, Benedict Sackey, Edward Yaw Afriyie, Isaac Acheampong, Prince Adoba,

John Kwaku Onipayede, Bright Antwi, Nathan Okai Odame, Samuel Kwasi Appiah, Samuel Wiafe Mintah.

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Data availability

All relevant data are within the article. Raw data cannot be made public but can be assessed upon formal request to authors.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) of the School of Medicine and Dentistry at the Kwame Nkrumah University of Science and Technology, Kumasi (CHRPE/AP/931/25). Permission was obtained from the management of Kwadaso SDA Hospital, in the Ashanti Region of Ghana. Written informed consent was obtained from adult participants, while assent and parental or guardian consent were obtained for participants younger than eighteen (18) years.

Conflicts of Interest

Authors declare no conflicts of interests exist.

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