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# Comparison of three rapid diagnostic tests for *Plasmodium falciparum* diagnosis in Ghana

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## Abstract

**Background** Accurate diagnosis and timely treatment are crucial in combating malaria.

**Methods** A total of 449 samples were screened for *Plasmodium falciparum* infection by expert microscopy, qPCR, and three RDTs, namely Rapigen Biocredit Malaria Ag Pf (detecting HRP2 and pLDH on separate bands), Abbott NxTek Eliminate Malaria Ag Pf (detecting HRP2), and SD Bioline Malaria Ag Pf (detecting HRP2). *hrp2/3* deletion typing was done by digital PCR.

**Results** 45.7% (205/449) individuals tested positive by qPCR for *P. falciparum* with a mean parasite density of 12.5 parasites/µL. Using qPCR as reference, the sensitivity of microscopy was 28.3% (58/205), the Biocredit RDT was 52.2% (107/205), the NxTek RDT was 49.3% (101/205), and the Bioline RDT was 39.5% (81/205). When only samples with densities > 20 parasites/µL were included (n = 89), sensitivity of 62.9% (56/89) by microscopy, 88.8% (79/89) by Biocredit, 88.8% (79/89) by NxTek, and 78.7% (70/89) by Bioline were obtained. All three RDTs demonstrated specificities > 95%. The limits of detection (95% probability that a sample tested positive) was 4393 parasites/µL (microscopy), 56 parasites/µL (Biocredit, considering either HRP2 or pLDH), 84 parasites/µL (NxTek), and 331 parasites/µL (Bioline). None of the three qPCR-confirmed *P. falciparum* positive samples, identified solely through the pLDH target, or eight samples negative for all RDTs but qPCR-positive at densities > 20 parasites/µL carried *hrp2/3* deletions.

**Conclusion** The Biocredit and NxTek RDTs demonstrated comparable diagnostic efficacies. All three RDTs performed better than microscopy.

**Keywords** Malaria diagnosis, Rapid diagnostic test, Ultrasensitive RDT, HRP2, PLDH

## Background

Malaria remains a significant public health concern in sub-Saharan Africa, including Ghana, where an estimated 5.3 million cases and 11,500 deaths were reported in 2022 [1]. Rapid and accurate diagnosis of malaria is crucial for effective treatment and control of the spread of the disease [2]. Rapid diagnostic tests (RDTs) are immunochromatographic assays widely used for malaria diagnosis, particularly in resource-limited settings. RDTs are easy to use, require minimal training, and provide results within 20–30 min [3]. High sensitivity of RDTs is crucial to detect low-density infections.

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RDTs for *Plasmodium falciparum* rely on detecting specific proteins such as histidine-rich proteins 2 (and histidine-rich proteins 3 as a result of HRP2 cross-reaction), parasite lactate dehydrogenase (LDH), or aldolase [4]. HRP2-based RDTs are considered the most sensitive [5–8]. However, deletions in the *hrp2* and *hrp3* genes will lead to false-negative RDT results, even in patients with high parasite density infections [4, 9]. These deletions have been observed in various countries, particularly in East Aftica [10–12], but also at low frequencies in Ghana [13].

Numerous studies have investigated the diagnostic performance of RDTs and found varying sensitivities [5–9, 14–18]. Variation in sensitivity can be as a result of differences in RDT design, characteristics of the study population (e.g. clinical vs. subclinical infections, or differences in age groups reflecting different levels of acquired immunity and thus different parasite densities), choice of the reference (e.g., microscopy or PCR), and differences among sample processing and PCR assays resulting in variation of the limit of detection and parasite quantification by qPCR [3, 17, 19–21]. As a result, data on sensitivity and Limit of Detection (LOD) of RDTs tested using different protocols are difficult to compare.

Here, the performance of the NxTek Eliminate Malaria Ag Pf, SD Bioline Malaria Ag Pf, and Biocredit Malaria Ag Pf RDTs in diagnosing clinical patients in Ghana was compared. The NxTek and Biocredit test are considered highly sensitive RDTs. Several studies showed them to be more sensitive compared to RDTs available previously [3, 22–33]. The NxTek and Bioline have one test band for HRP2. The Biocredit Malaria Ag Pf. (LDH/HRP2) has two separate test bands for HRP2 and *P. falciparum* specific LDH (pLDH). Having both targets as separate bands allow diagnosis in the case of *hrp2/3* deletion and enables surveillance of deletion status, as samples positive for pLDH but negative for HRP2/3 can be selected for molecular confirmation of deletion status.

## Methods

### Ethical approval

Prior to sample collection, informed written consent was obtained from each individual. For minors, assent was obtained in addition to consent obtained from legal guardians. This study was approved by the Committee on Human Research, Publications, and Ethics of the School of Medical Sciences, KNUST (CHRPE/AP/030/20), the University of Notre Dame Institutional Review Board (19–04-5321), and The Ohio State University Institutional Review Board (2020H0539).

### Study site and sample collection

Samples were collected from health centres in Mankranso (6.8181° N, 1.8635° W) and Agona (6.9347° N, 1.4870° W) in the Ashanti region of Ghana. The Ashanti region has a reported malaria prevalence of 22% by microscopy [34]. The samples were obtained during the rainy seasons, between August and September 2022, known to be periods of high malaria transmission [35]. All individuals above 1 year of age triaged to obtain malaria diagnosis were eligible to be enrolled. Blood samples (approximately 2 mL) from participants were collected in EDTA tubes, and malaria screening with the three RDTs was performed on-site. Study participants were treated as per the national guidelines by healthcare providers at the hospital.

### Rapid diagnostic tests kits and testing

Three different RDT kits were compared, the RDT, NxTek Eliminate Malaria Ag Pf. ((lot no. 05LDG008B, Product code: 05FK142), manufactured by Abbott, the Bioline Malaria Ag Pf. (lot no. 05CDH037C, Product code: 05FK51), also manufactured by Abbott, and the Biocredit Malaria Ag Pf. (LDH/HRP2) (lot no. H052BSA002, Product code: C13RHG25), manufactured by Rapigen. While no clear criteria exist on when an RDT should be labeled 'highly sensitive' or 'ultra-sensitive', the NxTek and Biocredit RDT were introduced to the market more recently and are considered highly sensitive, whereas the SD Bioline had been available for longer and is considered a conventional RDT. Test were conducted according to manufaturer's instructions. Tests were considered invalid and repeated if the control band was not positive.

### Diagnosis by microscopy

Thick and thin blood films, in duplicate, were prepared for each participant using 2µL and 6µL of whole blood on clean, frosted glass slides following established protocols [36]. Thin smears were fixed with absolute methanol and stained with a 10% Giemsa working solution (Biognost GM-OT-1L). Imaging was performed at the × 100 objective and detection of parasite was done by examining at least 100 high-power fields. Estimation of parasite quantity involved assessing between 200 to 500 white blood cells and then multiplied by 8000 white blood cells (WBCs), following established protocols [37]. Microscopic diagnosis was conducted by one WHO-certified (Level 1) expert blinded to RDT and qPCR results.

### DNA extraction, varATS qPCR, and hrp2/3 deletion typing

DNA was extracted from 100 µL blood and eluted in 100 µL elution buffer using the Macherey–Nagel NucleoMag extraction kit. To estimate parasite density, qPCR of the

*P. falciparum var*ATS multi-copy gene was carried out using a previously described protocol with 4 µL DNA as target resulting in a 95% limit of detection of 0.3 parasites/µL blood [38]. A standard curve, generated from quantified 3D7 parasites DNA using digital PCR (dPCR), was employed alongside the samples. *hrp2/3* deletion typing for qPCR positive samples that were (i) negative for HRP2 on RDTs but positive for pLDH and (ii) negative for all RDTs but with parasitemia of > 20 parasites/µL, was done by *hrp*2, *hrp*3 and tRNA multiplexed digital PCR as previously described [39].

### Data analysis

No formal sample size calculation was conducted. Sensitivity was calculated as the number of infections detected by an RDT divided by the number of infections detected by qPCR, and against thresholds of 2000, 200, and 20 parasites/µL (by qPCR). This was done to increase comparability with other studies, as different methods for sample collection, DNA extraction, and qPCR result in different limits of detection, and, thus, different numbers of positive samples [21]. For the Biocredit RDT, the HRP2 and pLDH targets were considered separately and in combination (i.e., an RDT was counted positive when either HRP2 or pLDH targets were positive). Specificity was calculated as the proportion of negative RDTs among individuals that tested negative by qPCR. The positive predictive value (PPV) was calculated as the probability that the infection is present when the RDT is positive and parasite density is > 20 parasites/µL [40]. Samples with densities of > 0 to 20 parasites/µL were exluded from the calculation of NPV and PPV. This threshold was set in line with our lowest threshold used to analyze RDT sensitivity. While it is impossible to determine whether any *P. falciparum* infection is the cause of fever, it is expected that many of the low-density infections < 20 parasites/µL are incidental. Given that RDTs are not expected to detect very low-density infections, and that many of them are not the cause of disease, their exclusion from diagnostic accuracy measures is justified. The negative predictive value (NPV) was calculated as the probability that qPCR is negative when the RDT is negative [40]. The limit of detection (LOD) was defined as the lowest parasite density where a qPCR-positive infection would be detected with 95% probability and logistic regression analysis was conducted to determine the LOD of each RDT target.

The area under the receiver operating characteristic curve (AUC) was calculated with a nonparametric analysis using 1000 bootstrap replications. As parasite density distributions were skewed, geometric mean densities are given whenever densities are reported. CI95 stands for the 95% confidence interval. The *p* values to compare groups for qPCR test positivity and RDT sensitivity were calculated by Chi-square and McNemar's test, while Kruskal–Wallis' test was used for parasite density.

## Results

### Study population demographics

A total of 449 clinical samples were collected and analysed. Table 1 provides the demographic information of the study participants. Among the participants, only 7.8% were below 5 years of age, while the majority (67.5%) were above 15 years of age. The majority of participants were female (71.7%).

205/449 (45.7%) clinical samples tested positive for *P. falciparum* by qPCR, with a mean parasite density of 12.5 parasites/µL. There were no statistically significant differences in positivity by qPCR based on participant's age or sex (Table 1). There was no significant difference in

**Table 1** Demographics of the study population, parasite density, test positivity (by qPCR) and RDT sensitivity by age group and sex

| Parameter | Category | N | qPCR Test positivity [95CI] (n/N) | p | Geometric Mean parasite density [95CI] | p | RDT sensitivity[1] [95CI] (n/N) | p |
|---|---|---|---|---|---|---|---|---|
| Age (years) | 0 to 5 | 35 (7.8%) | 48.6% [39.2, 58.0] (17/35) | 0.87 | 78.9 [4.0, 1556.8] | 0.02 | 70.6% [51.6, 89.5] (12/17) | < 0.01 |
| | 6 to 15 | 111 (24.7%) | 46.9% [37.4, 56.3] (52/111) | | 41.3 [11.9, 143.3] | | 73.1% [59.2, 86.9] (38/52) | |
| | > 15 | 303 (67.5%) | 44.9% [37.7, 52.1] (136/303) | | 6.3 [3.1, 12.8] | | 44.9% [35.5, 54.2] (61/136) | |
| Sex | Male | 127 (28.3%) | 49.6% [39.8, 59.4] (63/127) | 0.58 | 12.4 [3.8, 40.1] | 0.87 | 47.6% [35.8, 59.5] (30/63) | 0.21 |
| | Female | 322 (71.7%) | 44.1% [36.2, 52.0] (142/322) | | 12.6 [6.0, 26.3] | | 57.0% [46.6, 67.5] (81/142) | |
| Total | | 449 (100%) | 45.7% [38.7, 52.6] (205/449) | | 12.5 [6.7, 23.3] | | 54.2% [44.3, 63.9] (111/205) | |

[1] For the RDT data, the results from all three RDTs were combined, with either RDT and either target (HRP2 or pLDH) positive counting as a positive test

densities between male and female participants (p = 0.87, Table 1). Parasite density was significantly lower in participants older than 15 years (p = 0.02, Table 1).

### Parasite prevalence by microscopy and RDT

All 449 samples were screened for *P. falciparum* infection by microscopy and RDT. For the RDT, the results from all three RDTs were combined, with any RDT and any target (HRP2 or pLDH) positive counting as a positive test. Prevalence by RDT was 27.2% (122 out of 449), by microscopy 13.6% (61 out of 449). While 51.4% (231 out of 449) were negative for all diagnostic test (including qPCR), 12.5% (56 out of 449) were positive with all diagnostic tests (including qPCR).

### Diagnostic accuracy of RDT and Microscopy using qPCR as reference

Using qPCR as the reference, the sensitivity of RDT was 54.2% (111 of 205) whereas for microscopy it was 28.29% (58 of 205) (p < 0.01). False positive results were more frequent for RDTs (n = 11) than Microscopy (n = 3). One sample was false positive for both RDT and microscopy. Specificity for RDT was 95.5% and microscopy was 98.8%. PPV was higher for microscopy than RDT (94.9% vs. 87.8%) and NPV was lower in microscopy than RDT (87.9% vs. 95.9%).

Table 2 shows the sensitivity and specificity of the evaluated RDTs. The Biocredit and NxTek RDTs (considering either the HRP2 or pLDH band) showed similar sensitivity, detecting 52.2% and 49.3% of qPCR-confirmed infections (McNemar's test, p = 0.18). The Bioline RDT had lower sensitivity, compared to the Biocredit (McNemar's test, p < 0.01) and NxTek McNemar's test, (p < 0.01) RDTs, detecting 39.5% of qPCR-positive infections. As the threshold for parasite density decreased (from > 2000 parasites/μL to > 200 parasites/μL to > 20 parasites/μL), the sensitivity of the RDTs also decreased (Table 2). All RDTs demonstrated specificity levels above 95% (Table 2). The limit of detection (LoD) was determined as 4393 parasites/μL for microscopy, 56 parasites/μL for the Biocredit RDT (considering either the HRP2 or pLDH target), 84 parasites/μL for the NxTek RDT, and 331 parasites/μL for the Bioline RDT (Table 2). The Negative Predictive Value (NPV) was 95.9% for the Biocredit and NxTek RDTs, and 92.7% for the Bioline RDT. All RDTs achieved a test accuracy (area under the curve (AUC)) of > 0.85 (Table 2).

### Comparison of HRP2 vs. pLDH, and hrp2/3 Deletion Typing

The Biocredit RDT demonstrated higher sensitivity for the HRP2 target (88.8% at densities > 20 parasites/μL), compared to the pLDH target (74.2%) (McNemar's test, p < 0.01). When infections of all densities were considered, three qPCR-confirmed infections were detected by pLDH only (Fig. 1) thus, sensitivity for HRP2 only was minimally lower compared to when both HRP2 and pLDH targets were considered (Table 2). None of these three samples carried *hrp2* or *hrp3* deletions. The

**Table 2** Measure of diagnostic performance of Bioline, NxTek and Biocredit RDTs

| Diagnostic Measure | N | Microscopy [95CI] (n/N) | Bioline HRP2 [95CI] (n/N) | NxTek HRP2 [95CI] (n/N) | Biocredit HRP2 [95CI] (n/N) | Biocredit LDH [95CI] (n/N) | Biocredit HRP2/LDH [95CI] (n/N) |
|---|---|---|---|---|---|---|---|
| Sensitivity (all densities) | 205 | 28.3% [22.2, 34.9] (58/205) | 39.5% [32.8,46.6] (81/205) | 49.3% [42.2, 56.3] (101/205) | 50.7% [43.7, 57.8] (104/205) | 37.1% [30.5, 44.1] (76/205) | 52.2% [45.1, 59.2] (107/205) |
| Only samples with > 2000 parasites/μL | 37 | 70.3% [53.0, 84.1] (26/37) | 94.6 [81.8, 99.3] (35/37) | 100 [94.3, 100] (37/37) | 94.6 [81.8, 99.3] (35/37) | 94.6 [81.8, 99.3] (35/37) | 94.6 [81.8, 99.3] (35/37) |
| Only samples with > 200 parasites/μL | 63 | 76.2 [63.8, 86.0] (48/63) | 88.9 [78.4, 95.4] (56/63) | 95.2 [86.7, 99.0] (60/63) | 92.1 [82.4, 97.4] (58/63) | 90.5 [80.4, 96.4] (57/63) | 92.1 [82.4, 97.4] (58/63) |
| Only samples with > 20 parasites/μL | 89 | 62.9% [52.0, 72.9] (56/89) | 78.7 [68.7, 86.6] (70/89) | 88.8 [80.3, 94.5] (79/89) | 88.8 [80.3, 94.5] (79/89) | 74.2 [63.8, 82.9] (66/89) | 88.8 [80.3, 94.5] (79/89) |
| Specificity | 244 | 98.8% [96.5, 99.8] (241/244) | 98.8% [96.5, 99.8] (241/244) | 97.1% [94.2, 98.8] (237/244) | 97.1% [94.2, 98.8] (237/244) | 98.8% [96.5, 99.8] (241/244) | 96.3% [93.1, 98.3] (235/244) |
| Positive Predictive Value | 333 | 94.9% [85.7, 98.3] | 95.9% [88.3, 98.6] | 91.9% [84.4, 95.9] | 91.9% [84.4, 95.9] | 95.7% [87.8, 98.6] | 89.8% [82.2, 94.4] |
| Negative Predictive Value | 333 | 87.9% [84.8, 90.3] | 92.7% [89.5, 94.9] | 95.9% [92.9, 97.7] | 95.9% [92.9, 97.7] | 91.3% [88.1, 93.7] | 95.9% [92.9, 97.7] |
| Accuracy (AUC) | 449 | 0.892[0.85, 0.92] | 0.887 [0.84, 0.93] | 0.929 [0.89, 0.96] | 0.929 [0.89, 0.96] | 0.870 [0.82, 0.92] | 0.931 [0.90, 0.96] |
| 95% LOD (parasites/μL) | 205 | 4393 [2129, 9064] | 331 [148, 739] | 84 [40, 177] | 85 [43, 171] | 349 [142, 858] | 56 [28, 118] |

N: Sample size used for diagnostic measurement

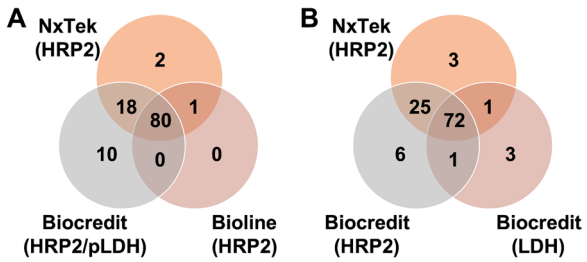


**Fig. 1** (A) The detection of qPCR-confirmed *P. falciparum* infections using three RDTs: Nxtek (HRP2), Biocredit (HRP2/pLDH), and Bioline (HRP2). (B) Comparison between the Nxtek and Biocredit RDT kits to accurately detect true positive *P. falciparum* infections with HRP2 and pLDH targets. A total of 111 out of 205 qPCR-confirmed infections were detected by these RDTs as true positive tests

parasite densities of these samples ranged from 2–5 parasites/μL. Also, none of eight samples that were positive by qPCR with parasite densities > 20 parasites/μL but negative for all RDTs carried *hrp*2/3 deletions.

## Discussion

This study showed similar sensitivities for the Biocredit and NxTek RDTs. These tests are considered highly sensitive, i.e., more sensitive than conventional tests such as the SB Bioline [3, 22–33]. Both the Biocredit and NxTek detected around 50% of all qPCR-positive infections (p = 0.18), compared to around 40% by the SD Bioline (p = 0.03). Excluding very low-density infections at < 20 parasites/μL, the sensitivities of NxTek and Biocredit RDTs were identical at 89%, compared to 79% for the SD Bioline. While this difference did not reach statistical significance (p = 0.09), it points to higher sensitivity of the NxTek and Biocredit.

The LoD of the Biocredit and NxTek RDTs, determined through logistic regression analysis as the minimum parasite density quantified by qPCR that the RDT could detect with a 95% probability, was approximately four-fold lower than the LOD for the SD Bioline. RDT sensitivity reached 73% in children aged 6–15 years and 71% in children under five, while older participants (> 15 years) had lower parasite densities, resulting in lower RDT sensitivity.

A limited number of previous studies compared the NxTek or Biocredit RDT to conventional RDTs among clinical patients. The NxTek was compared to the SD Bioline among over 3500 febrile patients in Tanzania, with a minimal difference in sensitivity observed (75% vs. 73% compared to qPCR) [28]. In this study, only 10% of patients tested positive by qPCR, and over 80% of participants were children. Possibly, the lower transmission intensity and enrolment of mostly children resulted in higher parasite density because of limited acquired immunity in the study population. At high densities, both RDTs are expected to yield similar results. Several studies compared the NxTek to the SD Bioline among asymptomatic individuals [23, 30, 32, 41–44] and pregnant women [25, 29, 45, 46]. In all studies, the NxTek was more sensitive. Using the NxTek as reference, the SD Bioline RDT reached a sensitivity of 73–97%, except for one study where the NxTek detected twice as many infections [42]. In the current study, the sensitivity of SD Bioline compared to NxTek was 80% (81 vs. 101 qPCR-positive infections detected), thus within the range observed in studies among asymptomatic populations.

Only one study, led by the same investigators as the current study, compared the Biocredit RDT tested to a conventional RDT, namely the CareStart HRP2 RDT [3]. Among febrile patients in Burundi with *P. falciparum* infection confirmed by qPCR, the Biocredit detected 80% of infections compared to 73% by CareStart. The reasons for the lower sensitivtiy of 52% of the Biocredit RDT in the current study are unknown. The very high tranmsision intensity in Burundi, possibly resulting in a higher pyrogenic threshold and higher parasite densities among patients presenting with fever, might play a role [47]. Indeed, the LoD of the Biocredit RDT, which would be expected to be affected less by differences in pyrogenic thresholds, was similar in Ghana (56 parasites/μL) and Burundi (34 parasites/μL) [3].

In accordance with WHO guidelines for genotyping *hrp2/3* deletions, samples that tested positive for pLDH but negative for HRP2 were typed. None of the three *P. falciparum* malaria-positive samples fulfilling these criteria carried *hrp*2/3 deletions. Also, eight samples positive by qPCR with parasite densities > 20 parasites/μL but negative for all three RDTs did not carry *hrp*2/3 deletions. The current data thus corroborated recent findings of very low frequency of *hrp*2/3 deletions in Ghana [48–50], including a set of over 200 infections collected at the same health centers and typed, where no deletions were detected [39].

The sensitivity of the pLDH target in the Biocredit RDT was found to be comparable to that of Bioline HRP2 RDT. Similar sensitivity for the pLDH target of the Biocredit RDT has been reported in Uganda and Djibouti [51–53]. This suggests that the Biocredit RDT, with its pLDH target, can be a suitable alternative to the Bioline RDT in regions where *hrp2* deletion is prevalent. In conclusion, the Biocredit and NxTek are more sensitive than the SD Bioline which is commonly used in in Ghana [54]. Shall *hrp2/3* deletions ever spread in the country, the Biocredit will be a reliable alternative for malaria diagnosis.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-024-05073-z.

Additional file1

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**Author contributions**
CK and KB conceived the study. AKA, TKA, AT, SOA, DAA, AS, and AKBT conducted sample collection and RDT testing. TK conducted PCR screening and hrp2/3 deletion typing. TK wrote the first draft of the manuscript. CK and KB edited the draft manuscript. All authors read and agreed with the final version of the manuscript.

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**Availability of data and materials**
All data is provided in supplementary File S1.

## Declarations

**Ethics approval and consent to participate**
Prior to sample collection, informed written consent was obtained from each individual. For minors, assent was obtained in addition to consent obtained from legal guardians. This study was approved by the Committee on Human Research, Publications, and Ethics of the School of Medical Sciences, KNUST (CHRPE/AP/030/20), the University of Notre Dame Institutional Review Board (19–04-5321), and The Ohio State University Institutional Review Board (2020H0539).

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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