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# False-positive and false-negative malaria rapid diagnostic test results: evidence from a nationally representative survey of children in Ghana

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## Abstract

**Background** Malaria remains a leading cause of morbidity and mortality among children under five in Ghana, where *Plasmodium falciparum* predominates. Rapid diagnostic tests (RDTs) are widely used for malaria case management and population surveillance; however, concerns about false-positive and false-negative results may compromise case detection and prevalence estimates.

**Objective** This study aimed to (i) estimate malaria prevalence using microscopy and RDTs, (ii) evaluate the surveillance performance of RDTs relative to microscopy, and (iii) identify socio-demographic and household-level predictors of false-positive and false-negative RDT results among Ghanaian children under 5 years.

**Methods** We analyzed data from 4,417 children who participated in the 2022 Ghana Demographic and Health Survey (DHS). Capillary blood samples were tested using HRP2-based RDTs and light microscopy. Weighted analyses accounted for the DHS sampling design. Malaria prevalence was estimated for both diagnostic methods, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for RDTs using microscopy as the reference. Logistic regression models were used to identify predictors of false-positive and false-negative results, reporting adjusted odds ratios (aORs) with 95% confidence intervals (CIs).

**Results** Malaria prevalence was 20.3% by RDT and 9.9% by microscopy. Compared to microscopy, RDTs demonstrated high sensitivity (89.7%, 95% CI: 86.5–92.2) and specificity (87.4%, 95% CI: 86.3–88.4), with a PPV of 43.9% and NPV of 98.7%. False-positive results were uncommon (1.0%) and not significantly associated with child or household characteristics. False-negative results were more likely among children living in dwellings sprayed with insecticide (aOR = 1.56, 95% CI: 1.05–2.30) and in rural areas (aOR = 0.54 for urban residence, 95% CI: 0.43–0.67), with significant regional variation higher odds in Upper East (aOR = 1.93, 95% CI: 1.16–3.21) and lower odds in Greater Accra (aOR = 0.21, 95% CI: 0.07–0.63).

**Conclusion** HRP2-based RDTs are highly sensitive and reliable for ruling out malaria in children under five in Ghana. However, their lower PPV may lead to overestimation of malaria prevalence, particularly in high-transmission rural areas and IRS-targeted districts. Programmatic use of RDT-based prevalence data should account for this limitation,

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and confirmatory microscopy or molecular data should be incorporated where feasible. Continued surveillance of pfhrp2/3 deletions and post-treatment HRP2 persistence is warranted to sustain diagnostic accuracy.

**Keywords** Malaria, Rapid diagnostic test, Microscopy, Sensitivity, Specificity, False positive, Ghana, Children under five, DHS

## Introduction

Malaria remains one of the leading causes of morbidity and mortality among children under five years of age, particularly in sub-Saharan Africa where transmission is intense and perennial [1, 2]. According to the World Health Organization (WHO), approximately 263 million malaria cases and 597,000 malaria-related deaths were reported worldwide in 2023, with more than 95% of this burden occurring in the WHO African Region [3]. Ghana is among the 15 highest-burden countries, accounting for an estimated 5.1 million malaria cases annually, and malaria continues to be the leading cause of outpatient visits and hospital admissions among children under five years of age [4–7]. In Ghana, *Plasmodium falciparum* is the predominant species, followed by *P. malariae* and *P. ovale* spp., which circulate at lower but significant prevalence levels [7, 8]. The presence of multiple malaria species has important implications for case detection and treatment, particularly in settings where diagnostic testing relies primarily on antigen detection.

Accurate diagnosis is essential for effective case management, monitoring transmission trends, and informing malaria control and elimination strategies [9–11]. WHO recommends parasitological confirmation of all suspected malaria cases before treatment, using either light microscopy or malaria rapid diagnostic tests (RDTs) [12]. Although microscopy remains the gold standard, it is labor-intensive, requires well-trained personnel, and may not be feasible in many primary health facilities, especially in rural and resource-limited settings [13]. As a result, RDTs have been widely adopted across Ghana for routine clinical diagnosis and are also used in population-based surveys, such as the Ghana Demographic and Health Survey (DHS), to estimate malaria prevalence at the national and regional levels [14, 15].

Despite their utility, RDTs are not infallible. False-positive results can occur due to persistent HRP2 antigenemia after parasite clearance, cross-reactivity with other infectious agents, or recent antimalarial treatment [16, 17]. Conversely, false-negative results may occur in cases of very low parasite density (below the test's detection threshold), improper storage or handling of test kits, operator error, or genetic deletions in the pfhrp2/pfhrp3 genes in *P. falciparum* parasites that encode the target antigens [18, 19]. Importantly, the presence of non-falciparum species such as *P. malariae* and *P. ovale*,

which may not be detected by HRP2-based RDTs, can also contribute to false-negative outcomes [20]. There have been anecdotal reports from frontline healthcare workers in Ghana noting instances where patients with clinical symptoms of malaria tested negative by RDT but were later confirmed positive by microscopy (*personal communication, Dr. Susanna Aba Abraham, University of Cape Coast*). These observations underscore the need for a better understanding of the reliability of RDT-based results under real-world conditions.

Understanding the magnitude and predictors of false RDT results is critical for strengthening diagnostic strategies, ensuring appropriate treatment, and improving the accuracy of malaria surveillance data. While previous studies have reported variable sensitivity and specificity of RDTs across settings, nationally representative data examining factors associated with diagnostic discordance in Ghana remain limited [21]. In particular, little is known about how socio-demographic factors (such as age, sex, and place of residence), household characteristics (such as insecticide-treated net ownership or indoor residual spraying), and anaemia status may influence the likelihood of false-positive or false-negative outcomes.

The present study addresses this evidence gap by analyzing nationally representative data from the 2022 Ghana DHS, focusing on children under five years of age. Specifically, we aimed to: (i) estimate malaria prevalence using both microscopy and RDTs, (ii) evaluate the surveillance performance of RDTs compared to microscopy, and (iii) identify socio-demographic and household-level predictors of false-positive and false-negative RDT results.

The findings from this study are expected to enhance understanding of RDT performance at the population level, improve the interpretation of malaria prevalence estimates derived from RDT-based data, and inform policies aimed at strengthening malaria surveillance systems in Ghana.

## Methods

### Study design and data source

This study utilized nationally representative data from the 2022 Ghana Demographic and Health Survey (DHS), a cross-sectional household survey that employed a two-stage stratified sampling design [22]. The DHS is conducted approximately every five years and provides

population-level estimates on key health indicators, including malaria prevalence. The 2022 DHS covered all 16 administrative regions of Ghana, allowing for regional-level disaggregation of findings.

### Study population

The analysis focused on children under five years of age who were de facto members of sampled households and who provided blood samples for both malaria RDT and light microscopy. Children were excluded if they were absent during the survey, if their parents or guardians did not consent to blood collection, or if either RDT or microscopy results were missing.

### Sample collection and laboratory procedures

Capillary blood samples were obtained via finger prick by trained DHS field technicians following standard procedures. Malaria infection was assessed using both RDT and microscopy:

1. RDT Testing: HRP2-based RDTs (SD Bioline Malaria Ag P.f, Standard Diagnostics, Inc., Republic of Korea) were used for field-based malaria diagnosis. Tests were performed according to the manufacturer's instructions, and results were read within 15–20 min.
2. Microscopy: Thick and thin blood smears were prepared for each child, stained with Giemsa, and examined under light microscopy by trained microscopists. A smear was considered positive if asexual parasites were detected in 200 fields on the thick film. Microscopy served as the reference ("gold standard") for surveillance performance evaluation.

### Variables

The primary outcome variables were malaria test results from RDT and microscopy. Using microscopy as the reference standard, children were classified into four diagnostic categories: true positive (TP), true negative (TN), false positive (FP), and false negative (FN).

Explanatory variables included:

1. Child characteristics: age category (<6 months, 6–11 months,  $\geq 12$  months), sex (male, female), anaemia status (not anemic, mild, moderate, severe).
2. Household characteristics: place of residence (urban, rural), ownership of insecticide-treated nets (yes, no), dwelling sprayed with insecticide in the last 12 months (yes, no, unsure).
3. Regional location: all 16 administrative regions were included, with Ahafo used as the reference category for regression analysis.

### Quality control for malaria diagnosis in Ghana DHS and MIS

The Ghana DHS and MIS implement rigorous quality control measures to ensure reliable malaria prevalence data. Data collection is conducted by the Ghana Statistical Service (GSS) using standardized protocols and field manuals developed with the National Malaria Control Programme (NMCP), the National Public Health Reference Laboratory (NPHRL), and technical support from The DHS Program.

Children aged 6–59 months are tested using both Rapid Diagnostic Tests (RDTs) and blood film microscopy. Blood slides are read in central laboratories, such as the Noguchi Memorial Institute for Medical Research, which also conducts external quality assurance by re-examining a subset of slides to verify accuracy and consistency. Internal quality control procedures, including standardized staining and careful handling, further help maintain slide integrity and reduce variability in microscopic readings.

Fieldworkers and laboratory technicians receive comprehensive training, with routine supervision, data audits, and automated quality checks during data entry to ensure protocol adherence. Ongoing monitoring of RDT kits including registration, storage, and performance checks ensures reliable results. Collectively, these measures support accurate, consistent, and comparable malaria prevalence estimates across regions and survey rounds. Detailed methodology is available in the DHS and MIS reports published by GSS and the DHS Program.

### Statistical analysis

All analyses were conducted using SPSS version 27 and GraphPad Prism version 10 for visualization. Sampling weights provided by the DHS were applied to account for the complex survey design and ensure nationally representative estimates. Descriptive statistics were computed to summarize socio-demographic and household characteristics of the study population. Categorical variables were expressed as frequencies and percentages, and continuous variables as means with standard deviations. Malaria prevalence was calculated separately for RDT and microscopy. Surveillance performance of RDT compared to microscopy was assessed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CIs). Agreement between the two methods was evaluated using the chi-square test. Predictors of false-positive and false-negative results were identified using logistic regression models. Univariable analyses and multivariable logistic regression models to adjust for potential confounders such as residence, region, the use of

mosquito nets or sprays. Adjusted odds ratios (aOR) with 95% CIs were reported, and p-values < 0.05 were considered statistically significant.

### Ethical considerations

The DHS protocol was reviewed and approved by the Institutional Review Board of ICF International and the Ghana Health Service Ethical Review Committee. Written informed consent was obtained from parents or guardians of all participating children prior to blood collection. For the present analysis, anonymized DHS datasets were obtained with permission from the DHS Program, and all analyses were conducted in accordance with ethical guidelines for secondary data use.

## Results

### Socio-demographic and household characteristics of the study population

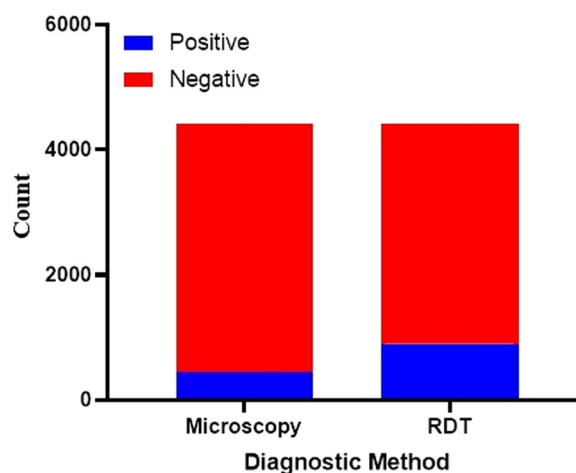
Data from 4,417 children under five years were analyzed from the 2022 Ghana Demographic and Health Survey (DHS) household dataset whose samples were collected for malaria analysis. The mean age of the study population was  $31.9 \pm 15.5$  months, with slightly more than half being male (2251/4417, 51.0%) and 2166/4417 (49.0%) female. The majority of children resided in rural areas (2587/4417, 58.6%), whereas 1830/4417 (41.4%) were from urban areas. Regarding malaria prevention, 81.2% (3588/4417) of children reported having a mosquito net available for sleeping, while 829/4417 (18.8%) did not. Only 19.6% (866/4417) of children lived in dwellings sprayed against mosquitoes in the 12 months preceding the survey, whereas 79.9% (3531/4417) reported no spraying, and 0.5% (20/4417) were unsure. With respect to anaemia status, nearly half of the children (2037/4417, 46.1%) were not anaemic, while 28.6% (1262/4417) had mild anaemia, 24.2% (1070/4417) had moderate anaemia, and 1.1% (48/4417) had severe anaemia. Regional distribution revealed that the largest proportion of children came from North East (462/4417, 10.5%) and Northern (452/4417, 10.2%) regions, followed by Savannah (376/4417, 8.5%), Upper West (309/4417, 7.0%), and Upper East (301/4417, 6.8%). The remaining regions contributed between 4.1% and 6.5% of the sampled children (Table 1).

### Malaria prevalence by diagnostic method

Malaria prevalence varied by diagnostic method. Based on blood smear microscopy (reference standard), 438 children (9.9%) tested positive for malaria, whereas 3979 children (90.1%) were negative. Using rapid diagnostic tests (RDTs), a higher prevalence of 20.3% (895/4417) was observed, with 3522/4417 (79.7%) testing negative (Fig. 1).

**Table 1** Socio-demographic and household characteristics of children

| Variable                                                  | Category             | N (%)             |
|-----------------------------------------------------------|----------------------|-------------------|
| Age (months), mean $\pm$ SD                               |                      | 31.90 $\pm$ 15.47 |
| Sex                                                       | Male                 | 2251 (51.0)       |
|                                                           | Female               | 2166 (49.0)       |
| Residence                                                 | Rural                | 2587 (58.6)       |
|                                                           | Urban                | 1830 (41.4)       |
| Has a mosquito net for sleeping                           | Yes                  | 3588 (81.2)       |
|                                                           | No                   | 829 (18.8)        |
| Dwelling sprayed against mosquitoes in the last 12 months | Yes                  | 866 (19.6)        |
|                                                           | No                   | 3531 (79.9)       |
|                                                           | Don't know           | 20 (0.5)          |
| Anaemia status                                            | Not anemic           | 2037 (46.1)       |
|                                                           | Mild anaemia         | 1262 (28.6)       |
|                                                           | Moderate anaemia     | 1070 (24.2)       |
|                                                           | Severe anaemia       | 48 (1.1)          |
| Region                                                    | Ahafo Region         | 237 (5.4)         |
|                                                           | Ashanti Region       | 289 (6.5)         |
|                                                           | Bono Region          | 194 (4.4)         |
|                                                           | Bono East Region     | 288 (6.5)         |
|                                                           | Central Region       | 239 (5.4)         |
|                                                           | Eastern Region       | 211 (4.8)         |
|                                                           | Greater Accra Region | 203 (4.6)         |
|                                                           | North East Region    | 462 (10.5)        |
|                                                           | Northern Region      | 452 (10.2)        |
|                                                           | Oti Region           | 265 (6.0)         |
|                                                           | Savannah Region      | 376 (8.5)         |
|                                                           | Upper East Region    | 301 (6.8)         |
|                                                           | Upper West Region    | 309 (7.0)         |
|                                                           | Volta Region         | 192 (4.3)         |
|                                                           | Western Region       | 217 (4.9)         |
|                                                           | Western North Region | 182 (4.1)         |



**Fig. 1** Malaria prevalence by diagnostic method

### Surveillance performance of RDT compared to microscopy

Cross-tabulation of RDT results against microscopy revealed that 393 children were true positives (TP), 3477 were true negatives (TN), 502 were false positives (FP), and 45 were false negatives (FN) (Table 2). There was a statistically significant association between the two diagnostic methods ( $\chi^2 = 1452.05$ ,  $p < 0.0001$ ). When evaluated against microscopy, the RDT demonstrated high sensitivity of 89.7% (95% CI: 86.5–92.2) and specificity of 87.4% (95% CI: 86.3–88.4). The positive predictive value (PPV) was 43.9% (95% CI: 40.7–47.2), whereas the negative predictive value (NPV) was very high at 98.7% (95% CI: 98.3–99.0). These results suggest that RDTs are reliable for ruling out malaria (high NPV) but may overestimate malaria prevalence due to a high false-positive rate (lower PPV) (Fig. 2).

### Factors associated with false-negative and false-positive RDT results

Logistic regression analysis was conducted to identify socio-demographic and household predictors of false-negative and false-positive RDT results using blood smear microscopy as the gold standard.

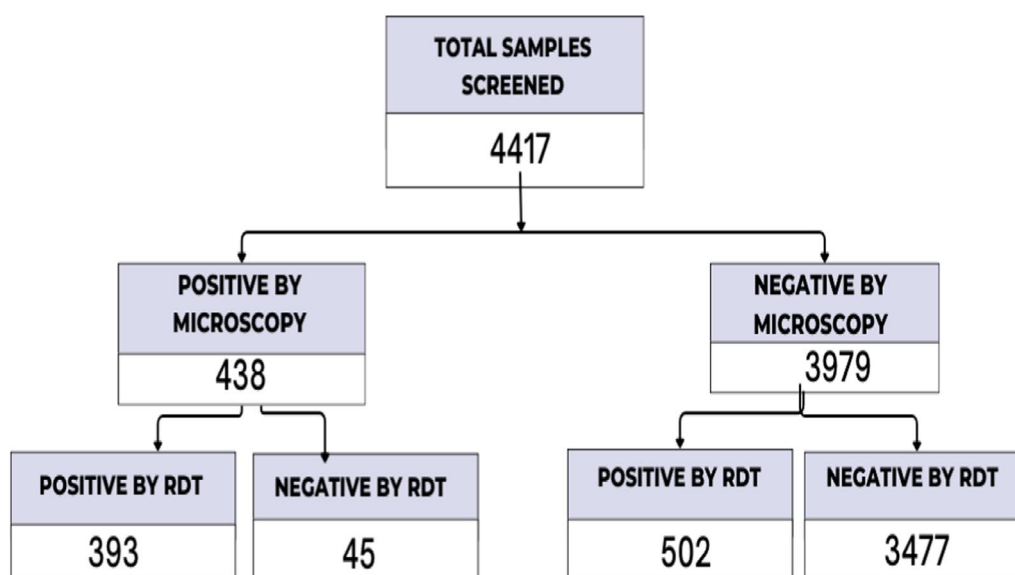
### False-positive results

No statistically significant associations were identified between false-positive RDT results and any of the socio-demographic or household characteristics assessed. In both univariable and multivariable logistic regression models, age category (less than 6 months, 6–11 months,  $\geq 12$  months) did not significantly predict false-positive outcomes (adjusted  $p > 0.05$ ). Similarly, anaemia status (anaemic vs. non-anaemic) was not associated with an increased likelihood of false positive

**Table 2** Surveillance performance of RDT Vs blood smear

|             | Smear +             | Smear -         | Total |
|-------------|---------------------|-----------------|-------|
| RDT + n (%) | 393 (43.9%)—TP      | 502 (56.1%)—FP  | 895   |
| RDT—n (%)   | 45 (1.3%)—FN        | 3477 (98.7%)—TN | 3522  |
| Total       | 438                 | 3979            | 4417  |
|             | <b>Estimate (%)</b> | <b>95% CI</b>   |       |
| Sensitivity | 89.7                | 86.5–92.2       |       |
| Specificity | 87.4                | 86.3–88.4       |       |
| PPV         | 43.9                | 40.7–47.2       |       |
| NPV         | 98.7                | 98.3–99.0       |       |

Chi-square = 1452.05, p-value < 0.0001



**Fig. 2** Chart showing agreement between microscopy and RDT (Total number surveyed = 69,684; Total excluded by reason as in a standard trial profile = 65,267; Total sample used = 4417 (contained malaria data))

after adjustment. Other variables, including sex (male vs. female), ownership of insecticide-treated nets (ITNs), dwelling spraying within the previous 12 months, and place of residence (urban vs. rural), showed no significant association with false-positive results. Furthermore, no regional differences were observed, as none of the 15 regions demonstrated statistically significant odds of false-positive outcomes compared to the reference region (Ahafo) after adjustment (all  $p > 0.05$ ). Overall, these findings suggest that false-positive RDT results were evenly distributed across socio-demographic groups and household characteristics, indicating that such diagnostic errors are unlikely to be driven by specific population-level risk factors (Table 3).

### False-negative results

Several socio-demographic and household characteristics were significantly associated with false-negative RDT outcomes. Children residing in dwellings sprayed with insecticide within the last 12 months had 56% higher odds of returning a false-negative result (aOR = 1.56, 95% CI: 1.05–2.30,  $p = 0.026$ ). Conversely, urban residence was associated with 46% lower odds of a false-negative result (aOR = 0.54, 95% CI: 0.43–0.67,  $p < 0.001$ ), indicating that false positives were more frequent in rural settings. Regional variations were also observed. Children from the Upper East Region had nearly two-fold higher odds of false-negative results compared to those from the Ahafo Region (aOR = 1.93, 95% CI: 1.16–3.21,  $p = 0.011$ ), whereas children from the Greater Accra Region were significantly less likely to have false-negative results (aOR = 0.21, 95% CI: 0.07–0.63,  $p = 0.005$ ). No statistically significant associations were found for other factors, including age, sex, anaemia status, insecticide-treated net (ITN) ownership, and most other regions ( $p > 0.05$ ) (Table 3).

### Discussion

This study provides nationally representative evidence on the surveillance performance of HRP2-based malaria RDTs compared with microscopy among children under five years of age in Ghana, using data from the 2022 Ghana DHS [23]. Our analysis revealed three key findings. First, malaria prevalence estimates derived from RDTs were approximately twice as high as those based on microscopy. Second, RDTs demonstrated high sensitivity and specificity compared to microscopy, but their positive predictive value was considerably lower, indicating a substantial burden of false-positive results [24]. Third, false-positive results were relatively uncommon and not significantly associated with any socio-demographic or household characteristics, whereas false-negative results were more frequent in rural areas, in dwellings that had

been sprayed with insecticide, and in certain regions such as Upper East.

The higher prevalence detected by RDTs relative to microscopy is consistent with earlier studies conducted in Ghana and other high-transmission settings [25, 26]. This discrepancy is likely explained by the persistence of HRP2 antigenemia after parasite clearance, which can remain detectable for several weeks following successful treatment [27]. Our finding of a low positive predictive value highlights the risk of overestimating malaria burden when relying solely on RDT-based prevalence data for surveillance or programmatic decision-making.

The high sensitivity and negative predictive value of the RDTs observed in this study support their utility for accurately identifying malaria infection status in community-based surveillance, reinforcing their value for reliable population-level detection and estimation of malaria prevalence [11, 12]. Importantly, false-positive results were rare and were not significantly associated with age, sex, anaemia status, household vector-control measures, or place of residence. The finding showed that a False-positive malaria RDT results arise in the absence of malaria parasites, mainly due to biological cross-reactivity and procedural errors [28, 29]. Rheumatoid factor, heterophile antibodies, co-infections (e.g., trypanosomiasis, hepatitis C, schistosomiasis, dengue), and persistent HRP2 antigens after treatment can all trigger false positivity [30]. In addition, improper sample handling or test procedures may compromise accuracy [31]. These false positives lead to overdiagnosis, unnecessary antimalarial use, increased costs, potential drug resistance, and delayed identification of the true cause of illness, underscoring the need for confirmatory microscopy or molecular testing, especially in endemic settings [31–33].

Our analysis also identified important predictors of false-negative results. Children living in rural areas and in dwellings sprayed with insecticide had significantly higher odds of false negative. This finding suggests that false-negative outcomes are largely stochastic, likely arising from low-density parasitemia below the RDT detection threshold, operator errors, or rare *pfhrp2/pfhrp3* gene deletions, which have been reported sporadically in West Africa [34, 35]. The absence of regional clustering of false negatives may indicate that *pfhrp2/3* deletions are not yet a major public health concern in Ghana, but continued molecular surveillance is warranted [36–38]. The association with rural residence may reflect higher malaria transmission intensity in these settings, resulting in more frequent HRP2 persistence after recent treatment, as previously described [39, 40]. The link between indoor residual spraying (IRS) and false positives may initially seem counterintuitive; however, households receiving IRS are typically located in high-burden districts

**Table 3** Logistic regression of socio-demographic factors associated with false-negative and false-positive malaria RDT results

| Variable                               | N    | False-negative malaria RDT results |                     |               |                     | False-positive malaria RDT results |                      |                     |         |
|----------------------------------------|------|------------------------------------|---------------------|---------------|---------------------|------------------------------------|----------------------|---------------------|---------|
|                                        |      | False-negative                     |                     | Multivariable |                     | False-positive                     |                      | Multivariable       |         |
|                                        |      | n (%)                              | OR (95% CI)         | p-value       | aOR (95% CI)        | n (%)                              | OR (95% CI)          | aOR (95% CI)        | p-value |
| Age                                    |      |                                    |                     |               |                     |                                    |                      |                     |         |
| Less than 6 months                     | 90   | 2 (4.4)                            | Reference           |               | Reference           | 11 (2.2)                           | Reference            | Reference           |         |
| 6 months–11 months                     | 401  | 2 (4.4)                            | 1.09<br>(0.55–2.19) | 0.8           | 1.21<br>(0.60–2.46) | 53 (10.6)                          | 0.22<br>(0.03–1.59)  | 0.24<br>(0.03–1.75) | 0.133   |
| 12 months and above                    | 3926 | 41 (91.1)                          | 0.9<br>(0.48–1.71)  | 0.751         | 0.98<br>(0.51–1.88) | 438 (87.3)                         | 0.46<br>(0.11–1.95)  | 0.51<br>(0.12–2.19) | 0.295   |
| Anemia status                          |      |                                    |                     |               |                     |                                    |                      |                     |         |
| Not Anemic                             | 2037 | 19 (42.2)                          | Reference           |               | Reference           | 204 (40.6)                         | Reference            | Reference           |         |
| Anemic                                 | 2380 | 26 (57.8)                          | 1.29<br>(1.07–1.55) | 0.009         | 1.08<br>(0.89–1.32) | 298 (59.4)                         | 1.17<br>(0.65–2.13)  | 1.22<br>(0.66–2.26) | 0.599   |
| Sex                                    |      |                                    |                     |               |                     |                                    |                      |                     |         |
| Male                                   | 2251 | 25 (55.6)                          | Reference           |               | Reference           | 253 (50.4)                         | Reference            | Reference           |         |
| Female                                 | 2166 | 20 (44.4)                          | 1.03<br>(0.85–1.24) | 0.788         | 1.01<br>(0.84–1.22) | 249 (49.5)                         | 0.83<br>(0.46–1.50)  | 0.83<br>(0.46–1.51) | 0.536   |
| ITN                                    |      |                                    |                     |               |                     |                                    |                      |                     |         |
| No                                     | 829  | 9 (20.0)                           | Reference           |               | Reference           | 91 (18.1)                          | Reference            | Reference           |         |
| Yes                                    | 3588 | 36 (80.0)                          | 1.05<br>(0.83–1.34) | 0.696         | 0.97<br>(0.75–1.25) | 411 (81.9)                         | 0.92<br>(0.44–1.93)  | 0.79<br>(0.37–1.70) | 0.832   |
| Dwelling sprayed in the last 12 months |      |                                    |                     |               |                     |                                    |                      |                     |         |
| No                                     | 3551 | 39 (86.7)                          | Reference           |               | Reference           | 359 (71.5)                         | Reference            | Reference           |         |
| Yes                                    | 866  | 6 (13.3)                           | 1.76<br>(1.43–2.17) | <0.001        | 1.56<br>(1.05–2.30) | 143 (28.5)                         | 0.628<br>(0.27–1.49) | 0.8<br>(0.20–3.18)  | 0.291   |
| Residence                              |      |                                    |                     |               |                     |                                    |                      |                     |         |
| Rural                                  | 2587 | 30 (66.7)                          | Reference           |               | Reference           | 371 (73.9)                         | Reference            | Reference           |         |
| Urban                                  | 1830 | 15 (33.3)                          | 0.46<br>(0.37–0.57) | <0.001        | 0.54<br>(0.43–0.67) | 131 (26.1)                         | 0.7<br>(0.38–1.31)   | 0.65<br>(0.34–1.25) | 0.27    |
| Region                                 |      |                                    |                     |               |                     |                                    |                      |                     |         |
| Ahafo                                  | 237  | 3 (6.7)                            | Reference           |               | Reference           | 25 (5.0)                           | Reference            | Reference           |         |
| Ashanti                                | 289  | 5 (11.1)                           | 0.56<br>(0.30–1.06) | 0.075         | 0.57<br>(0.30–1.08) | 18 (3.6)                           | 1.37<br>(0.33–5.81)  | 1.34<br>(0.31–5.69) | 0.666   |
| Bono                                   | 194  | 4 (8.9)                            | 0.71<br>(0.36–1.39) | 0.318         | 0.74<br>(0.38–1.45) | 15 (3.0)                           | 1.64<br>(0.36–7.43)  | 1.69<br>(0.37–7.69) | 0.519   |
| Bono East                              | 288  | 5 (11.1)                           | 1.21<br>(0.71–2.08) | 0.488         | 1.21<br>(0.70–2.08) | 36 (7.2)                           | 1.38<br>(0.33–5.83)  | 1.28<br>(0.30–5.44) | 0.663   |

**Table 3** (continued)

| Variable      | N   | False-negative malaria RDT results |                     |               |                     | False-positive malaria RDT results |                     |               |                     |
|---------------|-----|------------------------------------|---------------------|---------------|---------------------|------------------------------------|---------------------|---------------|---------------------|
|               |     | False-negative                     |                     | Multivariable |                     | False-positive                     |                     | Multivariable |                     |
|               |     | n (%)                              | OR (95% CI)         | p-value       | aOR (95% CI)        | n (%)                              | OR (95% CI)         | p-value       | aOR (95% CI)        |
| Central       | 239 | 3 (6.7)                            | 0.86<br>(0.47–1.57) | 0.623         | 0.89<br>(0.49–1.64) | 22 (4.4)                           | 0.99<br>(0.20–4.96) | 0.992         | 0.98<br>(0.20–4.95) |
| Eastern       | 211 | 0 (0.0)                            | 0.7<br>(0.36–1.34)  | 0.279         | 0.69<br>(0.36–1.34) | 16 (3.2)                           | 0                   | 0.995         | 0                   |
| Greater Accra | 203 | 1 (2.2)                            | 0.17<br>(0.06–0.50) | 0.001         | 0.21<br>(0.07–0.63) | 4 (0.8)                            | 0.39<br>(0.04–3.74) | 0.386         | 0.43<br>(0.04–4.21) |
| North East    | 462 | 4 (8.9)                            | 1.7<br>(1.05–2.74)  | 0.031         | 1.06<br>(0.58–1.93) | 77 (15.3)                          | 0.68<br>(0.15–3.07) | 0.617         | 0.69<br>(0.10–4.66) |
| Northern      | 452 | 6 (13.3)                           | 0.92<br>(0.55–1.54) | 0.735         | 0.79<br>(0.46–1.35) | 44 (8.8)                           | 1.05<br>(0.26–4.23) | 0.946         | 0.99<br>(0.23–4.22) |
| Oti           | 265 | 5 (11.1)                           | 0.96<br>(0.54–1.71) | 0.895         | 0.9<br>(0.50–1.61)  | 27 (5.4)                           | 1.5<br>(0.36–6.35)  | 0.582         | 1.39<br>(0.33–5.93) |
| Savannah      | 376 | 1 (2.2)                            | 1.42<br>(0.86–2.36) | 0.172         | 1.3<br>(0.78–2.17)  | 54 (10.8)                          | 0.21<br>(0.02–2.01) | 0.175         | 0.18<br>(0.02–3.68) |
| Upper East    | 301 | 2 (4.4)                            | 2.2<br>(1.34–3.63)  | 0.002         | 1.93<br>(1.16–3.21) | 62 (12.4)                          | 0.52<br>(0.09–3.15) | 0.478         | 0.47<br>(0.08–2.87) |
| Upper West    | 309 | 1 (2.2)                            | 1.56<br>(0.93–2.61) | 0.092         | 0.92<br>(0.49–1.76) | 48 (9.6)                           | 0.25<br>(0.03–2.45) | 0.236         | 0.27<br>(0.02–3.68) |
| Volta         | 192 | 1 (2.2)                            | 0.52<br>(0.25–1.08) | 0.078         | 0.51<br>(0.24–1.06) | 11 (2.2)                           | 0.41<br>(0.04–3.96) | 0.44          | 0.39<br>(0.04–3.77) |
| Western       | 399 | 4 (8.9)                            | 1.02<br>(0.61–1.73) | 0.928         | 1<br>(0.59–1.69)    | 43 (8.6)                           | 0.79<br>(0.18–3.56) | 0.759         | 0.75<br>(0.17–3.39) |

OR Unadjusted Odds Ratio, aOR Adjusted Odds Ratio

targeted for intensified vector control, which again may reflect a population with a higher likelihood of recent infection and lingering antigenemia [41–44]. Regional differences were also notable: Upper East recorded nearly double the odds of false-positive results relative to Ahafo, whereas Greater Accra had significantly lower odds. These findings mirror the known epidemiology of malaria in Ghana, where transmission is highest in the northern regions and lowest in urbanized southern regions [45–47].

Our findings align with prior analyses of DHS and Malaria Indicator Survey data from other sub-Saharan African countries, which have similarly reported RDT-based prevalence estimates exceeding those from microscopy and highlighted the need for careful interpretation of such data in population-level surveys [48, 49]. The high specificity observed in our study is reassuring but slightly lower than that reported in some facility-based evaluations (usually > 90%), possibly due to the inclusion of asymptomatic children with low-density infections and post-treatment antigenemia in the community-based DHS sample [50].

These findings have several implications for malaria control and elimination efforts in Ghana. First, programmatic decisions that rely on RDT-based prevalence estimates such as targeting of IRS, seasonal malaria chemoprevention, and allocation of commodities should consider the risk of overestimating malaria burden due to false positives. Where feasible, complementary microscopy or molecular diagnostic data should be used to validate prevalence trends. Second, the high NPV of RDTs supports their continued use as a reliable tool for excluding malaria in asymptomatic children during malaria. Third, the identification of geographic hotspots of false positives (e.g., Upper East) highlights areas where intensified monitoring of diagnostic accuracy and post-treatment antigen persistence would be beneficial.

HRP2-based RDTs have potential benefits that extend beyond their immediate diagnostic function. Although HRP2-RDTs do not precisely measure point prevalence, they capture period prevalence due to the persistence of HRP2 antigenemia, albeit with some degree of imprecision. This characteristic, often considered a limitation, can be advantageously exploited in population-based surveys, as the HRP2 “tail” may help identify areas experiencing recent or increased transmission. The results from this analysis suggest that HRP2-RDTs could therefore be useful for identifying factors associated with higher transmission intensity. Further comparative analyses could explore how malaria epidemiology differs when described using microscopy versus HRP2-RDTs, including whether HRP2-based results show stronger associations with markers of malaria burden such as anemia.

A major strength of this study is the use of nationally representative DHS data, which allows generalizability of findings to the entire population of Ghanaian children under five years. The use of microscopy as the gold standard strengthens the validity of surveillance performance estimates. However, some limitations should be acknowledged. First, microscopy, while considered the reference standard, can miss very low-density infections detectable by molecular methods, potentially leading to misclassification. Second, information on recent antimalarial use, which could help explain false-positive results, was not available for all children. Third, we did not have molecular data on *pfhrp2/3* deletions, which would have provided deeper insight into potential causes of false negatives. Finally, the study was limited to surveillance of asymptomatic malaria infections, and therefore the findings may not be generalizable to clinical case detection or symptomatic populations.

In conclusion, using nationally representative data from the 2022 Ghana Demographic and Health Survey, the study demonstrated that HRP2-based malaria RDTs substantially overestimate malaria prevalence among children under five years compared with microscopy, largely due to a high burden of false-positive results. Although RDTs showed high sensitivity, specificity, and an excellent negative predictive value supporting their continued use for ruling out malaria in community and primary healthcare settings their low positive predictive value underscores important limitations for surveillance and programmatic decision-making when used in isolation. False-positive RDT results were more frequent in rural settings, in households receiving indoor residual spraying, and in high-transmission regions such as the Upper East, reflecting persistent HRP2 antigenemia following recent infections rather than true parasitemia. In contrast, false-negative results were associated with socio-demographic or household characteristics, suggesting that factors such as low-density parasitemia or operational issues, rather than population-level risk factors or widespread *pfhrp2/3* deletions, are the primary drivers.

These findings highlight the need for cautious interpretation of RDT-based malaria prevalence estimates in national surveys and surveillance systems. Where feasible, complementary microscopy or molecular diagnostics should be used to validate prevalence trends, particularly in high-transmission settings. Nonetheless, the high negative predictive value of RDTs supports their continued role in reducing unnecessary antimalarial treatment. Importantly, the persistence of HRP2 antigenemia while limiting point prevalence estimation may offer value in identifying areas of recent or intense transmission. Integrating RDT results with other epidemiological

and laboratory data will be critical for improving malaria surveillance accuracy and informing targeted control and elimination strategies in Ghana.

#### Acknowledgements

We express our profound gratitude to Ghana Statistical Service (GSS) for permitting us to use DHS data for the studies. We also thank the staff of the Biomedical and Clinical Research Centre for supporting the study.

#### Author contributions

KKA, designed the experiment. KKA, YOA, MWM, MD, CA, HEF, NAB, and EA analyzed the data. KKA, and YOA, interpreted the results. KKA, and YOA, drafted the manuscript. All authors read and approved the final manuscript.

#### Funding

No funding was obtained for this work.

#### Data availability

All data supporting the conclusions of this article are included within the article.

#### Declarations

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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Received: 25 September 2025 Accepted: 22 December 2025

Published online: 03 January 2026

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