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Investigating the Abbott-Bioline™ malaria antigen Pf/Pv rapid diagnostic test

May Thu Thu Aung[1], Aung Myint Thu[1], Aung Pyae Phyo[1,3], Wai Linn Aung[1], Chanapat Pateekhum[1], Stephane Proux[1], Widi Yotyingaphiram[1], Laypaw Archusuksan[1], Mueanfan Wongaeka[1], Nicholas J. White[2,3], Nicholas P. Day[2,3] and Francois Nosten[1,3*]

Abstract

Background Rapid Diagnostic Tests (RDT) have become an essential tool for the control of malaria worldwide. Their simplicity of use and their reliability make them ideal for the diagnosis of malaria in endemic areas. Numerous brands are now available on the market. In South East Asia, where both *Plasmodium falciparum* and *Plasmodium vivax* are prevalent, the Abbott-Bioline™ Malaria Ag Pf/Pv rapid diagnostic test (for the detection of Pf HRP2 and Pv LDH) is deployed widely but, after years of satisfactory performance, its recent sensitivity has been questioned after multiple false negative results were reported.

Methods and results The study was conducted between October 2024 and January 2025. A field comparison with the First Response® Malaria Ag pLDH/HRP2 RDT (for the detection of Pf HRP2 and *Plasmodium* Pan LDH) and microscopy (i.e. the gold standard) was conducted on the Thailand-Myanmar border where, until recent conflict, falciparum malaria was close to elimination. Overall (combining all field specimen), the Bioline RDT had a sensitivity of 0.18 and a specificity of 0.99 for *P. falciparum*. The corresponding figures for the First Response RDT were 0.89 and 0.93 respectively. For *P. vivax* malaria, the Bioline RDT had a sensitivity of 0.44 and a specificity of 0.99, while the First Response RDT had a sensitivity of 0.59 and a specificity of 0.98. In laboratory studies, using samples from patients or standard antigen panels (NIBSC antigens including histidine-rich protein 2 (HRP2 and *P. vivax* lactate dehydrogenase (PvLDH)), Bioline RDT consistently showed fainter result lines compared to the other brands of RDTs, at parasite densities between 208 and 1993/µL, and some tests had no visible lines at all. The Bioline RDT detected only 45.0% (9 of 20) cases of acute falciparum malaria and 74.0% (37 of 50) cases of acute vivax malaria whereas the First-response RDT, identified 90.3% (18 of 20) of *P. falciparum* and 84.0% (42 of 50) *P. vivax* cases.

Conclusion The Abbott-Bioline™ Malaria Ag Pf/Pv RDT that were obtained in 2024 failed to detect microscopically confirmed cases of malaria and is not fit for purpose. This test should no longer be used and should be replaced by one with adequate performance.

Keywords Rapid diagnostic tests, The Abbott-Bioline™ Malaria Ag Pf/Pv, The First Response® Malaria Ag pLDH/HRP2 RDT, *P. falciparum*, *P. vivax*

*Correspondence:
Francois Nosten
francois@tropmedres.ac
Full list of author information is available at the end of the article

Background

Clinical symptoms alone cannot be used to diagnose malaria. Rapid Diagnostic Tests (RDT) have revolutionized the capabilities of malaria control programmes worldwide. Before they became widely available three decades ago, the diagnosis of malaria required microscopy examination of stained blood smears. Malaria RDTs rely on the detection of parasite antigens [1]. They do not quantitate parasites or assess their stage of development. Their main advantage is their ease and speed of use allowing early diagnosis and immediate treatment in resource limited settings. Health workers can use them after a short training, making their deployment possible in remote areas [2]. Current RDTs have approximately the same limit of detection as microscopy [3, 4]. In South East Asia, RDTs have been important in driving progress to elimination and reducing the overuse of antimalarial drugs [5]. The increasing global demand for malaria RDTs meant that many products of variable quality became available on the international market. The World Health Organization (WHO) provides prequalification assessment of malaria RDTs and gives guidance on acceptable performance characteristics [6–8]. With the notable exception of Myanmar, the Greater Mekong sub-region has been remarkably successful in progressing towards the elimination of malaria over the past two decades. Before the civil war in the east of Myanmar, close the Thailand border, falciparum malaria was all but eliminated. This was achieved mainly by supporting village health workers to use RDTs and providing artemisinin-based combination treatments. In Kayin State, a successful *Plasmodium falciparum* elimination project was conducted by the Shoklo Malaria Research Unit (SMRU) in over 1,000 villages since 2014 [9]. This programme, the Malaria Elimination Task Force (METF), has used the SD BIOLINE™ Malaria Ag (Pf/Pv) (Standard Diagnostic Korea) RDT since 2014. In 2023, the tests were replaced by the Abbott-Bioline™ Malaria Ag Pf/Pv, manufactured by the Abbott Diagnostic Korea. These tests detect the HRP2 (histidine rich protein 2) antigen produced by *P. falciparum* and the lactate dehydrogenase (LDH) produced by *P. vivax.* The performance of these tests, manufactured by Abbott Diagnostic (Korea) has been described previously and generally considered satisfactory [10]. Large quantities were procured by the National Malaria Programmes of several Asian and African countries [11, 12]. In June 2024, several false negative tests in patients with *P. falciparum* and *P. vivax* were reported to SMRU and this triggered a root cause investigation. This manuscript reports the findings of this evaluation.

Methods

Field screening

The malaria rapid tests in patients who presented to the SMRU clinics with symptoms were evaluated. In addition, cross-sectional surveys were conducted and RDTs were tested in the laboratory on preserved samples from microscopy positive samples of known parasite density. Three different brands of RDT were used in the evaluations: the Abbott-Bioline™ Malaria Ag (Pf/Pv) that detects *P. falciparum* and *P. vivax*, the First Response® Malaria Ag (pLDH/HRP2) that detects *P. falciparum* and *non-falciparum,* and STANDARD Q™ Malaria Ag (Pf/Pan) which also detects *P. falciparum* and *non-falciparum* malaria. The results were compared with those of microscopy examination of blood slides by experienced microscopists. Slides were read against 500 white blood cells (WBC) and the parasite density was calculated assuming 8000 WBC per µL [13]. All RDTs were stored at room temperature (< 40 °C) and used as per manufacturer's instructions by well-trained experienced health workers. In the field evaluation, blood samples were collected by finger prick to perform two RDTs as in routine diagnosis, thick and thin blood film for microscopy examination and, in some cases, dried blood spots for molecular analysis. Different lot numbers of Abbott-Bioline™ Malaria Ag (Pf/Pv) were tested (Table S1). Diagnosis of malaria was confirmed by microscopy examination for identification of the species, stages of malaria parasite development and determination of parasite density. The RDT results were recorded as either positive or negative by species. When the RDT result-line was faint (i.e. the intensity of the line colour was visibly lower than the RDT control line), the result was assessed by two readers before recording it into the report.

Laboratory testing

Several evaluations were conducted in SMRU laboratory using stored samples of known parasite density from malaria patients. There was no blinding of the specimens in the assessments. The Abbott-Bioline™ Malaria RDTs were also sent to two external independent malaria laboratories: the Pasteur Institute of Cambodia and the *Unité de Recherche* UR3073 at the Institute of Bacteriology and Parasitology of the University of Strasbourg (France). In addition, some samples of the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDTs used in the comparison were sent to the Research Institute for Tropical Medicine (Philippines) for a standard quality control evaluation following the World Health Organization (WHO) protocol [7, 14]. After alerting the company to the potential problem, a joint evaluation was conducted by Abbott and SMRU using a commonly agreed protocol to test the National Institute

of Biological Standards and Control (NIBSC) malaria antigen panels, which are the WHO International Standard antigens, as well as ten stored samples from malaria patients, using a previously undistributed colour chart shown in Fig. S1. This WHO standard antigen panel containing histidine-rich protein 2 (HRP2) (NIBSC code: 16/376) and *P. vivax* lactate dehydrogenase (PvLDH) (NIBSC code: 19/116) is used for the standardization, and evaluation of performance and sensitivity as well as for the calibration of secondary reference materials. The details of the method used during this joint evaluation are given in the appendix.

Data analysis

Data were entered into the Microsoft Excel® database (Microsoft Corporation, USA) and Stata version 16.0 (Stata Corp, 2023) was used for the data management. The statistical tests were performed using R studio (version 2024.09.1 + 394, Posit Software, PBC). The performance of the RDT tests was calculated by comparing the results with those obtained by microscopy, including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Their diagnostic accuracy was evaluated through receiver-operating characteristics (ROC) curves. Parasite densities are logarithmically distributed and reported as geometric means with 95% confidence intervals.

Ethics

The protocol was reviewed and approved by the local health authority of the Democratic Karen Benevolent Army Medical Department and by the Tak Province Border Community Ethnics Advisory Board.

Results

Diagnostic performance in field testing of patient samples

The first discrepancies in the malaria diagnostic test results between the two RDT brands (Abbott-Bioline™ Malaria Ag (Pf/Pv) and First Response® Malaria Ag pLDH/HRP2) were reported in June 2024. The first case was a young male aged 20 years old who presented with high fever at a malaria clinic and had been tested for malaria by the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT with a negative result for 3 consecutive days. On the third day of fever, the symptoms had worsened and the patient's level of consciousness deteriorated. The Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT remained negative while the First Response® Malaria Ag pLDH/HRP2 test was *P. falciparum* positive. The diagnosis was confirmed by microscopy with a parasite density of 35,168 per µL (circa 7 parasites/1,000 red blood cells (RBC)). The patient was treated for severe *P. falciparum* malaria with intravenous artesunate and recovered with no sequelae. In the following months several other negative results using the same RDT were reported from Myanmar. To investigate this potential problem further, two RDTs (Abbott-Bioline™ Malaria Ag (Pf/Pv) and First Response® Malaria Ag pLDH/HRP2) were compared in malariometric surveys (mostly asymptomatic people) and routine diagnosis in clinics (in febrile patients) (Fig. 1, Table 1). A total of 338 cases were included in the final analysis, consisting of 258 (76.4%) from the malaria surveys and 80 (23.6%) from routine malaria diagnosis in febrile patients. Overall, 170 (50.3%) were positive by microscopy of which 49.4% (84/170) were *P. falciparum*, 42.9% (73/170) were *P. vivax* and 7.6% (13/170) were mixed infections (*P. falciparum* + *P. vivax*) (Table 1). The overall geometric mean parasite density for *P. falciparum* was 491 (95% CI: 302–795; range: 2–75,360) parasites per µL and *P. vivax* was 558 (95% CI: 291–1070; range: 2–95,456) parasites per µL. *P. falciparum* parasite densities were ten times higher in the acute malaria group detected at the malaria clinic: 2,800 (95% CI: 1153–6,800; range 16–75,360) parasites per µL compared to cases in the malariometric surveys: 285 (95% CI: 172–471; range 2–12,416) and were also substantially higher for *P. vivax*: 2376 (95% CI: 1314–4293; range 16–95,456) versus 24 (95% CI: 16–37; range: 16–112) parasites per µL (Fig. 2).

Detection of low parasite densities in malariometric surveys

In the malaria surveys (active case detection), 258 individuals were screened and 97 (37.6%) were positive for malaria by microscopy (64 *P. falciparum*, 23 *P. vivax*, 10 mixed infections). The geometric mean parasite densities were *P. falciparum* 285 (95% CI, 172–471; range: 2–12,416) parasites per µL and P. *vivax* 24 (95% CI, 16–36; range: 2–112) parasites per µL (Fig. 2). Of these malaria positives, the Abbott-Bioline™ Malaria Ag (Pf/Pv) detected only 4 (6.2%) *P. falciparum* (Table 2) and no *P. vivax* while First Response® Malaria Ag pLDH/HRP2 RDT identified 55 (85.9%) *P. falciparum* and 8 (34.8%) *P. vivax* (Table 3). Among 10 mixed infections, 6 (60.0%) were diagnosed accurately whereas 2 (20.0%) cases were reported only as *P. falciparum* and 1 (10.0%) as non-*falciparum* infection. The thresholds for positivity of the two tests were compared. The Bioline RDT remained negative with *P. falciparum* geometric mean parasite densities of 266 parasites per µL (95% CI: 159–443; range: 2–9856) (Fig. 3A), compared with the First Response RDT geometric mean for negative results of 28 parasites per µL (95% CI: 9–84; range: 2–526) (Fig. 3B). In this evaluation, the geometric mean parasite densities in RDT negative *P. vivax* infections were similar for both tests: 24 parasites per µL (95% CI: 16–36; range: 2–112) vs 21 parasites per

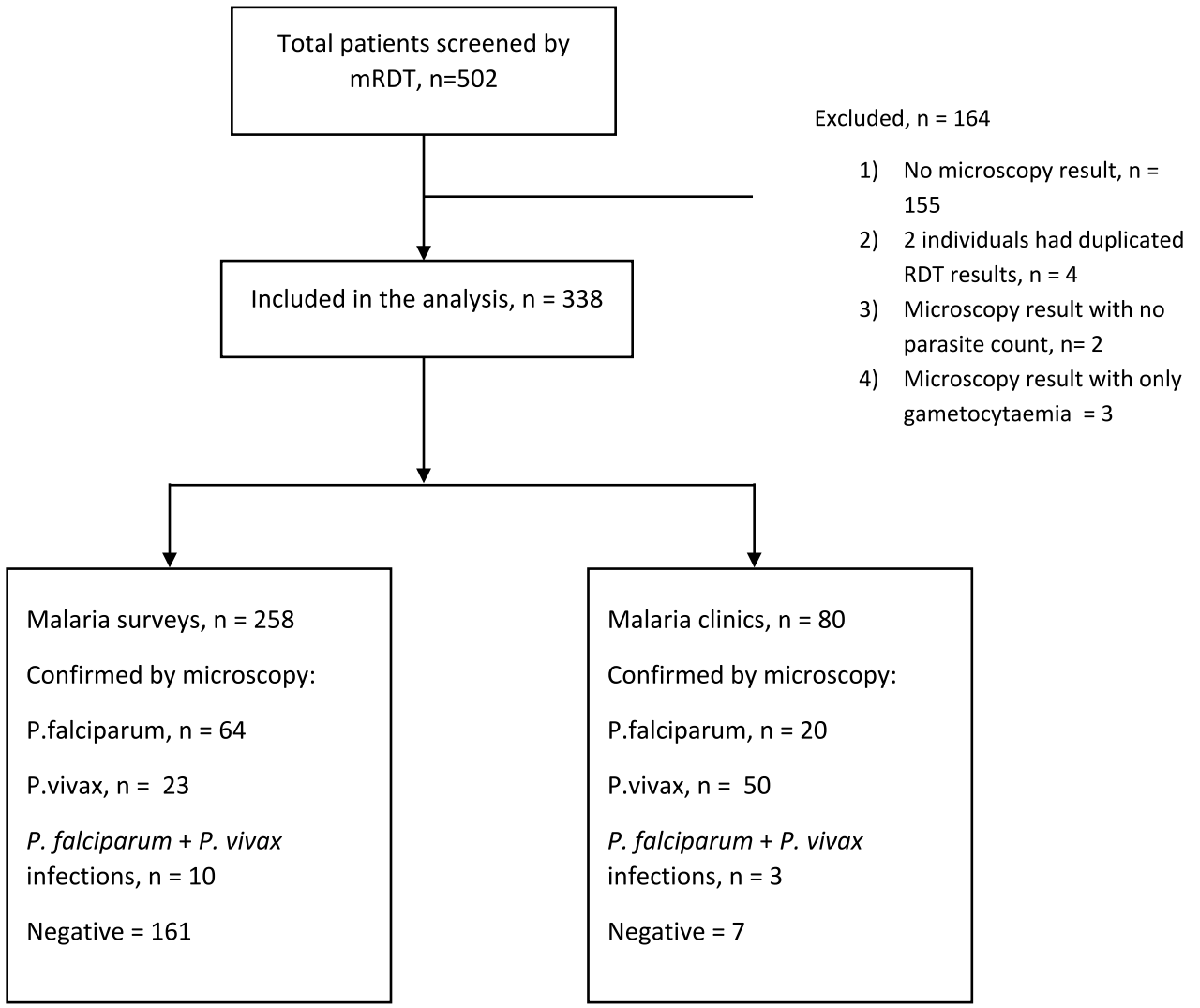

Fig. 1 Study flow chart

µL (95% CI: 11–38; range: 2–112) for Abbott-Bioline™ and First Response® RDTs (Fig. 4).

Diagnosis performance for clinical malaria at malaria clinic

In eighty febrile patients who attended the malaria clinic, 73 were positive by microscopy (20 *P. falciparum*, 50 *P. vivax*, 3 *Plasmodium* mixed infections). The geometric mean parasite densities were 2800 (95% CI: 1153–6800; range: 16–75,360) parasites per µL for *P. falciparum* and 2,376 (95% CI: 1314–4293; range: 16–95,456) parasites per µL for *P. vivax*. Among the microscopy *P. falciparum* positive infections, the Bioline RDT detected only 9 (45%) whereas the First Response RDT detected all 20 cases (100%). For *P.vivax*, the RDTs detected 37 (74%) and 42 (84%) for the Bioline and First Response tests, respectively (Tables 4 and 5). Thus, the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT missed half the symptomatic *P. falciparum* infections and one quarter of the symptomatic *P. vivax* infections presenting to the clinic. The geometric mean parasite density was 3,488 (95% CI: 1267–9604; range: 512–42,320) parasites per µL in the 11 Bioline RDT negative *P. falciparum* infections. All of the Bioline RDT negative *P. falciparum* infections had parasite densities above 200 parasites per µL, the WHO recommended threshold of RDT performance for *P.falciparum*. Among the RDT negative *P. vivax* infections, the geometric mean densities were 168 (95% CI: 74–381; range: 16–2512) for the Bioline RDTs and 154 (95% CI: 51–465; range: 16–2496) for the First Response RDTs. Six out of 13 (46%) of *P. vivax* infections that were negative by the Bioline RDT had parasite density above 200 parasites per µL.

Table 1 Characteristics of the patients

Type of detection	Surveys	Clinics
Number of participants	258	80
Gender		
Male, n (%)	221 (85.6)	52 (65.0)
Female, n (%)	37 (14.4)	28 (35.0)
Age (years)		
Median (Q1–Q3)	21 (19–25)	22 (15–30)
Malaria symptoms reported		
Yes, n (%)	136 (52.7)	80 (100)
No, n (%)	122 (47.3)	–
Temperature* (Celsius)		
Median (Q1-Q3; range)	36.3 (36.1–36.5)	37.1 (36.5–38.4)
Mean (min–max)	36.4 (35.4–38.0)	37.5 (35.2–40.2)
Malaria positive, n (%)		
Microscopy	97 (37.6)	73 (91.3)
Abbott RDT (Pf/Pv)	6 (2.3)	52 (65.0)
First Response RDT (Pf/Pan)	88 (34.1)	71 (88.8)
Parasite density microscopy positive**		
Median (Q1-Q3)	128 (32–720)	2384 (672–10,400)
Geometric Mean (min–max)	157 (2–18,240)	2498 (16–95,456)

*228 patients, **170 patients, IQR—Interquartile range

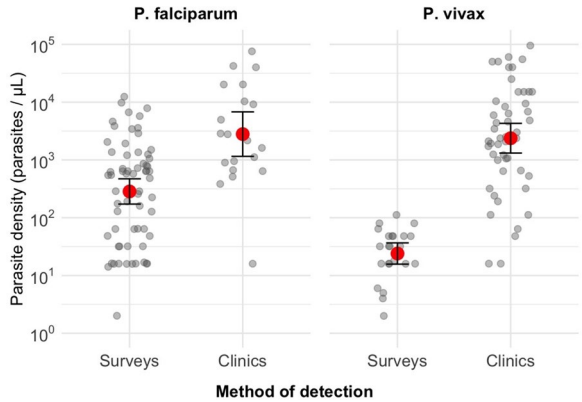

Fig. 2 The parasite densities (parasites per µL) *P. falciparum* (left) *and P. vivax* (right) infections detected in the surveys and febrile patients at the clinics. Geometric mean (red) and 95% CI (black bar)

The faint line issue

In febrile patients, the Bioline RDT detected 74% (37/50) of microscopy confirmed *P. vivax* infections. Of these, 38% (19/37) showed a faint positive line result while only 4% (2/45) of the First Response RDT tests had a faint line result. The geometric mean *P. vivax* density in the Bioline RDT faint line positives was 2,165 (95% CI: 1420–3300; range: 528–40,192) parasites per µL. For *P. falciparum*, where the Bioline RDT detected only half of the microscopy confirmed positives (9/20, 45%), two of the positives also showed a faint result line (parasite densities 16 and 1120 parasites per µL, respectively).

Diagnostic sensitivity

Overall, when considering all samples whether from symptomatic or asymptomatic participants, for *P. falciparum*, the Bioline RDT had a sensitivity of 0.18 and a specificity of 0.99 and the First Response RDT had a sensitivity of 0.89 and a specificity of 0.93 (Table 6). For *P. vivax* malaria, the Bioline RDT had a sensitivity of 0.44 and a specificity of 0.99, while the First Response RDT had a sensitivity of 0.59 and a specificity of 0.98 (Table 6). Comparing performances across the parasite density range from ≤200 to ≥2000 parasites per µL for *P. falciparum*, the Bioline RDT consistently showed poor sensitivity below 2000 parasites per µL, ranging from 10 to 20% (Table 7). For *P.vivax*, the Bioline RDT sensitivity was very low (less than 20%) below 500 parasites per µL (Table 8). Overall, ROC analysis of Bioline RDT and First Response RDTs against microscopy for *P. falciparum* detection gave accuracies of 58.1% and 91.0% respectively (Fig. 5A and B). A similar result was seen for *P. vivax* with accuracies of 50.0% and 78.4%, respectively (Fig. 5A and B). The Bioline RDT failed to detect *P. falciparum* parasite densities above 200 parasites per µL in 65.7% (46/70) of microscopy positive infections (Fig. 6). In positive tests, 39.6% (23/58) of Bioline tests had a faint result line (4 of 20 *P. falciparum* and 19 of 38 *P. vivax* cases, respectively). Incomplete migration of blood was observed frequently within the recommended reading time of 15 to 30 min (Fig. S2). Of note, the majority (88%, 52/59) of the

Table 2 Microscopy and Abbott-Bioline™ Malaria Ag (Pf/Pv) result comparison in surveys

		Microscopy, n (%)			
		Pf	Pv	Pf + Pv	Negative
Abbott-Bioline™ Malaria Ag (Pf/Pv)	*P. falciparum* (Pf)	4 (6.2)	0	0	0
	P. vivax (Pv)	0	0	0	1 (0.6)
	Pf + Pv	1 (1.6)	0	0	0
	Negative	59 (92.2)	23 (100)	10 (100)	160 (99.4)
	Total	64 (100)	23 (100)	10 (100)	161 (100)

Table 3 Microscopy and First Response® Malaria Ag pLDH/HRP2 result comparison in surveys

		Microscopy, n (%)			
		Pf	**Pv**	**Pf + Pv**	**Negative**
First Response® Malaria Ag pLDH/HRP2	*P. falciparum* (Pf)	2 (3.1)	0	2 (20.0)	3 (1.9)
	Non-falciparum (Pan)	3 (4.7)	8 (34.8)	1 (10.0)	3 (1.9)
	Pf + Pan	53 (82.8)	1 (4.3)	6 (60.0)	6 (3.7)
	Negative	6 (9.4)	14 (60.9)	1 (10.0)	149 (92.5)
	Total	64 (100)	23 (100)	10 (100)	161 (100)

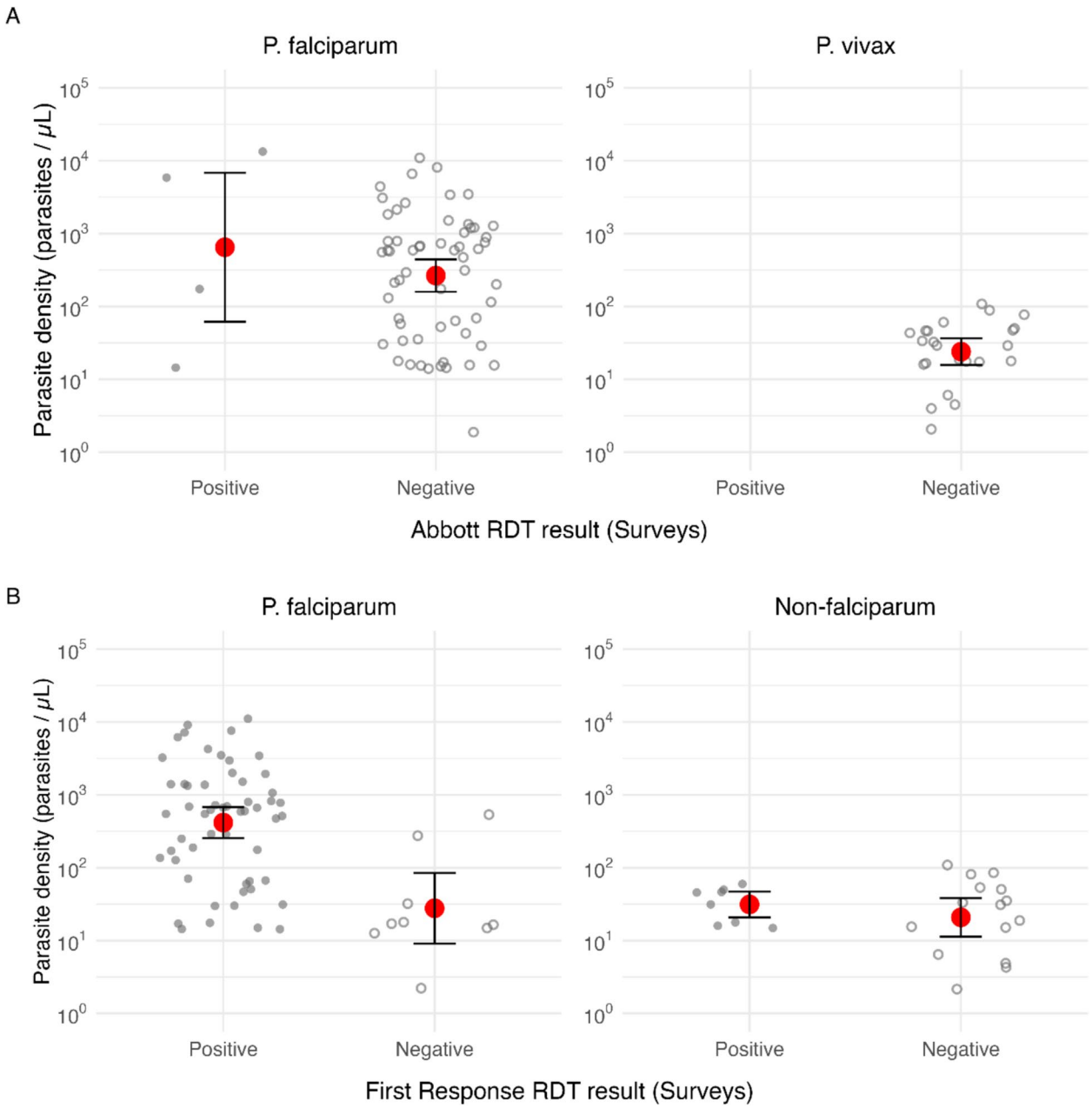

Fig. 3 Parasite densities (parasites per µL) of microscopy-positive *P. falciparum* and *P. vivax* diagnosed by (**A**) Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT and (**B**) First Response® Malaria Ag pLDH/HRP2 RDT from surveys. Geometric mean (red) and 95% CI (black bar)

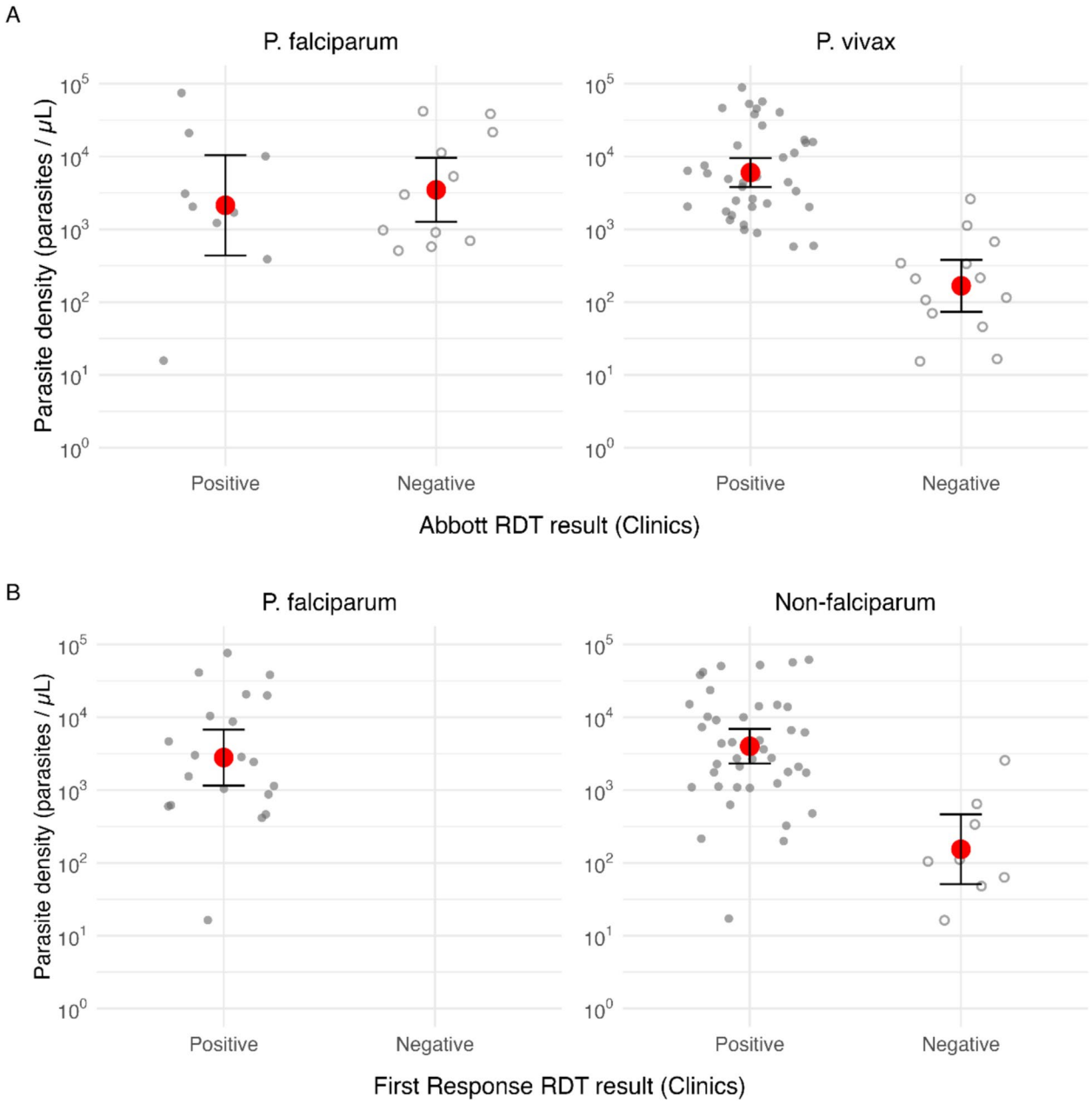

Fig. 4 Parasite densities (parasites per µL) of microscopy-positive *P. falciparum* and *P. vivax* diagnosed by (**A**) Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT and (**B**) First Response® Malaria Ag pLDH/HRP2 from clinics. Geometric mean (red) and 95% CI (black bar)

Table 4 Microscopy and Abbott-Bioline™ Malaria Ag (Pf/Pv) result comparison in clinics

		Microscopy, n (%)			
		Pf	**Pv**	**Pf + Pv**	**Negative**
Abbott-Bioline™ Malaria Ag (Pf/Pv)	*P. falciparum* (Pf)	9 (45.0)	0	2 (66.7)	3 (42.9)
	P. vivax (Pv)	0	37 (74.0)	0	0
	Pf + Pv	0	0	1 (33.3)	0
	Negative	11 (55.0)	13 (26.0)	0	4 (57.1)
	Total	20 (100)	50 (100)	3 (100)	7 (100)

Table 5 Microscopy and First Response® Malaria Ag pLDH/HRP2 result comparison in clinics

		Microscopy, n (%)			
		Pf	**Pv**	**Pf + Pv**	**Negative**
First Response® Malaria Ag pLDH/HRP2	*P. falciparum* (Pf)	2 (10.0)	0	0	3 (42.9)
	Non-falciparum (Pan)	0	42 (84.0)	0	0
	Pf + Pan	18 (90.0)	3 (6.0)	3 (100)	0
	Negative	0	5 (10.0)	0	4 (57.1)
	Total	20 (100)	50 (100)	3 (100)	7 (100)

Table 6 The overall performance of the Abbott-Bioline™ (Pf/Pv) and First Response® Malaria pLDH/HRP2

		Sensitivity (95% CI)	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Abbott Bioline RDT P.f/P.v	*P. falciparum* (Pf)	0.18 (0.11, 0.27)	0.99 (0.96, 1.00)	0.85 (0.64, 0.97)	0.75 (0.69, 0.79)
	P. vivax (Pv)	0.44 (0.33, 0.55)	0.99 (0.97, 1.00)	0.95 (0.80, 0.99)	0.84 (0.79, 0.88)
First Response RDT Pan/P.f	*P. falciparum* (Pf)*	0.89 (0.81, 0.94)	0.93 (0.89, 0.96)	0.84 (0.76, 0.91)	0.95 (0.92, 0.98)
	Non-falciparum (Pan)	0.59 (0.48, 0.70)	0.98 (0.95, 0.99)	0.89 (0.78, 0.96)	0.88 (0.83, 0.91)

**P.falciparum* is counted if Pf line or Pf + Pan line positive

Table 7 The overall sensitivity and specificity of Abbott-Bioline™ Malaria Ag (Pf/Pv) for *P. falciparum* by parasite concentration

	Parasite count per µL	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Abbott Bioline RDT P.f/P.v	≤ 200	0.10 (0.02, 0.26)	1.00 (0.88, 1.00)	1.00 (0.29, 1.00)	0.52 (0.38, 0.65)
	201–500	0.20 (0.03, 0.56)	1.00 (0.29, 1.00)	1.00 (0.16, 1.00)	0.27 (0.06, 0.61)
	501–1000	0.05 (0.00, 0.25)	1.00 (0.40, 1.00)	1.00 (0.03, 1.00)	0.17 (0.05, 0.39)
	1001–2000	0.18 (0.02, 0.52)	1.00 (0.59, 1.00)	1.00 (0.16, 1.00)	0.44 (0.20, 0.70)
	> 2000	0.36 (0.18, 0.57)	1.00 (0.88, 1.00)	1.00 (0.66, 1.00)	0.64 (0.49, 0.78)

Table 8 The overall sensitivity and specificity of Abbott-Bioline™ Malaria Ag (Pf/Pv) for *P. vivax* by parasite concentration

	Parasite count per µL	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Abbott Bioline RDT P.f/P.v	≤ 200	0.10 (0.02, 0.26)	1.00 (0.88, 1.00)	1.00 (0.29, 1.00)	0.52 (0.38, 0.65)
	201–500	0.14 (0.00, 0.58)	1.00 (0.54, 1.00)	1.00 (0.03, 1.00)	0.50 (0.21, 0.79)
	501–1000	0.75 (0.19, 0.99)	0.95 (0.75, 1.00)	0.75 (0.19, 0.99)	0.95 (0.75, 1.00)
	1001–2000	0.67 (0.30, 0.93)	1.00 (0.66, 1.00)	1.00 (0.54, 1.00)	0.75 (0.43, 0.95)
	> 2000	0.88 (0.71, 0.96)	1.00 (0.85, 1.00)	1.00 (0.88, 1.00)	0.85 (0.65, 0.96)

true positive results diagnosed by the Bioline RDT were obtained from one specific Lot (05DDI040AA) which had been stored in an air-conditioned room since the arrival of the test to the SMRU central facility.

RDT evaluations in external laboratories

Two batches of Abbott-Bioline™ (Pf/Pv) (05DDI040AA and 05DDI020BA) were sent to the Institute Pasteur in Phnom Penh (Cambodia). The RDTs were tested on frozen blood samples from confirmed *P. vivax* patients. Five different samples with parasite densities ranging from 4500 to 20,000 parasites per µL were tested. Of these, the sample with 20,000 parasites per µL was positive for both lots, a sample with 6052 parasites per µL was positive for lot 05DDI040AA but negative for lot 05DDI020BA while samples with *P. vivax* densities of 4500, 7800, and 11,200 parasites per µL were negative for both lots. A migration issue was also reported in this evaluation. A complete report with photographs is available in the following link: https://drive.google.

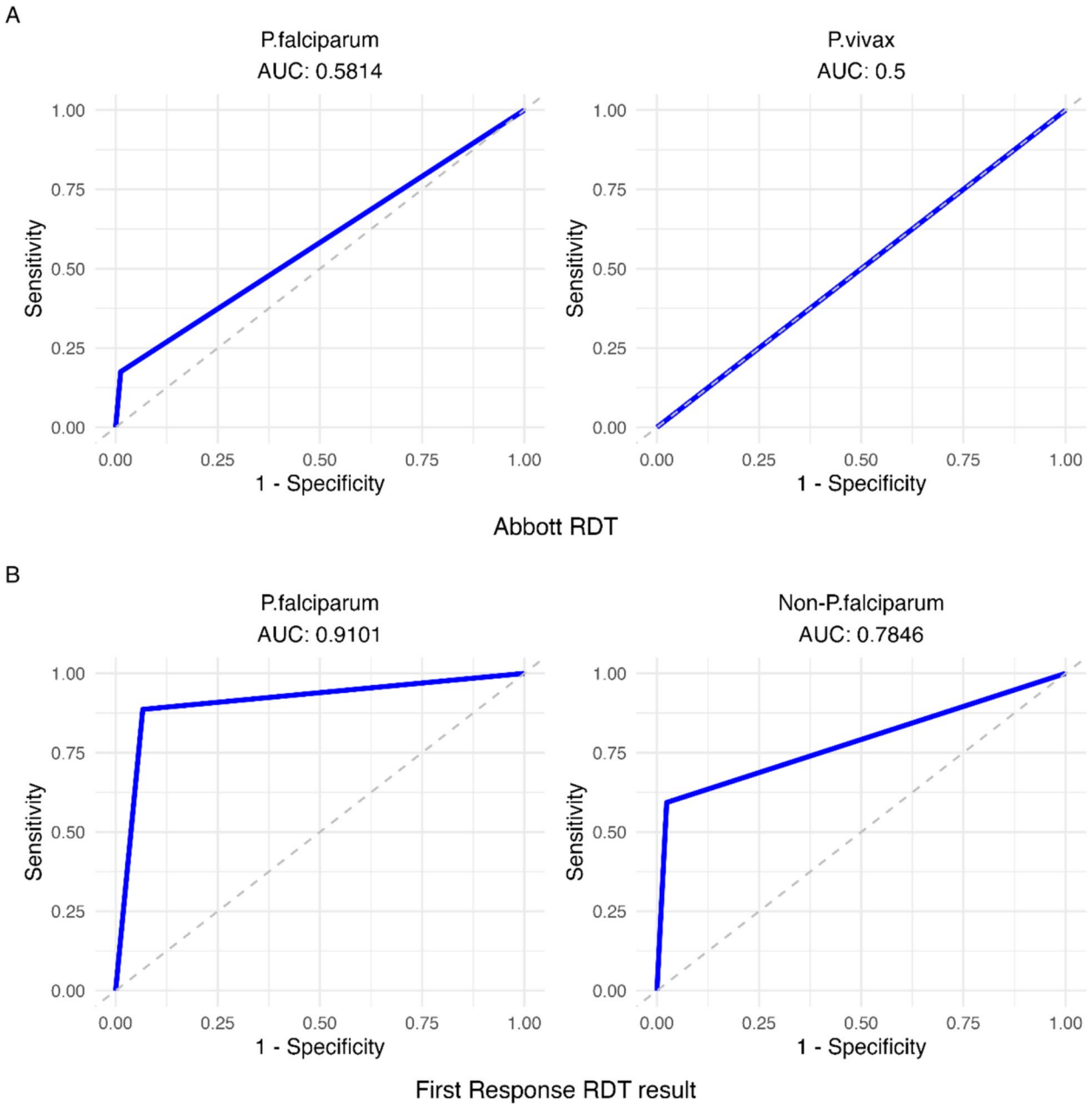

Fig. 5 ROC analysis of (**A**) the Abbott-Bioline™ Malaria Ag (Pf/Pv) for *P. falciparum* (left) and *P. vivax* (right) against microscopy result (**B**) the First Response® Malaria Ag pLDH/HRP2 for *P. falciparum* (left) and *P. vivax* (right) against microscopy result

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Four lots of Abbott-Bioline™ (Pf/Pv) (05DDI018BH, 05DDI020BA, 05DDI040AA and 05DDI041AB) were sent to the Institute of Bacteriology and Parasitology of the University of Strasbourg (France) for evaluation. The Abbott-Bioline™ (Pf/Pv) was tested using diluted *P. falciparum* specimens with parasite densities ranging from 19 to 60,874 parasites per µL. The same lots were also tested in malaria patients presenting at clinics in Madagascar. This evaluation reported that the RDTs tested gave "normal" results. The full results will be reported in full elsewhere.

Two lots of Abbott-Bioline™ (Pf/Pv) (05DDI039CE and 05DDI041AB) were evaluated by the Malaria RDT Quality Assurance Laboratory, Research Institute for Tropical Medicine, Department of Health, Philippines. The RDTs were tested using laboratory samples of positive

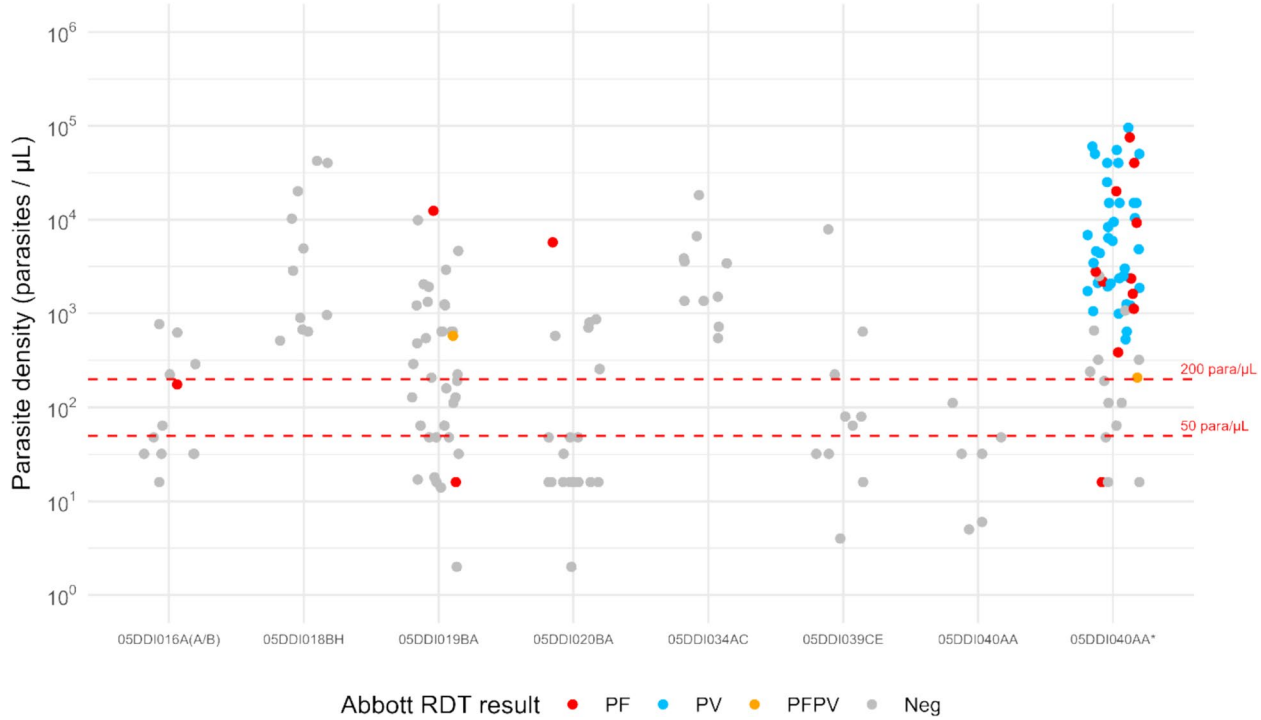

Fig. 6 Results from the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT across various lots, shown against parasite counts by microscopy. Dotted lines indicate the standard detection level (50 parasites per µL) and the WHO evaluation limit (200 parasites per µL)

controls (calibrated to 200 parasites per µL) and a negative control which was a malaria negative fresh whole blood. These RDT lots were reported to pass the quality control test, although faint lines and incomplete migration were reported. Most of the positive lines results were very faint, and the retrospective reading with the Abbott-Bioline™ colour chart fell between G1 to G3 (Figure S1). A complete report with photos is available here: https://drive.google.com/drive/folders/148gqor8L4ZOv9sZCoQQ-JC2ewblmCHDO?usp=sharing

Joint assessment of the malaria RDTs

NIBSC control antigens

In the assessment of the NIBSC antigen panels, samples with high concentrations of *P. falciparum* antigens (573.34 IU/ml, roughly equivalent to 2,000 parasites/µL), the average band intensity scores, assessed by three readers, were consistently higher for the First Response® Malaria Ag pLDH/HRP2; (mean ± standard deviation) 19.7 ± 0.5 and STANDARD Q™ Malaria Ag (Pf/Pan); 14.0 ± 3.9 than for the Abbott-Bioline™ Malaria Ag Pf/Pan and Pf/Pv RDTs; 8.4 ± 2.6 and 10.1 ± 3.1, respectively. Similar differences in band intensity were observed when the RDTs were tested with lower concentrations of *P. falciparum* antigens (57.34 IU/ml, approximately equivalent to 200 parasites/µL); average line intensities for these tests were 7.2 ± 4.0 for First Response® Malaria Ag pLDH/HRP2, 5.5 ± 2.0 for STANDARD Q™ Malaria Ag (Pf/Pan) compared with 2.2 ± 1.5 for Abbott-Bioline™ (Pf/Pan) and 2.5 ± 1.4 for Abbott-Bioline™ (Pf/Pv). Nearly half the (31/68) Abbott-Bioline™ results had a very low (i.e. faint) result line, also scoring between G1-G3. For specimens with high *P. vivax* parasite counts (1773.34 IU/ml, roughly equivalent to 800 parasites/µL), the average positive line scores for the RDTs were higher for First Response® Malaria Ag pLDH/HRP2 and STANDARD Q™ Malaria Ag (Pf/Pan): 20.0 ± 0 and 16.3 ± 5.7 respectively, compared to Abbott-Bioline™ Malaria Ag (Pf/Pan) and Abbott-Bioline™ Malaria Ag (Pf/Pv): 6.2 ± 3.0 and 10.7 ± 2.9 respectively (Fig. 7A). Significant differences were also observed in specimens with lower concentrations of *P. vivax* antigens (443.34 IU/ml, approximately 200 parasites per µL). First Response® Malaria Ag pLDH/HRP2 and STANDARD Q™ Malaria Ag (Pf/Pan) scored 10.5 ± 2.8 and 11.0 ± 3.6, respectively while Abbott-Bioline™ Malaria Ag (Pf/Pan) and Abbott-Bioline™ Malaria Ag (Pf/Pv) scored lower: 2.5 ± 1.5 and 3.7 ± 1.6 respectively. Two Abbott-Bioline™ Malaria RDTs were reported negative, and 4 were invalid due to unclear backgrounds at the 15-min mark (Table S2). The evaluation of NIBSC antigen panels showed that First Response® Malaria Ag pLDH/HRP2 and STANDARD Q™ Malaria Ag (Pf/Pan)

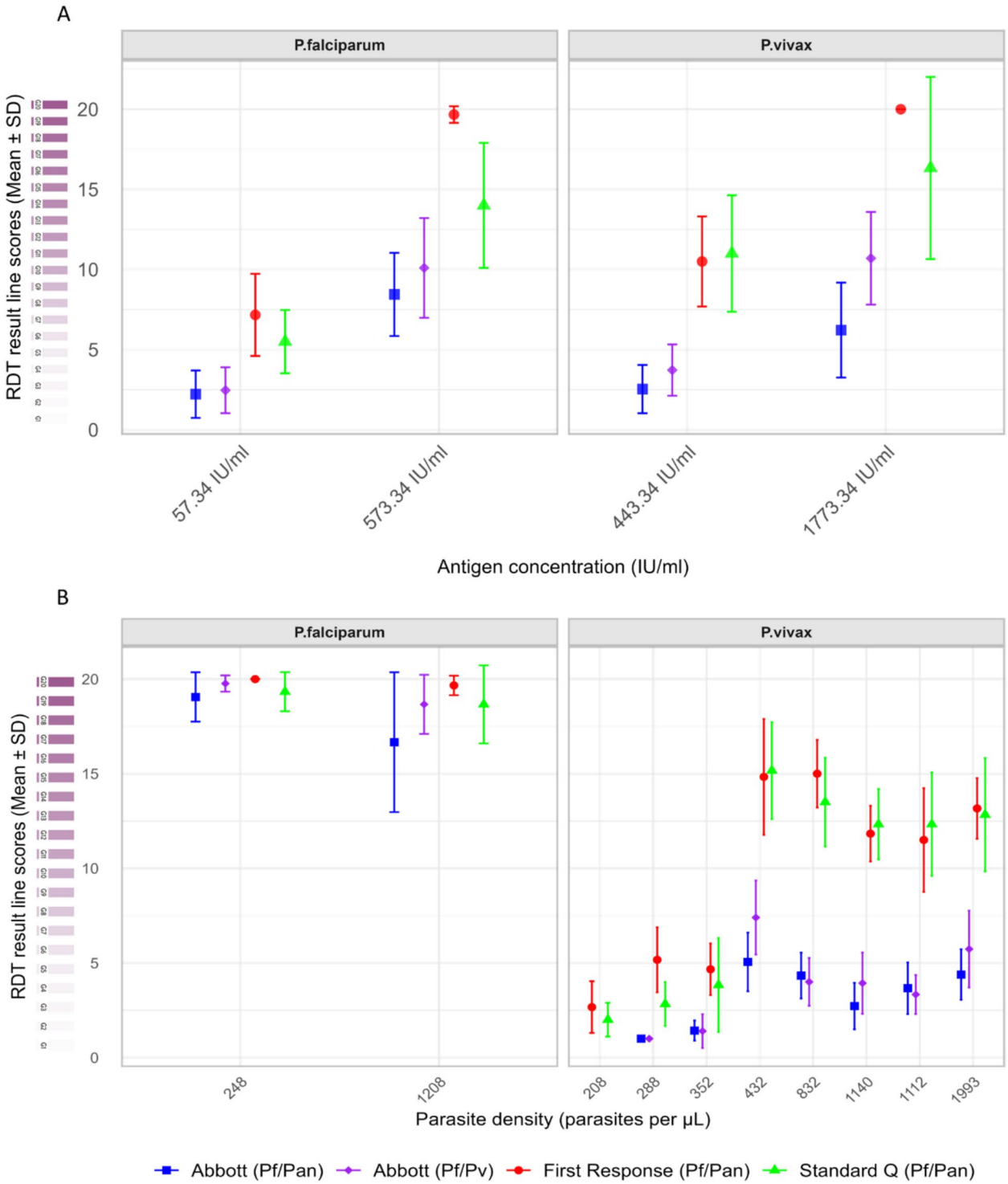

Fig. 7 Grading of RDT test line intensity using the Abbott color chart for (**A**) NIBSC panels and (**B**) patient specimens

RDTs consistently had higher band intensity scores compared to Abbott-Bioline™ Malaria Ag Pf/Pan and Pf/Pv RDTs. This was particularly evident at both high and low concentrations of *P. falciparum* and *P. vivax* antigens.

Stored patient specimens

All RDT lots showed strong performance with high-intensity scores for the two *P. falciparum* specimens (248 and 1208 parasites per µL), with an average intensity

score of 18.9 ± 2. Both Abbott-Bioline™ Malaria Ag Pf/Pv and Pf/Pan RDTs exhibited significantly lower band intensity scores for the *P. vivax* specimens; 5.2 ± 2.5 and 3.8 ± 1.7 for Abbott-Bioline™ compared to 9.9 ± 5.0 and 9.4 ± 5.5 for First Response® and STANDARD Q™, respectively (Fig. 7B). The average result line scoring for *P. vivax* with Abbott-Bioline™ RDTs was 2–3 times lower than that for First Response® or STANDARD Q™ (Table S3). All readers consistently noted this low intensity in *P. vivax* specimens. Notably, in three specimens with parasite densities between 208 and 352 parasites per µL, the majority of Abbott Bioline™ RDT tests (34 out of 48, 70.8%) were negative, contrasting with the First Response® Malaria Ag pLDH/HRP2 and STANDARD Q™ Malaria Ag (Pf/Pan) RDTs (Table S3) which detected all the parasites specimens tested.

Discussion

Rapid diagnostic tests for malaria provide a qualitative binary result based on the presence or absence of a colour band. False positive and false negative tests both have serious consequences. This investigation was triggered by a series of observations of false negative results in patients with malaria tested with a widely used RDT, the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT which was being used in the malaria elimination programme on the Thailand-Myanmar border. Previous studies have demonstrated the high sensitivity and specificity of the Abbott-Bioline™ (previously branded as SD-Bioline) malaria RDT [15, 16]. Budodo et al. [17] claimed that the Abbott-Bioline™ exhibited performance comparable to qPCR, surpassing that of microscopy, although the accuracy of the microscopy was questioned. The evaluation conducted by Rakotoarisoa and colleagues in Madagascar found that Abbott-Bioline™ Malaria Ag Pf/Pv and Pf/Pan RDTs both have high diagnostic sensitivity (over 90%) for parasite densities above 100 parasites per µL [10]. The Abbott-Bioline™ RDTs used in the Rakotoarisoa et al. study was manufactured at Abbott Point of Care in Princeton, NJ, (USA) whereas the Abbott-Bioline™ evaluated in this study was produced at Abbott Diagnostic in Korea. It is possible that the Abbott-Bioline™ RDT products originating from two different manufacturing sites have different performances. In contrast, a recent study from Cameroon showed that the Abbott-Bioline™ Ag (Pf) had only 45% sensitivity compared to microscopy in the detection of *P. falciparum* malaria [18]. Mandamet et al. [19] also reported that Abbott-Bioline™ performed less well than the comparator brand, Acro Malaria Pf/Pv/Pan Rapid Test Cassette (Acro Biotech, Montclair, CA, USA). Histidine Rich Protein 2 and 3 (HRP2/3) gene deletion mutations in *P. falciparum* can impair the performance of HRP2 based malaria rapid diagnostic tests, but the prevalence of this polymorphism is low along the China-Myanmar border [20, 21]. To rule this potential factor out, 69 *P. falciparum* specimens were analysed between 2017 and 2023 collected as part of a separate study on the molecular markers of antimalarial drug resistance [22, 23]. No HRP2/3 deletions were detected in this separate investigation conducted in the same area. Incorrect storage of RDTs can also impair their performance. The effects of environmental conditions on the Abbott-Bioline™ RDT storage were assessed by comparing two lots of RDT stored at SMRU and two recently manufactured lots from Abbott Diagnostic Korean (ADK) stored in Korea. All the tests demonstrated equivalent performance during the joined Abbott-SMRU evaluation using stored patient specimens. This suggests that issues with the transportation and storage of Abbott-Bioline™ RDTs at SMRU operation sites did not explain the poor test results.

Although malaria RDTs have a binary result, the positivity depends on the colour intensity in the malaria antigen detection band which can be misinterpreted as false negative for very faint color (see G1 on the colour chart) This investigation shows that the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT provided fainter bands at low densities than the other tests. A faint band may not be detected or recognised as positive. This extensive evaluation in the field and the laboratory shows that the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT provided to us in 2024 is not fit for purpose. The test lacks sensitivity which is not explained by storage conditions, blood volume or reader experience. The rigorous evaluation in the field which was confirmed in the laboratory, found a high percentage of false negative results. Using the WHO recommended detection threshold of 200 parasites/µL for *P. falciparum*, 46 of 70 samples which should have been positive were not detected by the Abbott-Bioline™ Malaria Ag (Pf/Pv) RDT that were received from Korea. As expected for a binary test operating close to its limits of detection test performance was not consistent across batches or even within a given batch of RDTs. The reasons behind the low test sensitivity of this widely deployed RDT have not yet been determined and are under investigation. Continued use of this particular test will risk patient safety, reduce community confidence and increase malaria transmission. Delayed or missed diagnosis of falciparum malaria may have fatal consequences.

The joint laboratory evaluation with the Abbott team sent from Korea was open and convivial but our conclusions differed. The manufacturer conceded that their product produced fainter lines than other RDTs but concluded that their RDT performance was still within the specifications, and that the issues might have arisen from the users. The conclusions were diametrically opposite.

The clinical consequences of this loss of malaria diagnostic sensitivity are huge, as these particular malaria RDTs are used by several National Malaria Control Programmes in countries such as Vietnam, Cambodia, Laos PDR, Myanmar and Pakistan. In our malaria elimination programme in Karen state, Eastern Myanmar, the impact has been limited (from August 2024) by replacing the Abbott product with another brand (First Response® Malaria Ag pLDH/HRP2) in all the malaria posts. Other malaria service providers may not have been able to do this. No fatalities directly related to a false negative result have been reported but given the current situation in Myanmar, which bears the vast majority of malaria burden in the Greater Mekong sub-region, with conflict and health service disruption, this negative result does not provide reassurance. It is important to emphasize that a patient who presents with a serious febrile illness in a remote rural area and is incorrectly diagnosed as not having falciparum malaria, usually has no other option for diagnosis and treatment. Untreated severe falciparum malaria is usually fatal. In March 2025, the WHO issued a cautionary note related to the use of malaria RDTs in general, it did not mention the Abbott product specifically and it did not give any useful guidance to potential users of this test [24].

Conclusion

The conclusion from the evidence gathered in the field and the laboratory, is that the Abbott-Bioline™ Malaria Ag Pf/Pv RDT provided in 2024 is insensitive and not fit for purpose. Too many cases of confirmed clinical malaria are missed by this test. It is urgent that the use of this test is discontinued until its low sensitivity has been corrected. In the meantime, other validated brands should be used in the field. In the future, all deployed malaria RDT should be periodically evaluated in the field against a "gold standard".

Appendix

Method

Joined assessment of the RDT with a team from Abbott

The procedure involved testing multiple lots of Abbott-Bioline™ Malaria Ag Pf/Pv and Pf/Pan, First Response® Malaria Ag pLDH/HRP2, and STANDARD Q™ Malaria Ag P.f/Pan using (1) NIBSC antigen panels for *P. falciparum* (NIBSC code: 16/376) and *P. vivax* (NIBSC code: 19/116) which are the WHO International Standard antigens, (2) stored malaria positive patient specimens and (3) the observation of the RDT screening of the malaria patients at the SMRU clinic. The results were interpreted by the readers independently using a colour scale chart provided by Abbott (Figure S1).

NIBSC antigens were diluted with negative human whole blood according to the signed evaluation protocol between SMRU and Abbott. The malaria negative human whole blood for dilution was used as a negative control sample. The Abbott scientist prepared 2 concentrations each for *P. falciparum, P. vivax* antigens and a negative control as below.

NIBSC *P. falciparum* antigens

- 57.34 IU/ml (approx.200 parasites per µL)
- 573.34 IU/ml (approx.2,000 parasites per µL)

NIBSC *P. vivax* antigen

- 443.34 IU/ml (approx.200 parasites per µL)
- 1,773.34 IU/ml (approx.800 parasites per µL)

Negative control sample

A total of 10 different RDT lots including 8 lots of Abbott-Bioline™ Malaria RDT, one lot of First Response® Malaria Ag, and one lot of STANDARD Q™ Malaria RDT (appendix, Table S1). Two RDT devices from each lot were selected to test for 2 concentrations each of *P. falciparum* antigen and *P. vivax* antigen and a negative control specimen. The specimen preparation and RDT testing procedure were performed by an expert from Abbott and the results were independently interpreted by an observer from Abbott and 2 laboratory staffs from SMRU.

For the patient's samples, a total of 10 malaria-positive stored specimens with parasitaemia (determined by microscopy), ranging from 208 to 1993 parasites per µL were tested across 10 different lots of RDTs. The malaria RDT were interpreted using a colour chart provided by Abbott and graduated from G1 to G20 (Figure S1).

Abbreviations

ACT	Artemisinin-based combination therapy
EDT	Early diagnosis and treatment
HRP2	Histidine rich protein 2
LDH	Lactate dehydrogenase
METF	Malaria elimination task force
µL	Microliter
RDT	Rapid diagnostic test
ROC	Receiver operating characteristic
SMRU	Shoklo Malaria research unit

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05577-2.

Additional file 1.

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Author contributions
FN, NJW and ND contributed to the conceptualization of the study. MTTA, WY, WLA and APP contributed to the field screening and data collection. SP, LA and MW contributed to the laboratory work. MTTA, CP and AMT contributed to the data analysis. MTTA, FN and AMT wrote the original draft of the manuscript. APP, NJW and ND contributed to the writing and editing of the manuscript. All authors read, edited and approved the final manuscript.

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Data availability
All relevant data are within the manuscript. Upon reasonable request via the [MORU website] (https:/www.tropmedres.ac/units/moru-bangkok/bioethics-engagement/data-sharing/moru-tropical-network-policy-on-sharing-data-and-other-outputs) or from MORU data sharing committee [datasharing@tropmedres.ac] (mailto:datasharing@tropmedres.ac) the raw data set will be available.

Declarations

Competing interests
The authors declare no competing interests.

Author details
[1]Shoklo Malaria Research Unit (SMRU), Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University Mae Sot, Mae Sot, Thailand. [2]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand. [3]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7BN, UK.

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