



**HAL**  
open science

# Performance of ParaHIT® HRP2-Based Rapid Diagnostic Test and Proportions of Plasmodium falciparum Histidine-Rich Protein 2/3 Gene Deletions in Togo

Diwaba Carmel Teou, Ameyo Monique Dorkenoo, Essoham Ataba, Kossi Yakpa, Efoe Sossou, Laurence Ma, Emmanuelle Caspar, Manani Hemou, Aguerregna Abdou-Kerim, Didier Ménard

► **To cite this version:**

Diwaba Carmel Teou, Ameyo Monique Dorkenoo, Essoham Ataba, Kossi Yakpa, Efoe Sossou, et al.. Performance of ParaHIT® HRP2-Based Rapid Diagnostic Test and Proportions of Plasmodium falciparum Histidine-Rich Protein 2/3 Gene Deletions in Togo. American Journal of Tropical Medicine and Hygiene, 2024, 111 (5), pp.977-983. 10.4269/ajtmh.24-0197. hal-04738642

**HAL Id: hal-04738642**

**<https://hal.science/hal-04738642v1>**

Submitted on 24 Oct 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



24 <sup>8</sup> Institut National d'Hygiène, ministère de la Santé de l'Hygiène Publique et de l'Accès Universel  
25 Aux Soins, Quartier Administratif, 01BP 1396 Lomé, Togo ;

26 <sup>9</sup> Laboratory of Parasitology and Medical Mycology, Centre Hospitalier Universitaire Strasbourg,  
27 F-67000 Strasbourg, France.

28 <sup>10</sup> Malaria Parasite Biology and Vaccines Unit, Institut Pasteur, Université Paris Cité, F-75015  
29 Paris, France.

30

31 **\*Corresponding authors:**

32 Diwaba Carmel TEOU - E-mail: [dicarmelteou@gmail.com](mailto:dicarmelteou@gmail.com) 01BP1515, Lomé, Togo

33 Didier MENARD - E-mail: [dmenard@unistra.fr](mailto:dmenard@unistra.fr) / [dmenard@pasteur.fr](mailto:dmenard@pasteur.fr)

34

35 **Author e-mails:**

36 Diwaba Carmel TEOU: [dicarmelteou@gmail.com](mailto:dicarmelteou@gmail.com)

37 Ameyo Monique DORKENOO: [monicadork@yahoo.fr](mailto:monicadork@yahoo.fr)

38 Essoham ATABA: [fidelcab10@gmail.com](mailto:fidelcab10@gmail.com)

39 Kossi YAKPA: [yakpakossi@yahoo.fr](mailto:yakpakossi@yahoo.fr)

40 Efoe SOSSOU: [sossoustephane@yahoo.fr](mailto:sossoustephane@yahoo.fr)

41 Laurence MA: [laurence.ma@pasteur.fr](mailto:laurence.ma@pasteur.fr),

42 Emmanuelle CASPAR: [emmanuellecaspar@unistra.fr](mailto:emmanuellecaspar@unistra.fr)

43 Manani HEMOU: [mhemou@yahoo.fr](mailto:mhemou@yahoo.fr)

44 Agueregna ABDON-KERIM: [aagueregnasabitiou@yahoo.fr](mailto:aagueregnasabitiou@yahoo.fr)

45 Didier MENARD: [dmenard@unistra.fr](mailto:dmenard@unistra.fr) / [dmenard@pasteur.fr](mailto:dmenard@pasteur.fr)

46

47

## 48 **Abstract**

49 In areas where malaria is endemic, and microscopes are unavailable, rapid diagnostic  
50 tests (RDTs) are essential tools for early diagnosis and prompt and effective treatment.

51 However, HRP2-based RDTs are threatened by the emergence of *Plasmodium*  
52 *falciparum* parasites that do not carry the *pfhrp2* or *pfhrp3* genes, leading to false-  
53 negative results. Therefore, the aim of this study was to evaluate the performance of the  
54 ParaHIT® RDT together with the proportion of *pfhrp2/3* gene-deleted *P. falciparum*  
55 parasites in Togo.

56 The performance of RDT compared with microscopy and PCR was determined using  
57 capillary blood collected by finger prick during a cross-sectional study conducted from  
58 September 2021 to January 2022, in children aged 6–59 months at two sentinel sites.  
59 Blood spots were collected for molecular analysis. Amplicons from the target regions  
60 (exon 2 of *hrp2* and *hrp3* genes) were generated by multiplex nested PCR and  
61 sequenced using Illumina's MiSeq protocol.

62 A total of 278 samples were analyzed for ParaHIT® RDT evaluation. The sensitivity and  
63 specificity of the RDT test compared to microscopy were 96.4% and 85.7%, respectively,  
64 which increased to 97.9% and 90.7%, respectively, when compared to PCR. Of the  
65 microscopically and PCR-positive *P. falciparum* samples, 138 were sequenced to detect  
66 *pfhrp2/3* deletions. None of the parasites had a single *pfhrp2* deletion or a single *pfhrp3*  
67 deletion.

68 The ParaHIT® RDT demonstrated an acceptable level of performance in this evaluation,  
69 confirming the use of HRP2-based RDTs for the detection of *P. falciparum* infection in  
70 areas where microscopy is not available in Togo.

71 **Keywords:** RDT, malaria, *Plasmodium falciparum*, diagnostic performance, *pfhrp2*,  
72 *pfhrp3*, Togo.

73

74

## 75 **Background**

76 Over the past two decades, recent reports have shown a significant decline in the global  
77 burden of malaria. The incidence of malaria (*i.e.*, cases per 1000 population at risk)  
78 decreased from 81 in 2000 to 57 in 2019, before increasing to 60 in 2020[1]. However,  
79 the African Region of the World Health Organization (WHO) has a higher burden of  
80 malaria cases, accounting for 94% of global malaria cases (233 million) and 95% (580  
81 000) of malaria deaths in 2022. Children under five years of age are the most affected,  
82 accounting for about 80% of all malaria deaths in the region [2].

83 WHO recommends that all suspected cases of malaria should be confirmed by  
84 microscopy or rapid diagnostic tests (RDTs) before treatment. Although microscopy of  
85 Giemsa-stained thick/thin blood smears (TTBS) remains the reference method for  
86 malaria diagnosis [3], it requires a trained microscope technician, meticulous  
87 preparation, and a constant power supply to operate the equipment. These conditions  
88 are not always available in most remote malaria-endemic areas, limiting its use [4].

89 Therefore, RDTs have been proposed as an alternative for malaria diagnosis. These tests  
90 can be used by staff, including laypersons, but not necessarily by laboratory technicians  
91 after limited training [5]. Their use has increased significantly in recent years with an  
92 estimated 412 million RDTs distributed worldwide in 2018 [6]. The majority of these tests  
93 (64%) detected only *P. falciparum* and were supplied to sub-Saharan Africa, with HRP2  
94 being the predominant target. HRP2-based RDTs tend to be more sensitive and heat  
95 stable than RDTs that detect lactate dehydrogenase (LDH) or aldolase [7]. Although the  
96 antibodies on the test strip are designed to recognize the HRP2 antigen, they may also  
97 cross-react with another antigen in the HRP family, namely HRP3, due to strong  
98 similarities in the amino acid sequences. However, their performance is affected by a  
99 number of factors, including low parasite density (DP) infections, particularly below 100  
100 parasites/ $\mu$ L, and parasites with *pfhrp2* and *pfhrp3* gene deletions, which can lead to  
101 false-negative results in infected individuals. Indeed, these deletion cases have been  
102 reported in several West African countries, such as Mali, Senegal, Ghana, Nigeria, and  
103 Burkina Faso [8–12]. This situation could contribute to an increase in the risk of malaria-  
104 related morbidity and mortality and poses a challenge for individual patient management  
105 and for control and elimination efforts [13]. WHO has therefore recommended that  
106 countries conduct representative baseline surveys of suspected malaria cases to  
107 determine whether the prevalence of parasites with *pfhrp2/3* deletions has reached a  
108 threshold for RDT change (>5% *pfhrp2* deletions causing false-negative RDT results  
109 [14]). Since 2004, Togo has introduced RDTs into its diagnostic arsenal, initially in health  
110 facilities without microscopy and in the community by health workers, and more recently

111 in health facilities with microscopy because of their ease of use and rapid availability of  
112 results.

113 Several brands of RDTs have been used and evaluated with acceptable levels of  
114 performance according to the WHO criteria. In 2021, ARKRAY Healthcare's PARAHIT®  
115 RDT was introduced in Togo and made available to providers. Therefore, this study was  
116 conducted to evaluate the performance of this RDT compared to microscopy and PCR  
117 for the diagnosis of malaria in children aged 6–59 months seeking antimalarial treatment.  
118 Detection of *Plasmodium falciparum* parasites with deletions in the *pfhrp2/pfhrp3* gene  
119 was also carried out.

120

121

## 122 **Methods**

### 123 **Study design and sites**

124 This cross-sectional analytical study was conducted in Togo from September 14, 2021,  
125 to January 29, 2022. Togo is a West African country with stable malaria transmission and  
126 two predominant facies: sub-equatorial with two rainy seasons in the southern part of the  
127 country and the tropical with a single rainy season in the northern part. The evaluation  
128 used two sentinel sites to monitor the effectiveness of ACTs were used: The "la  
129 Providence" hospital in Kouvé, a religious institution in the Maritime region, about 76 km  
130 from the capital Lomé, and the Anié district hospital in the Plateaux region, 188 km from  
131 Lomé. The two sites were sub-equatorial and were located in rural and urban areas,  
132 respectively.

133 **Study population and sampling**

134 The target population was children aged 6–59 months, with an axillary temperature  $\geq$   
135 37.5°C, and/or a history of fever in the previous 24 hours, suspected of having malaria,  
136 consulted at one of the two participating sentinel sites, and prescribed TTBS.

137 The calculation methods of Buderer *et al.* (1996) [15] were used to define the size of our

138 sample. To estimate sensitivity and specificity, the formulae,  $Np = \frac{Z_{\alpha/2}^2 se(1-se)}{E^2}$  and

139  $Nn = \frac{Z_{\alpha/2}^2 sp*(1-sp)}{E^2}$  were used to determine the total number of positive cases to be

140 included and the number of negative cases in the control group. Thus, for an estimated

141 sensitivity and specificity of 90%, with a tolerated margin of error (E) of 5% and an

142 accepted risk of error ( $\alpha$ ) of 5% ( $Z_{\alpha/2}$  of 1.96), 139 blood smear-positive patients for *P.*

143 *falciparum* and an additional 139 blood smear-negative patients were required, for a total

144 sample size of 278 children.

145 The sensitivity of RDTs varies with parasite density [16,17]. When in good condition,

146 some products can achieve sensitivities similar to those typically obtained by field

147 microscopy (~100 parasites/ $\mu$ L) and may vary from product to product [18]. Thus,

148 malaria TTBS-positive patients were divided into two groups: a low parasite density

149 group (patients with asexual parasitemia counts per microliter between 50 and 1000)

150 and a high parasite density group (patients with asexual parasitemia per microliter

151 between 2000 and 10,000) [19–21]. The control group included patients who were

152 negative for all *Plasmodium* species.

153

154

155 **Inclusion and exclusion criteria**

156 Inclusion criteria were as follows: (a) a monospecific *P. falciparum* infection detected by  
157 microscopic examination; (b) inclusion of patients with 50 to 1000 asexual parasites per  
158 microliter in the low parasitemia group; 2000 to 10000 asexual parasites per microliter in  
159 the high parasitemia group; individuals with a negative blood smear in the control group  
160 and (c) signed written consent obtained from the parent/guardian of the children.

161 Children who did not meet the above criteria and whose parents/guardians voluntarily  
162 refused to participate were not included in the study.

163

164 **Data collection**

165 A structured questionnaire was used to collect socio-demographic characteristics of the  
166 enrolled children, clinical symptoms, medical history and presence of other diseases, if  
167 any.

168

169 **Laboratory tests**

170 Each patient had a capillary blood sample taken for TTBS. Once the microscopy results  
171 were known (parasitemia within a certain range or malaria negative), a second sample  
172 was taken from the enrolled children for RDT evaluation and dried blood spots (DBS) on  
173 Wattman type III paper for molecular analysis.

174

175 **Thick and thin blood smears**

176 Thick and thin blood smears were made on the same slide stained with 3% Giemsa for  
177 45 minutes after fixing the thin blood smear with methanol for a few seconds [22]. The  
178 slides were dried and examined under an immersion microscope at 100× magnification.  
179 Thick smears were used to determine positivity and for parasite density counting, and  
180 thin smears were examined to confirm the parasite species for positive samples. Parasite  
181 density was calculated as: number of parasites counted × 6000/number of leukocytes  
182 counted using the WHO method [22].

183

#### 184 **Rapid diagnostic test**

185 ParaHIT® f ver. 1.0 de ARKRAH Healthcare Pvt. Ltd., which is a WHO prequalified RDT  
186 for the diagnosis of *P. falciparum* malaria [21], is based on the principle of  
187 immunochromatography, in which a nitrocellulose membrane is coated with an anti-HRP  
188 II antibody (capture antibody) specific for *P. falciparum*. The test was performed in the  
189 laboratory by the study staff for each enrolled subject according to the manufacturer's  
190 instructions and the results were interpreted. Eight µl of capillary blood from the finger  
191 prick was immediately transferred with the micropipette, to the square window “A” of the  
192 test device, and then four drops of reaction buffer were transferred to the round window  
193 “B.” The results were read after 20 to 30 minutes.

194

#### 195 **Real-time PCR assays**

196 DBS was cut into small pieces and transferred to 2 mL microtubes. Genomic DNA was  
197 extracted using the QIAamp DNA Mini Blood Kit according to the manufacturer’s

198 protocol with some minor modifications [23]. Elution plates containing the extracted DNA  
199 were stored at - 80 °C until use. Samples were screened for the presence of  
200 *Plasmodium* DNA using a qualitative real-time PCR assay targeting the *cytochrome b*  
201 gene. DNA samples identified as positive for *Plasmodium* were then diluted 1:10 and  
202 analyzed using a nested real-time PCR assay with primers targeting the same gene  
203 fragment and specific for *P. falciparum*. All real-time PCR assays were performed using  
204 SYBR green ready-to-use PCR mix (Solis Biodyne, Tartu, Estonia) on a 5 µL DNA  
205 template on a BioRad CFX96 real-time PCR system (BioRad) [24]. Details of the PCR  
206 program, mix composition, and primer sequences were previously described by Canier  
207 *et al.* [25].

208

### 209 **Detection of pfhrp2/3 gene deletions**

210 TTBS- and PCR-positive samples for *P. falciparum* were selected for testing in the  
211 absence of the *phrp2* and *phrp3* genes [8]. Amplicons from the targeted regions (exon 2  
212 of the *hrp2* and *hrp3* genes) were generated using multiplexed nested PCR assays with  
213 indexed primers containing specific tags (barcodes) [26,27]. A total of 4 µl of PCR  
214 reactions from each sample were pooled (96 samples) to increase the sample volume  
215 and minimize sample consumption for downstream protocol steps. For each pool,  
216 amplicons were purified using AMPure XP beads (Beckman Coulter) according to the  
217 manufacturer's protocol to remove dNTPs, salts, primers, and primer dimers. The quality  
218 of the purified PCR products was assessed by analysing the eluates containing the  
219 purified amplicons on a Bioanalyzer Agilent 2100 (Agilent). The concentrations of the

220 pooled fragments were assessed by fluorometric quantification (Qubit, Thermo Fisher).  
221 The pooled libraries were denatured with NaOH to a final concentration of 0.1 N and  
222 diluted with hybridization buffer prior to sequencing. Sequencing was performed using  
223 the MiSeq v2 reagent and a 300-cycle kit (Illumina) according to the manufacturer's  
224 recommendations. Raw sequences were demultiplexed and quality trimmed to a Phred  
225 score of 30. Primer sequences were trimmed from the 5'-end of the sequences to avoid  
226 primer bias in the sequenced fragments. Base calling was performed by comparing the  
227 reads to a custom database consisting of the 3D7 reference sequence (PlasmoDB v45).  
228 Bioinformatic analyses were performed using the CLC Genomics Workbench 24  
229 software (Qiagen). To detect a deletion of the hrp2 or hrp3 genes, we compared the  
230 coverage of each target gene with the coverage of the amplicons generated for hrp2 and  
231 hrp3. If the coverage of the hrp2 or hrp3 gene was zero or <1% relative to the coverage  
232 of the other target genes, we considered the gene to be deleted.  
233 For each plate, we tested DNA from laboratory reference parasite strains for which the  
234 presence or absence of hrp2 and hrp3 deletions was known, as controls: a Cambodian  
235 strain 3601 and 7G8 without deletions, Dd2 with an hrp2 deletion and HB3 with an hrp3  
236 deletion).

237

### 238 **Quality control**

239 Duplicate readings were performed on each slide by two experienced microscopists  
240 [28], one of whom is WHO level 2 accredited for malaria diagnosis, and the other is from  
241 Togo National Reference Laboratory (Institut National d'Hygiène). If the coefficient of

242 variation of the estimated parasitemia was > 5% [29], a third reading was performed by  
243 an independent microscopist [22]. If there was a difference between the study site  
244 parasitemia and the results found by quality control, parasitemia of the quality control  
245 was considered. The estimated parasitemia was used to create the three groups.  
246 To ensure the reliability of the RDT results, the kit instructions were followed. The control  
247 band should appear regardless of whether the sample is reactive or non-reactive.  
248 Visualization of the control band indicated the successful migration of the  
249 reaction mixture.  
250 For the real-time PCR screening runs, a set of four controls (two DNA positive controls: a  
251 high positive control, Pf 3D7 DNA extract at 0.1 ng/μl and a low positive control, Pf 3D7  
252 DNA extract at 0.001 ng/μl and two negatives: water) were added to the 96-well plate  
253 before performing the real-time PCR run. Laboratory reference parasite strains (Dd2,  
254 7G8, HB3, and the 3601 strain, a Cambodian culture-adapted parasite) with known  
255 alleles of each gene were used as controls for the analysis of *pfhrp2* and *pfhrp3* gene  
256 deletions [27].  
257 Training of field team members and supervisors was conducted to standardize working  
258 methods and ensure the smooth running of the activity, especially for completing  
259 questionnaires and conducting RDT, TTBS, and filter paper sampling.

260

## 261 **Endpoints**

262 Thick/thin blood smear and PCR results were used as reference methods for this  
263 evaluation against which RDT results were compared. Sensitivity, specificity, positive

264 predictive value (PPV), and negative predictive value (NPV) are the estimated  
265 performance indicators of RDT. Sensitivity and PPV were calculated for low and high  
266 parasitemia, respectively.

267

### 268 **Data management and analysis**

269 Data were recorded on registration forms, entered into a Microsoft Excel database  
270 (Microsoft Corp, Redmond, Washington, USA), and analyzed using MedCalc® version  
271 20.218 (MedCalc Software Ltd, Ostende, Belgium; <https://www.medcalc.org> ; 2023). The  
272 sensitivity, specificity, and positive and negative predictive values of the RDTs were  
273 determined by microscopy or PCR using 2 × 2 contingency tables. Exact 95%  
274 confidence intervals (95% CI) were calculated for each of the above measures.

275 Bioinformatic analyses were performed using CLC Genomics Workbench 22 software  
276 (Qiagen).

277

### 278 **Ethical considerations**

279 The study protocol was approved by the Bioethics Committee for Health Research  
280 (CBRS) of Togo (N°021/2021/CBRS of May 27, 2021) before its implementation. In  
281 addition, signed informed consent was obtained from the parents or guardians of the  
282 children. Any patient testing positive by at least one of the methods was referred to the  
283 site clinicians for free treatment with an antimalarial drug available through the National  
284 Malaria Control Program.

285

286

## 287 **Results**

### 288 **Characteristics of the study participants**

289 Of the 369 children screened at the 2 sites, 287 were included in the study, for an  
290 inclusion rate of 77.8%. Nine patients were excluded for quality control (parasitemia  
291 outside the accepted range). The final number of patients for analysis was 278 (**Figure**  
292 **1**). The mean age of the included patients was 31 ( $\pm 1$ ) months, and the sex ratio (M/F)  
293 was 0.9. Children with a body temperature  $\geq 37.5$  °C represented 79.5%, and the others  
294 reported having had a febrile episode in the previous 24 hours. The socio-demographic  
295 characteristics of the children are summarized in **Table 1**.

296 The evaluation of the ParaHIT® RDT compared to microscopy included 278 patients, of  
297 which 140 were in the control group, 41 in the low parasitemia group, and 97 in the high  
298 parasitemia group (**Table 2**).

299

### 300 **Performance of RDT evaluated compared to microscopy**

301 The sensitivity and specificity of the ParaHIT® RDT compared with TTBS for the  
302 detection of *P. falciparum* were 96.4% [CI = 91.7-98.8] and 85.7% [CI = 78.8-91.1],  
303 respectively; the PPV and NPV values were 86.9% [CI = 81.6-90.9] and 96.0% [CI = 91.0-  
304 98.3], respectively (**Table 3**).

### 305 **Performance of evaluated RDTs compared to PCR**

306 For the PCR analysis, plasmodial DNA was not successfully amplified in five samples  
307 due to the poor quality of the DBS, leading to DNA degradation; these were microscopy-  
308 negative samples. Therefore, the estimation of the performance of the evaluated RDT

309 compared with PCR was estimated for a total of 273 patients divided into 129 for the  
310 PCR-negative group and 144 for the *P. falciparum* PCR-positive group (**Table 2**). The  
311 sensitivity of the ParaHIT® RDT compared to PCR for the detection of *P. falciparum*  
312 infection was 97.9% [CI = 94.0-99.6], and the specificity was 90.7% [CI = 84.3-95.1]. The  
313 PPV and NPV values were 92.2% [CI = 87.3-95.3] and 97.5% [CI = 92.7-99.2],  
314 respectively (**Table 3**).

315

### 316 **Detection of *pfhrp2/3* gene deletions**

317 All 138 microscopy-positive samples were positive by *P. falciparum* species-specific  
318 PCR and were analyzed for *pfhrp2* and *pfhrp3* deletions (**Figure 1**). The extracted DNA  
319 isolates were successfully sequenced to detect *phrp2* and *phrp3* deletions. None of the  
320 parasites had a single *pfhrp2* deletion or a single *pfhrp3* deletion.

321

### 322 **Relationship between RDT results and *pfhrp2/3* gene deletions**

323 Five samples were microscopy-positive and RDT-negative, and none showed a deletion  
324 of *pfhrp2/3*. Of these, three had parasite densities of 70, 329, and 781 parasites/ $\mu$ l,  
325 respectively, and would likely have been missed by the RDT due to low parasitemia. The  
326 other samples had high parasite densities (2,176 trophozoites/ $\mu$ l and 2,187  
327 trophozoites/ $\mu$ l).

328

329

330

## 331 **Discussion**

332 RDTs have been developed for their ease of use in the early diagnosis of malaria,  
333 especially in endemic countries where microscopy is not available [30]. Although the  
334 WHO and the Foundation for Innovative New Diagnostics (FIND) conduct detailed  
335 performance evaluations of these tests, it is important that field evaluations are carried  
336 out in endemic areas. For example, the WHO requires all RDTs to have a minimum  
337 sensitivity of 95% compared to microscopy and a minimum specificity of 90% for all  
338 malaria species [31]. Therefore, this study evaluated the diagnostic performance of the  
339 PARAHIT® ver. 1.0 RDT used in health facilities in Togo and the prevalence of associated  
340 *pfhrp2/pfhrp3* gene deletions in samples.

341 The results showed a sensitivity of 96.4% and a specificity of 85.7% for the PARAHIT®  
342 RDT, using microscopy as the reference method. These performance levels are similar  
343 to those found in Uganda, where an evaluation of the same RDT in field use found a  
344 sensitivity of 97.4% and a specificity of 88.1% in field use [32]. These values are slightly  
345 lower than those reported in the WHO prequalification report for this RDT (sensitivity :  
346 99.42%, specificity : 99.59%) [33]. The difference observed between our results and  
347 those of the WHO prequalification report may be explained by the difference in the  
348 samples used to test the RDT (a series of *Plasmodium falciparum* wild-type parasites and  
349 negative panels in the WHO prequalification study and field isolates in our study). In our  
350 evaluation, sensitivity was proportional to parasitemia, reaching 97.9% when parasitemia  
351 was up to 2000 parasites/ $\mu$ L, whereas sensitivity was only 92.7% when parasitemia was  
352 less than 1000 parasites/ $\mu$ L. Our results corroborate those of an evaluation of the

353 CareStart™ RDT in an area of unstable malaria, where the highest sensitivity (95.8%)  
354 was found for parasite densities > 5000 parasites/μL [17,34]. However, another study  
355 conducted in a hyperendemic area of *P. falciparum* malaria showed no change in  
356 sensitivity with decreasing parasitemia [5]. The specificity observed in our evaluation  
357 (85.7%) was slightly lower than that recommended by the WHO (90%). These false  
358 positives could be due to the persistence of the HRP2 antigen after treatment. Indeed,  
359 Grandesso *et al.* [35] showed that the median time to become negative for HRP2-based  
360 tests was 35–42 days. Another study supporting this finding showed that this persistence  
361 can be up to 52 days [36]. It has also been suggested that rheumatoid factor may cause  
362 false positives due to binding to IgG [37]. Although the use of IgM is thought to reduce  
363 the problem of rheumatoid factor cross-reactions [15], it is not known which type of  
364 antibody is used to coat the PARAHIT® RDT [31].

365 Regarding the predictive accuracy of the RDT used for malaria diagnosis in the current  
366 study, the PPV was 86.9%, while the NPV was relatively high at 96.0%. Bouah-Kamon *et*  
367 *al.* in their study in 2018 showed a similar PPV (87.1%) for "SD Bioline Malaria Antigen Pf  
368 ® (HRP2)" [38]. However, it should be noted that PPVs are a function of disease  
369 prevalence. Indeed, a study conducted in Burkina Faso found that the PPV and NPV of  
370 RDT in the dry season were 9% and 99.8%, respectively, compared with 82% and 84%  
371 in the rainy season for infants and over 99% for adults [39].

372 When PCR was used as the reference method, higher performance levels were  
373 observed for the PARAHIT® RDT. The sensitivity and specificity of the test were 97.9%  
374 (96.4% compared to microscopy) and 90.7% (85.7% compared to microscopy),

375 respectively. The same result was reported by Diallo *et al.* in Senegal (sensitivity: 97.3%  
376 and specificity: 94.1) [40]. In our study, some RDT-positive but microscopy negative  
377 results could indicate submicroscopic infections. Indeed, PCR can detect parasites at  
378 levels as low as 0.002 P/ $\mu$ L, thus detecting submicroscopic infections in the population  
379 [17].

380 This study also provides data on parasites with *pfhrp2/3* gene deletions. None of the  
381 isolates had a single *pfhrp2* deletion or a single *pfhrp3* deletion. The proportion of false-  
382 negative RDTs compared with microscopy (3.6%) found in our evaluation was probably  
383 not related to the *pfhrp2/3* deletion, but rather to their low parasitemia, below the  
384 detection threshold of the HRP2-based RDT. Other factors, such as the lot-to-lot  
385 variability of the RDT used, operating error or potential epitope variations that could  
386 account for these unexpected false negatives in RDT result. [41–44]. In the Democratic  
387 Republic of Congo, a study of asymptomatic children did not find parasites with a  
388 *pfhrp2/3* deletion [45]. In West Africa, several studies have shown the presence of this  
389 deletion: Funwei *et al.* in Nigeria (10.6% *pfhrp2* deletion, 6.0% *pfhrp3* deletion, and 6.1%  
390 combined *pfhrp2/3* deletion) [10] and Tarama *et al.* in Burkina Faso (4.4% *pfhrp2*  
391 deletion) [8]. The variability in prevalence suggests that geographical and  
392 methodological differences influence the diversity of *pfhrp2* prevalence in malaria-  
393 endemic regions. In Ghana, Amoah *et al.* reported *pfhrp2* gene deletions in 33.3% and  
394 36.2% of microscopically confirmed and PCR-confirmed RDT-positive samples,  
395 respectively, in Accra [11], whereas Addai-Mensah *et al.* found no deletions in the Volta  
396 region [46].

397 Our study had several limitations. The sample size was smaller than that recommended  
398 by the WHO in the surveillance template protocol for *pfhrp2/pfhrp3* gene deletions,  
399 which is 370 confirmed cases of *P. falciparum* malaria per region, although the sample  
400 size may vary depending on logistical and budgetary constraints [7]. Nevertheless, it is  
401 worth noting that the target population is consistent, i.e., individuals with fever seeking  
402 care at health care facilities. Indeed, our study included febrile children with suspected  
403 malaria who presented to health facilities. Therefore, another study with a larger sample  
404 size would better estimate the proportion of *pfhrp2/pfhrp3* gene deletions in Togo.

405

406

## 407 **Conclusion**

408 The PARAHIT<sup>®</sup> RDT showed acceptable levels of performance in this evaluation  
409 compared to TTBS as the reference method. Higher levels of test performance were  
410 observed when PCR was used as the reference method due to its higher sensitivity than  
411 microscopy. In addition, none of the isolates showed a deletion of the *pfhrp2/3* gene in  
412 the samples evaluated. The results of this study support the use of HRP2-based RDTs to  
413 confirm *P. falciparum* infections in Togo.

414

## 415 **Authors' contributions**

416 DCT, AMD, EA, and KY conceived of and designed the study. AMD led to training and  
417 oversaw its implementation in Togo. AMD, EA, KY, ES, and MH supervised the data  
418 collection. DCT, AMD, EA, and DM drafted the analysis plan; provided data analysis and

419 interpretation; and wrote the first draft of the manuscript. All authors have read, revised,  
420 and approved the final manuscript.

421

## 422 **Acknowledgments**

423 This study received support from the Laboratory Division and National Malaria Control  
424 Program of Togo, University of Lomé, and the University of Strasbourg. The study was  
425 made possible by the Global Fund to Fight HIV/AIDS, TB, and Malaria (GFATM) for  
426 financial support at the country level and the Bill and Melinda Gates Foundation for their  
427 financial support through the World Health Organization in Geneva. The authors thank  
428 the regional and prefectural health directors of the two study sites, the research team,  
429 and members of the local teams at the sites for their involvement at all levels. The  
430 authors also thank the study participants and their parents and guardians for their  
431 participation in the study.

432

## 433 **Competing interests**

434 The authors declare that they have no conflict of interest.

435

## 436 **Availability of data and materials**

437 All the data used to draw the conclusions of the study are provided in the manuscript.

438

## 439 **Consent for publication**

440 Not applicable.

## 441 **Funding**

442 The therapeutic efficacy trial in which our study was nested was funded by the Global  
443 Fund, while the molecular biology was supported by the University of Strasbourg and  
444 France Génomique (ANR-10-INBS-09) and IBISA (Platform, C2RT, Institut Pasteur, Paris,  
445 France). The RDTs evaluated were supplied by the central purchase of the essential and  
446 generic medicines of Togo.

447

448

## 449 **References**

- 450 1. WHO. World malaria report 2023 [Internet]. 2023 [cited 2024 May 9]. Available from:  
451 <https://www.who.int/publications-detail-redirect/9789240086173>
- 452 2. OMS. Données et tendances régionales : rapport 2022 sur le paludisme dans le monde  
453 [Internet]. 2022 [cited 2023 Aug 16]. Available from:  
454 <https://www.who.int/fr/publications/m/item/WHO-UCN-GMP-2022.08>
- 455 3. WHO. Malaria microscopy quality assurance manual, version II [Internet]. 2016 [cited 2023 Aug  
456 16]. Available from:  
457 [https://scholar.google.com/scholar\\_lookup?title=Malaria%20microscopy%20quality%20assurance%20manual%2C%20version%20II&publication\\_year=2016](https://scholar.google.com/scholar_lookup?title=Malaria%20microscopy%20quality%20assurance%20manual%2C%20version%20II&publication_year=2016)  
458
- 459 4. Moyeh MN, Ali IM, Njimoh DL, Nji AM, Netongo PM, Evehe MS, et al. Comparison of the  
460 accuracy of four malaria diagnostic methods in a high transmission setting in coastal  
461 Cameroon. *J Parasitol Res.* 2019;2019.
- 462 5. Gerstl S, Dunkley S, Mukhtar A, De Smet M, Baker S, Maikere J. Assessment of two malaria  
463 rapid diagnostic tests in children under five years of age, with follow-up of false-positive  
464 pLDH test results, in a hyperendemic falciparum malaria area, Sierra Leone. *Malar J.*  
465 2010;9:1–10.
- 466 6. WHO. World malaria report 2019 [Internet]. 2019 [cited 2023 Aug 17]. Available from:  
467 <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2019>
- 468 7. WHO. Template protocols to support surveillance and research for pfhrp2/pfhrp3 gene  
469 deletions [Internet]. 2020 [cited 2024 Jan 21]. Available from:  
470 <https://www.who.int/publications-detail-redirect/9789240002036>

- 471 8. Tarama CW, Soré H, Siribié M, Débé S, Kinda R, Nonkani WG, et al. Assessing the histidine-  
472 rich protein 2/3 gene deletion in Plasmodium falciparum isolates from Burkina Faso. *Malar*  
473 *J.* 2023;22:363.
- 474 9. Koita OA, Doumbo OK, Ouattara A, Tall LK, Konaré A, Diakité M, et al. False-Negative Rapid  
475 Diagnostic Tests for Malaria and Deletion of the Histidine-Rich Repeat Region of the hrp2  
476 Gene. *Am J Trop Med Hyg.* 2012;86:194–8.
- 477 10. Funwei R, Nderu D, Nguetse CN, Thomas BN, Falade CO, Velavan TP, et al. Molecular  
478 surveillance of pfhrrp2 and pfhrrp3 genes deletion in Plasmodium falciparum isolates and the  
479 implications for rapid diagnostic tests in Nigeria. *Acta Trop.* 2019;196:121–5.
- 480 11. Amoah LE, Abankwa J, Oppong A. Plasmodium falciparum histidine rich protein-2 diversity  
481 and the implications for PfHRP 2: based malaria rapid diagnostic tests in Ghana. *Malar J.*  
482 2016;15:101.
- 483 12. Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C, et al. Pfhrrp2 and pfhrrp3 polymorphisms  
484 in Plasmodium falciparum isolates from Dakar, Senegal: impact on rapid malaria diagnostic  
485 tests. *Malar J.* 2013;12:34.
- 486 13. Kreidenweiss A, Trauner F, Rodi M, Koehne E, Held J, Wyndorps L, et al. Monitoring the  
487 threatened utility of malaria rapid diagnostic tests by novel high-throughput detection of  
488 Plasmodium falciparum hrp2 and hrp3 deletions: A cross-sectional, diagnostic accuracy  
489 study. *EBioMedicine.* 2019;50:14–22.
- 490 14. WHO. World malaria report 2020 [Internet]. 2020 [cited 2024 Jan 21]. Available from:  
491 <https://www.who.int/publications-detail-redirect/9789240015791>
- 492 15. Buderer NMF. Statistical methodology: I. Incorporating the prevalence of disease into the  
493 sample size calculation for sensitivity and specificity. *Acad Emerg Med.* 1996;3:895–900.
- 494 16. Dorkenoo D, Kouassi KC, Koura AK, Adams ML, Gbada K, Katawa G, et al. The use of dried  
495 tube specimens of Plasmodium falciparum in an External Quality Assessment program to  
496 evaluate health worker performance for malaria rapid diagnostic testing in healthcare  
497 centers in Togo. 2020;
- 498 17. Wanja EW, Kuya N, Moranga C, Hickman M, Johnson JD, Mosei C, et al. Field evaluation of  
499 diagnostic performance of malaria rapid diagnostic tests in western Kenya. *Malar J.*  
500 2016;15:1–10.
- 501 18. Bell D, Organization WH. L'utilisation des tests diagnostiques rapides du paludisme. 2004;
- 502 19. Fransisca L, Kusnanto JH, Satoto TBT, Sebayang B, Supriyanto , Andriyan E, et al.  
503 Comparison of rapid diagnostic test Plasmotec Malaria-3, microscopy, and quantitative real-  
504 time PCR for diagnoses of Plasmodium falciparum and Plasmodium vivax infections in  
505 Mimika Regency, Papua, Indonesia. *Malar J.* 2015;14:1–11.
- 506 20. Madkhali AM, Ghzwani AH, Al-Mekhlafi HM. Comparison of Rapid Diagnostic Test,  
507 Microscopy, and Polymerase Chain Reaction for the Detection of Plasmodium falciparum

- 508 Malaria in a Low-Transmission Area, Jazan Region, Southwestern Saudi Arabia.  
509 Diagnostics. 2022;12:1485.
- 510 21. OMS. Performance des tests de diagnostic rapide du paludisme: bilan des résultats  
511 d'évaluation des produits par l'OMS: séries 1–7 (2008-2016). 2018;
- 512 22. OMS. Méthodes de surveillance de l'efficacité des médicaments antipaludiques. Genève :  
513 OMS ; 2009. [Internet]. 2009 [cited 2023 Dec 26]. Available from:  
514 [https://www.google.com/search?q=OMS.+M%C3%A9thodes+de+surveillance+de+l%E2%80%99efficacit%C3%A9+des+m%C3%A9dicaments+antipaludiques.+Gen%C3%A8ve+%3A+OMS+%3B+2009.&rlz=1C1GCEA\\_enCA1088CA1088&oq=OMS.+M%C3%A9thodes+de+surveillance+de+l%E2%80%99efficacit%C3%A9+des+m%C3%A9dicaments+antipaludiques.+Gen%C3%A8ve+%3A+OMS+%3B+2009.&gs\\_lcrp=EgZjaHJvbWUyBggAEEUYOTIGCAEQRRg80gEIMTA3N2owajeoAgCwAgA&sourceid=chrome&ie=UTF-8](https://www.google.com/search?q=OMS.+M%C3%A9thodes+de+surveillance+de+l%E2%80%99efficacit%C3%A9+des+m%C3%A9dicaments+antipaludiques.+Gen%C3%A8ve+%3A+OMS+%3B+2009.&rlz=1C1GCEA_enCA1088CA1088&oq=OMS.+M%C3%A9thodes+de+surveillance+de+l%E2%80%99efficacit%C3%A9+des+m%C3%A9dicaments+antipaludiques.+Gen%C3%A8ve+%3A+OMS+%3B+2009.&gs_lcrp=EgZjaHJvbWUyBggAEEUYOTIGCAEQRRg80gEIMTA3N2owajeoAgCwAgA&sourceid=chrome&ie=UTF-8)  
515  
516  
517  
518  
519
- 520 23. Abate A, Bouyssou I, Mabilotte S, Doderer-Lang C, Dembele L, Menard D, et al. Vivax malaria  
521 in Duffy-negative patients shows invariably low asexual parasitaemia: implication towards  
522 malaria control in Ethiopia. *Malar J.* 2022;21:230.
- 523 24. Canier L, Khim N, Kim S, Eam R, Khean C, Loch K, et al. Malaria PCR detection in  
524 Cambodian low-transmission settings: dried blood spots versus venous blood samples. *Am*  
525 *J Trop Med Hyg.* 2015;92:573.
- 526 25. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, et al. An innovative tool for moving  
527 malaria PCR detection of parasite reservoir into the field. *Malar J.* 2013;12:1–12.
- 528 26. Koko VS, Warsame M, Vonhm B, Jeuronlon MK, Menard D, Ma L, et al. Artesunate–  
529 amodiaquine and artemether–lumefantrine for the treatment of uncomplicated falciparum  
530 malaria in Liberia: in vivo efficacy and frequency of molecular markers. *Malar J.* 2022;21:1–  
531 15.
- 532 27. Mihreteab S, Platon L, Berhane A, Stokes BH, Warsame M, Campagne P, et al. Increasing  
533 Prevalence of Artemisinin-Resistant HRP2-Negative Malaria in Eritrea. *N Engl J Med.*  
534 2023;389:1191–202.
- 535 28. OMS O. Diagnostic microscopique du paludisme: manuel d'assurance qualité [Internet].  
536 Organisation mondiale de la Santé; 2017 [cited 2023 Oct 22]. Available from:  
537 <https://apps.who.int/iris/bitstream/handle/10665/258702/9789242549393-fre.pdf>
- 538 29. Dorkenoo AM, Layibo Y, Agbo YM, Morgah K, Agbèrè D. Numération leucocytaire et densité  
539 parasitaire dans le paludisme simple chez les enfants âgés de 6 mois à 5 ans en milieu  
540 urbain au Togo. *Médecine Santé Trop.* 2013;23:412–6.
- 541 30. Abba K, Deeks JJ, Olliaro PL, Naing C, Jackson SM, Takwoingi Y, et al. Rapid diagnostic tests  
542 for diagnosing uncomplicated *P. falciparum* malaria in endemic countries. *Cochrane*  
543 *Database Syst Rev.* 2011;2011:CD008122.
- 544 31. Mouatcho JC, Goldring JD. Malaria rapid diagnostic tests: challenges and prospects. *J Med*  
545 *Microbiol.* 2013;62:1491–505.

- 546 32. Guthmann JP, Ruiz A, Priotto G, Kiguli J, Bonte L, Legros D. Validity, reliability and ease of use  
547 in the field of five rapid tests for the diagnosis of Plasmodium falciparum malaria in Uganda.  
548 Trans R Soc Trop Med Hyg. 2002;96:254–7.
- 549 33. WHO. Public Report for ParaHIT f Ver. 1.0 Rapid Test for P. falciparum Malaria Device, (PQDx  
550 0062-023-00) | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and  
551 Immunization Devices, Vector Control) [Internet]. 2016 [cited 2024 Jan 25]. Available from:  
552 [https://extranet.who.int/prequal/WHOPR/public-report-parahit-f-ver-10-rapid-test-p-](https://extranet.who.int/prequal/WHOPR/public-report-parahit-f-ver-10-rapid-test-p-falciparum-malaria-device-pqdx-0062-023-00)  
553 [falciparum-malaria-device-pqdx-0062-023-00](https://extranet.who.int/prequal/WHOPR/public-report-parahit-f-ver-10-rapid-test-p-falciparum-malaria-device-pqdx-0062-023-00)
- 554 34. Ashton RA, Kefyalew T, Tesfaye G, Counihan H, Yadeta D, Cundill B, et al. Performance of  
555 three multi-species rapid diagnostic tests for diagnosis of Plasmodium falciparum and  
556 Plasmodium vivax malaria in Oromia Regional State, Ethiopia. Malar J. 2010;9:297.
- 557 35. Grandesso F, Guindo O, Woi Messe L, Makarimi R, Traore A, Dama S, et al. Efficacy of  
558 artesunate–amodiaquine, dihydroartemisinin–piperazine and artemether–lumefantrine for  
559 the treatment of uncomplicated Plasmodium falciparum malaria in Maradi, Niger. Malar J.  
560 2018;17:1–9.
- 561 36. Markwalter CF, Gibson LE, Mudenda L, Kimmel DW, Mbambara S, Thuma PE, et al.  
562 Characterization of Plasmodium lactate dehydrogenase and histidine-rich protein 2  
563 clearance patterns via rapid on-bead detection from a single dried blood spot. Am J Trop  
564 Med Hyg. 2018;98:1389.
- 565 37. Stauffer WM, Cartwright CP, Olson DA, Juni BA, Taylor CM, Bowers SH, et al. Diagnostic  
566 performance of rapid diagnostic tests versus blood smears for malaria in US clinical  
567 practice. Clin Infect Dis Off Publ Infect Dis Soc Am. 2009;49:908–13.
- 568 38. Bouah-Kamon E, Niamien-Attai C, Konaté A, Adonis-Koffy L. Evaluation du test «SD Bioline  
569 Malaria Antigen pf®(HRP2)» dans le diagnostic du paludisme à Plasmodium falciparum de  
570 l'enfant au CHU de Yopougon (Côte d'Ivoire)[Evaluation of “SD Bioline Malaria Antigen  
571 pf®(HRP2)” test in Plasmodium falciparum malaria diagnosis in child at the Yopougon  
572 teaching hospital (Côte d'Ivoire)]. Bull Soc Pathol Exot. 2018;111:289–94.
- 573 39. Bisoffi Z, Sirima SB, Menten J, Pattaro C, Angheben A, Gobbi F, et al. Accuracy of a rapid  
574 diagnostic test on the diagnosis of malaria infection and of malaria - attributable fever during  
575 low and high transmission season in Burkina Faso. Malar J. 2010;9:192.
- 576 40. Diallo MA, Diongue K, Ndiaye M, Gaye A, Deme A, Badiane AS, et al. Evaluation of  
577 CareStart™ Malaria HRP2/pLDH (Pf/pan) Combo Test in a malaria low transmission region  
578 of Senegal. Malar J. 2017;16:328.
- 579 41. Orish VN, De-Gaulle VF, Sanyaolu AO. Interpreting rapid diagnostic test (RDT) for  
580 Plasmodium falciparum. BMC Res Notes. 2018;11:1–6.
- 581 42. Watson OJ, Sumner KM, Janko M, Goel V, Winskill P, Slater HC, et al. False-negative malaria  
582 rapid diagnostic test results and their impact on community-based malaria surveys in sub-  
583 Saharan Africa. BMJ Glob Health. 2019;4:e001582.

- 584 43. Wu L, van den Hoogen LL, Slater H, Walker PG, Ghani AC, Drakeley CJ, et al. Comparison of  
585 diagnostics for the detection of asymptomatic Plasmodium falciparum infections to inform  
586 control and elimination strategies. *Nature*. 2015;528:S86–93.
- 587 44. Pasquier G, Azoury V, Sasso M, Laroche L, Varlet-Marie E, Houzé S, et al. Rapid diagnostic  
588 tests failing to detect infections by Plasmodium falciparum encoding pfhrp2 and pfhrp3  
589 genes in a non-endemic setting. *Malar J*. 2020;19:179.
- 590 45. Parr JB, Kieto E, Phanzu F, Mansiangi P, Mwandagalirwa K, Mvuama N, et al. Analysis of false-  
591 negative rapid diagnostic tests for symptomatic malaria in the Democratic Republic of the  
592 Congo. *Sci Rep*. 2021;11:6495.
- 593 46. Addai-Mensah O, Dinko B, Noagbe M, Ameke SL, Annani-Akollor ME, Owiredu E-W, et al.  
594 Plasmodium falciparum histidine-rich protein 2 diversity in Ghana. *Malar J*. 2020;19:256.

595

596

597

598

599

600

601

602

603

604

605

606

607

608

609 **Figure legend**

610 **Figure 1:** Flow diagram for the selecting samples

611

612

613 **Tables**

614

615 **Table 1:** Patients' sociodemographic characteristics and clinical signs

---

Baseline characteristics (N = 278)	
Sex, n (%)	
Female	141 (50.7)
Male	137 (49.3)
Age in months, n (%)	
6-59	278 (100)
Median temperature, °C (range)	37.8 (36-40.3)
Residence, n (%)	
Rural	100 (36.0)
Urban	178 (64.0)

---

616

617

618

619

620

621

622

623

624 **Table 2:** Total number of samples included by defined site

<b>Sites</b>	<b>Anié, n (%)</b>	<b>Kouvé, n (%)</b>
<b>Microscopy (TTBS)</b>		
Negative (n = 140)	75 (27.0)	65 (23.4)
Positive		
<i>Low parasitemia (n = 41)</i>	26 (09.3)	15 (05.4)
<i>High parasitemia (n = 97)</i>	77 (27.7)	20 (07.2)
Total (n = 278)	178 (64.0)	100 (36.0)
<b>PCR</b>		
Negative (n = 129)	66 (24.2)	63 (23.1)
Positive (n = 144)	110 (40.3)	34 (12.4)
Total (n = 273)	176 (64.5)	97 (35.5)

625

626 **Table 3:** ParaHIT® performance compared to TTBS and PCR for detecting *P. falciparum*.

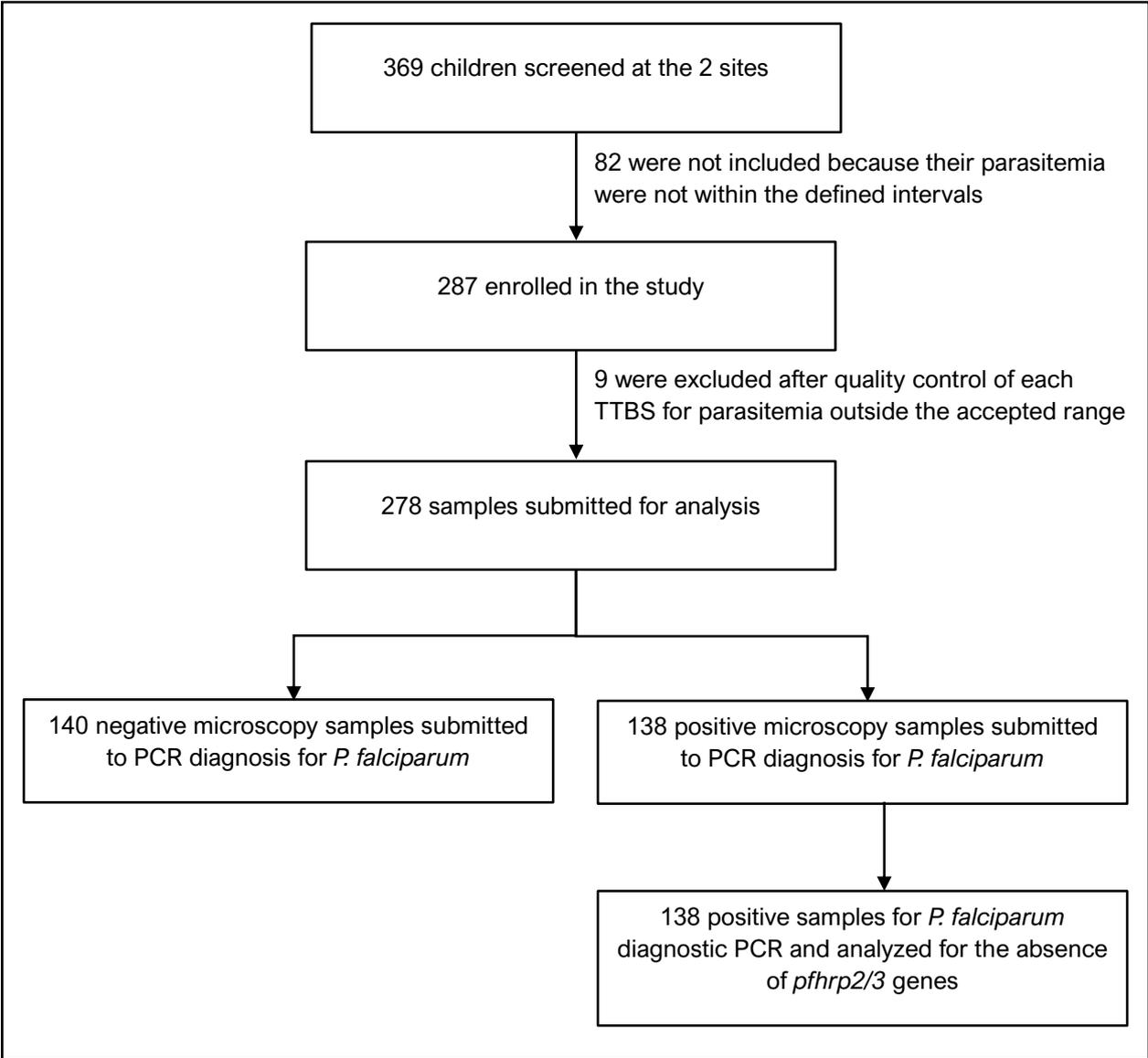
	Positive RDT	Negative RDT	*SE% [95% CI]	*SP% [95% CI]	*PPV% [95% CI]	*NPV% [95% CI]
<b>Microscopy</b>						
<b>Low parasitemia</b>						
Positive (n=41)	38	3				
Negative (n=140)	20	120	92.7 [80.1-98.5]	-	65.5 [55.7-74.2]	-
<b>High parasitemia</b>						
Positive (n=97)	95	2				
Negative (n=140)	20	120	97.9 [92.8-99.7]	-	82.6 [76.0-87.7]	-
<b>Total</b>						
Positive (n=138)	133	5				
Negative (n=140)	20	120	96.4 [91.7-98.8]	85.7 [78.8-91.1]	86.9 [81.6-90.9]	96.0 [91.0-98.3]
<b>PCR</b>						
Positive (144)	141	3				
Negative (129)	12	117	97.9 [94.0-99.6]	90.7 [84.3-95.1]	92.2 [87.3-95.3]	97.5 [92.7-99.2]

627 \*: SE=sensitivity; SP=specificity; PPV=positive predictive value; NPV=negative predictive value

628 #: Low parasitemia =50-1000 asexual parasitaemia/μl; high parasitemia=2000-10000 asexual parasitaemia/μl

629

630



**Figure 1:** Flow diagram for the selecting samples