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

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1 **Performance of the ParaHIT® HRP2-based rapid diagnostic test and**
2 **proportions of *Plasmodium falciparum* histidine-rich protein 2/3 gene**
3 **deletions in Togo**

4
5 Running title: Performance of ParaHIT® for malaria diagnostic in Togo

6
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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/ μ 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 (α) 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/ μ 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/ μ 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/ μ l and 2,187
327 trophozoites/ μ 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/ μ L, whereas sensitivity was only 92.7% when parasitemia was
352 less than 1000 parasites/ μ 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/ μ 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[®] 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

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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.

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609 **Figure legend**

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

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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)

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

Sites	Anié, n (%)	Kouvé, n (%)
Microscopy (TTBS)		
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)
PCR		
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]
Microscopy						
Low parasitemia						
Positive (n=41)	38	3				
Negative (n=140)	20	120	92.7 [80.1-98.5]	-	65.5 [55.7-74.2]	-
High parasitemia						
Positive (n=97)	95	2				
Negative (n=140)	20	120	97.9 [92.8-99.7]	-	82.6 [76.0-87.7]	-
Total						
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]
PCR						
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

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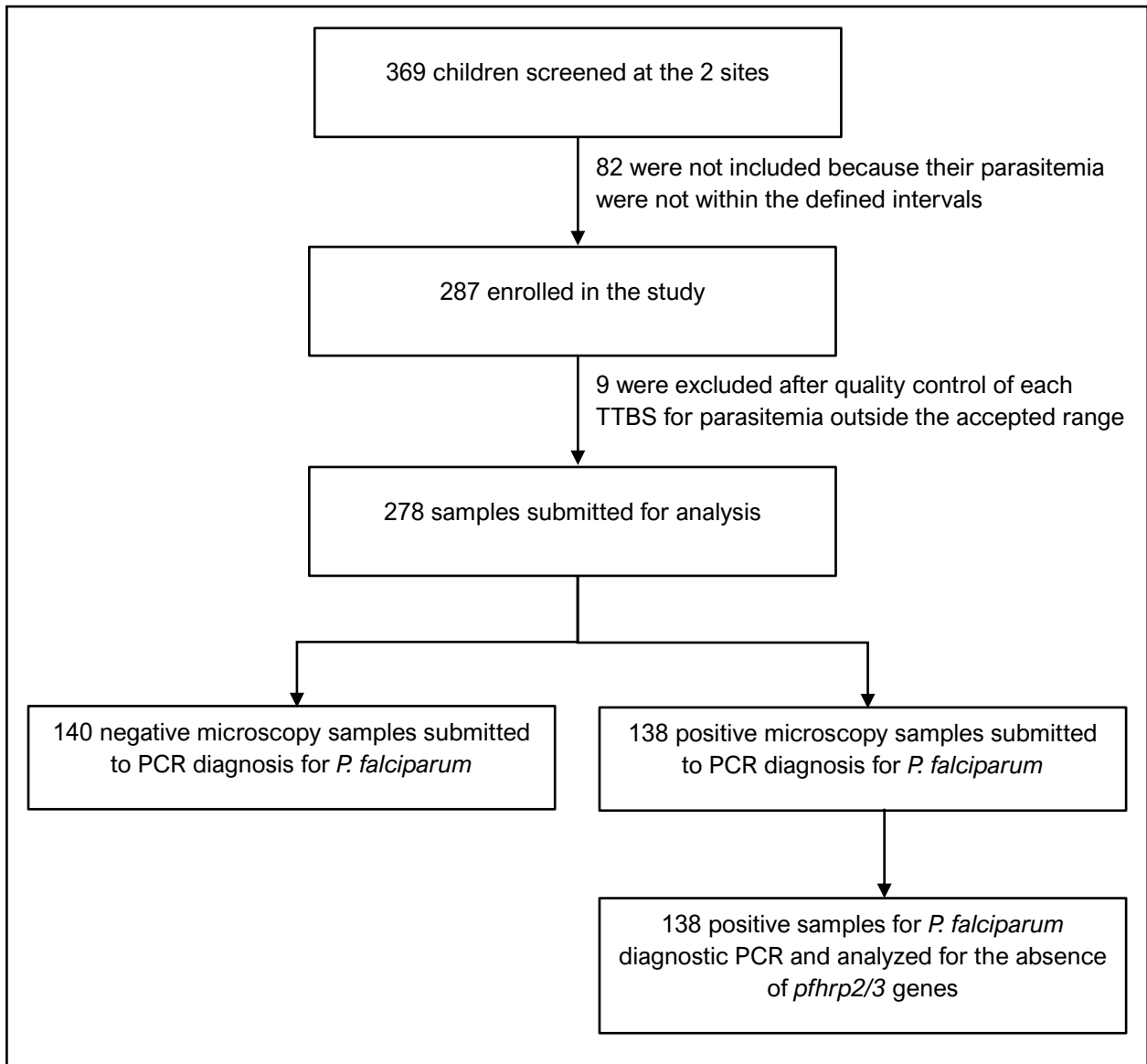


Figure 1: Flow diagram for the selecting samples