Performance of PURE-LAMP to Detect Declining Prevalence of Malaria in Haiti

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Research

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Abstract

Background Malaria continues to cause burden in various parts of the world. Endemic countries are developing schemes to end this nuisance in accordance with the World Health Organization's Global Technical Strategy for Malaria 2016–2030. Haiti, a Caribbean country, is among those aiming to eliminate malaria within a few years. Two surveys were conducted in Haiti in the summers of 2017 and 2018, respectively, during which we aimed to evaluate the performance of the simple and rapid PURE-LAMP (procedure for ultra-rapid extraction–loop-mediated isothermal amplification) method with dried blood spots as an alternative diagnostic method for malaria in the context of low to very low rates of transmission.

Methods Febrile and asymptomatic subjects were recruited from three administrative divisions within Haiti. Their blood samples were tested by microscopy, rapid diagnostic tests (RDT), PURE-LAMP and nested PCR to detect *Plasmodium* infection. The positive rates for the two study periods as determined by the different methods were compared. Sensitivity, specificity, positive and negative predictive values and kappa statistics were estimated with the nested PCR results as the gold standard.

Results Among 1074 samples analyzed, a positive rate of 8.3% was calculated based on the nested PCR results. Among symptomatic subjects, the rates in 2017 and 2018 were 14.6% and 1.4%, respectively. Three positives were detected among 172 asymptomatic participants in 2018 by PURE-LAMP and nested PCR, and all three were from the same locality. No asymptomatic subjects were recruited in 2017. The PURE-LAMP, RDT and microscopy had respective sensitivities of 100%, 85.4% and 49.4%. All of the testing methods had specificities over 99%.

Conclusion The prevalence of malaria was found to have substantially decreased in 2018 in Haiti. A specific focus of infection appeared to require intervention with a sensitive malaria diagnostic method and treatment. This study confirmed the high performance of the PURE-LAMP method with dried blood spots and recommends its use in targeted mass screening and treatment activities in low endemic areas of malaria.

Background

The World Health Organization estimated 228 million cases of malaria worldwide in 2018. Nonetheless, some regional progress has been made: from the E-2020 countries (identified as having the potential to be malaria free by 2020), China, El Salvador, Iran, Malaysia and Timor-Leste did not register indigenous cases. In the Americas, Paraguay and Argentina achieved malaria elimination [1, 2]. Apart from El Salvador, there remain 18 malaria-endemic countries and territories in the Americas, which includes only 2 in the Caribbean: the Dominican Republic and Haiti, on the sole island of Hispaniola on which malaria elimination is also considered feasible [3]. *Plasmodium falciparum* is the endemic species, transmission is mainly focal, and the annual incidence was < 1% in Haiti and reached < 0.1% in 2018 whereas < 1000
cases were reported for the Dominican Republic [1, 4]. Both countries have a population of approximately 11 million inhabitants.

When transmission reaches a very low level, it is important to have sensitive tools that help detect the remaining reservoirs that need to be cleared to avoid the reestablishment of high positive rates within the population [5, 6]. Nucleic acid amplification techniques are the most accurate diagnostic choice [6]. Although polymerase chain reaction (PCR) assays to diagnose malaria have been developed since the 1990s, they remain centralized and too demanding for regular use in the endemic field [7]. The loop-mediated isothermal amplification method (LAMP) is becoming a formidable alternative [8–10]. LAMP is fast, robust and less expensive than PCR and, subsequently, is applicable in less well-equipped laboratories although the current cost per sample is still higher than ideal.

Among the different malaria LAMP assays developed, two commercial kits have become available: the Loopamp™ MALARIA Pan/Pf Detection Kit (Eiken Chemical, Tokyo, Japan) [11–21] and the Illumigene Malaria LAMP (Meridian Bioscience Inc., Cincinnati, OH, USA) [22, 23]. The Loopamp™ MALARIA Pan/Pf Detection Kit has been evaluated with a significant number of samples in various studies conducted in both endemic [12–15, 20, 21] and non-endemic settings [11, 16], using whole blood [11–13] or dried blood spots [15, 16], and coupled with diverse DNA extraction methods. One of these methods is the procedure for ultra-rapid extraction (PURE), a quick and simple DNA extraction method, also developed by Eiken Chemical to prepare DNA solution suitable for the LAMP reaction. We previously evaluated the accuracy of the combination of PURE and Loopamp™ MALARIA Pan/Pf (PURE-LAMP) methods on dried blood spots of suspected and confirmed cases of malaria imported to Japan [16]. The performance of this system was proved at a Japanese laboratory. However, the greater impact of a LAMP-based system is expected in malaria-endemic areas through its application in screening and confirmation of a malaria diagnostic or parasitic clearance. Thus, the present study aimed to evaluate the PURE-LAMP malaria detection method in the low transmission context of Haiti along with concurrent observation of the evolving epidemiological situation.

**Methods**

**Study sites**

Based on data from the national malaria control program, three departments (main administrative divisions in Haiti) with the highest positivity rates in 2016 and early 2017 were selected as study sites. They were Nippes, Sud, and Grand’Anse in the southwestern part of the country (Fig. 1). Sud and Grand’Anse shared 63.6% of the malaria cases registered in 2016 in the country. Although a descending or at least stable incidence trend has been registered in the other 7 departments since 2015, the three chosen departments have shown an increasing or stable trend. Recruitment was carried out in small coastal cities with less than 50,000 inhabitants and which were located remotely from the capital cities of each department. At the start of the study, the sequels of hurricane Matthew that had hit 10 months earlier (October 2016) could still be spotted in a recovering environment.
Light microscopy is dictated as the gold standard for malaria diagnosis by the Haitian Ministry of Health; however, after allowing the alternative use of rapid diagnostic tests (RDT) in recent years, they are more widely used [3, 24, 25].

**Recruitment**

Subjects aged 1 year and over were recruited following two approaches: 1. hospital-based recruitment targeting participants with fever on the day of their hospital visit or anytime up to 2 weeks earlier, and 2. community-based recruitment targeting the general population during social gatherings. There was no criterion for fever. Most social group participants were asymptomatic, but some subjects with a history of fever were also recruited there.

Recruitment was carried out simultaneously at the same three hospitals during the summers of 2017 (early August to early September) and 2018 (late July to late August). At the point of recruitment, participants had to submit a consent form, answers to a short questionnaire about demography, and their fever history and preventive practices, and venous blood samples were collected by a qualified staff member either in EDTA- or heparin-containing blood tubes. After blood collection, subjects were tested immediately with an SD Bioline Malaria Ag Pf/Pan RDT (Standard Diagnostics, Inc., Suwon, South Korea) in accordance with the manufacturer's guidelines, and blood smears were prepared and 100 µL of blood was seeded on filter paper (Whatman™ FTA™ classic cards; GE Healthcare, Tokyo, Japan) and air dried. After drying, each card was stored in a plastic bag containing a small desiccant bag. The subjects were treated immediately if the RDT result was positive. Blood smears were fixed, stained and transported to the same laboratory along with the filter papers. Other tests were conducted afterwards.

This study used venous blood to avoid double invasion of the participants as the majority seeking care were also predicted to require other blood tests. A small volume (100 µL) was used to prepare dried blood spots to approximate capillary blood sampling.

**Microscopy**

Microscopists based at a Haitian reference laboratory conducted double lecture on thick and thin Giemsa-stained smears using a × 1000 light microscope. Three hundred microscopic fields were visualized before declaring a negative sample. Quality control was conducted on positives and 10% of the negatives by a third staff member. The microscopists were blinded to the other test results of the subjects.

**PURE-LAMP**

DNA was extracted with the Loopamp™ PURE DNA Extraction Kit from three dried blood spots with a diameter of 3 mm following the manufacturer's guidelines and as reported elsewhere [16]. Reactions were carried in two tubes for all samples with the Loopamp™ MALARIA Pan/Pf Detection Kit, a pan (targeting *Plasmodium* spp.) and a pf (*P. falciparum*-specific) tube for each. Negative and positive controls (provided in the kit) were also included in each run.
The presence of amplified DNA inside the reaction tubes may be recognized in two speedy ways: 1. by fluorescence under UV excitation due to calcein unquenched during reactions, and 2. by turbidity due to the precipitation of magnesium pyrophosphate [26]. In this study, evaluation of the result was conducted by fluorescence under UV excitation for all samples. However, for the hospital samples collected in EDTA blood tubes, evaluation was also conducted by turbidity with a Loopamp EXIA real-time turbidimeter (Eiken Chemical), and the diagnosis was categorized in favor of the turbidity results if there was a discrepancy with results from fluorescence. This was done to exclude false positives that may arise by fluorescence due to EDTA chelating properties: calcein is pre-added to the reaction tubes in the quenched state by binding to manganese ions. The EXIA real-time turbidimeter returns a quantitative parameter for each sample, the turbidity threshold time (tt), which was expected to correlate with the amount of amplified DNA.

**DNA extraction for PCR**

DNA samples were extracted from three dried blood spots with a diameter of 3 mm using a Maxwell® RSC DNA FFPE Kit (Promega, Madison, WI, USA) and the automated Maxwell® RSC Instrument (Promega). Minor modifications to the manufacturer’s guidelines can be found in a previous report [27]. DNA was eluted in 50 µL of elution buffer and stored at 4 °C.

**Nested PCR**

The reference test in this study was a nested PCR system targeting the *Plasmodium* 18 s ribosomal RNA gene [28]. This method generally implies a secondary step with species-specific primer sets for all human *Plasmodium*. However, secondary PCRs in this study were conducted only with a primer set specific to *P. falciparum*, which is the species expected in Haiti. For discrepant samples between PURE-LAMP (+) and nested PCR (-), both tests were repeated, and the number of secondary PCR cycles was increased from 20 to 30. The nested PCR diagnosis was updated if a previously negative sample became positive at this step.

**Statistical analysis**

Data analysis was performed with Stata (StataCorp, TX, USA). Sensitivity, specificity, positive predictive value and negative predictive value with their respective 95% confidence intervals (CIs) and kappa statistics were estimated with nested PCR as the reference.

**Results**

**Characteristics of the study participants**

In total, 1115 subjects gave consent to participate in this study: 265 in community settings and 850 at the hospitals. After exclusions due to missing samples, 554 blood samples were analyzed from those recruited during the summer of 2017, all from hospitals, and 520 from those of summer 2018, including 242 from community-based recruitment (Fig. 1). The subjects ranged in age from 1 to 85 years old.
(mean: 31.0; SD: 20.5 years) and included 366 (34.7%) males and 690 (65.3%) females. Among them, 225 (21.1%) had a personal history of fever on the day of recruitment, 488 (45.7%) from a day before up to 7 days earlier and 183 (17.1%) from 8 days up to 2 weeks earlier, and 284 (26.9%) reported having a household member with fever and 92 (8.7%) a close neighbor. The history of having a relative with fever was significantly related to being tested positive for malaria by nested PCR or PURE-LAMP (P value: 0.000). Preventive behaviors were quite common: 948 (88.3%) subjects reported at least one, with the use of a bed net being the most reported (584 subjects) (Table 1).
Table 1
Characteristics of the study participants

| Characteristic                        | 2017 Symptomatic | 2018 Symptomatic | 2018 Asymptomatic | Total (%) |
|--------------------------------------|------------------|------------------|-------------------|-----------|
| Number of participants               | 554 (51.6)       | 348 (32.4)       | 172 (16.0)        | 1074 (100) |
| Age (mean [SD])                      | 27.4 (18.3)      | 29.1 (18.6)      | 46.4 (23.5)       | 31.0 (20.5) |
| Last episode of fever                |                  |                  |                   |           |
| - Day 0                              | 84 (15.3)        | 141 (40.8)       | Not applicable    | 225 (21.1) |
| - 1–7 days                           | 315 (57.2)       | 173 (50.0)       |                   | 488 (45.7) |
| - 8–14 days                          | 151 (27.4)       | 32 (9.2)         |                   | 183 (17.1) |
| Known relative with fever            |                  |                  |                   |           |
| - No                                 | 296 (54.5)       | 248 (71.5)       | 138 (82.6)        | 682 (64.5) |
| - At home                            | 173 (31.9)       | 91 (26.2)        | 20 (12.0)         | 284 (26.9) |
| - Neighborhood                       | 74 (13.6)        | 8 (2.3)          | 10 (6.0)          | 92 (8.7)  |
| Preventive practices                 |                  |                  |                   |           |
| - Bed net                            | 255 (48.2)       | 234 (70.5)       | 95 (78.5)         | 584 (59.5) |
| - Insecticide (spray)                | 24 (4.5)         | 16 (4.8)         | 19 (15.7)         | 59 (6.0)  |
| - Window screen                      | 215 (40.6)       | 91 (27.4)        | 32 (26.4)         | 338 (34.4) |
| - Fumigation                         | 1 (0.2)          | 0                | 2 (1.7)           | 3 (0.3)   |
| - Mosquito repellent                 | 1 (0.2)          | 0                | 0                 | 1 (0.1)   |
| - Chemoprophylaxis                   |                  |                  |                   |           |
| Positive rates                       |                  |                  |                   |           |
| - Nested PCR                         | 81 (14.6)        | 5 (1.4)          | 3 (1.7)           | 89 (8.3)  |
| - Microscopy                         | 47 (8.5)         | 4 (1.1)          | 0                 | 51 (4.7)  |
| - RDT Pan                            | 54 (9.7)         | 2 (0.6)          | 0                 | 56 (5.2)  |
| - RDT Pf                             | 76 (13.7)        | 5 (1.4)          | 0                 | 81 (7.5)  |
| - PURE-LAMP Pan                      | 90 (16.2)        | 5 (1.4)          | 3 (1.7)           | 98 (9.1)  |
| - PURE-LAMP Pf                       | 88 (15.9)        | 5 (1.4)          | 3 (1.7)           | 96 (8.9)  |

**Abbreviations:** RDT Pan: rapid diagnostic test band to detect *Plasmodium* lactodehydrogenase; RDT Pf: band for detection of *P. falciparum*-specific Histidine Rich Protein II; PURE-LAMP: procedure for ultra-rapid extraction–loop-mediated isothermal amplification; Pan: tubes for diagnosis of *Plasmodium* genus; Pf: *P. falciparum*-specific tubes.
Rates of positivity

The rates of positivity for malaria as estimated by each method and by year are summarized in Table 1. As concluded by nested PCR, there were 89 (8.3%) positives among 1074 samples that had been tested by all methods. The positive rate varied from 14.6% among samples collected in 2017 to 1.5% for samples from 2018. Although all participants in 2017 were symptomatic, the positive rate among symptomatic subjects in 2018 was 1.4%. The positive rate among the asymptomatic subjects was 1.74%. RDT and microscopy did not detect any *Plasmodium* infection among the asymptomatic subjects. Microscopic parasitemia varied, ranging from 0.02–20.0% (mean: 2.3, SD: 3.2).

Performance parameters

Almost perfect agreement was calculated between the nested-PCR and PURE-LAMP (*kappa*: 0.95). The PURE-LAMP Pan and PURE-LAMP Pf had respective sensitivities of 100% [95% CI 95.9–100] and 98.9% [95% CI 93.9–100], and their specificities were 99.1% [95% CI 98.3–99.6] and 99.2% [95% CI 98.4–99.6]. The RDT Pf band had a sensitivity of 85.4% [95% CI 76.3–92.0] and specificity of 99.5% [95% CI 98.8–99.8], whereas for the RDT Pan band, values of 60.7% [95% CI 49.7–70.9] and 99.8% [95% CI 99.3–100], respectively, were calculated. Microscopy sensitivity was 49.4% [95% CI 38.7–60.2], and its specificity was 99.3% [95% CI 98.5–99.7] (Fig. 2 and Table 2).

|                          | Sensitivity % [95% CI] | Specificity % [95% CI] | PPV % [95% CI] | NPV % [95% CI] | Kappa |
|--------------------------|------------------------|------------------------|----------------|----------------|-------|
| PURE-LAMP Pan            | 100 [95.9–100]         | 99.1 [98.3–99.6]       | 90.8 [83.3–95.7] | 100 [99.6–100] | 0.95  |
| PURE-LAMP Pf             | 98.9 [93.9–100]        | 99.2 [98.4–99.6]       | 91.7 [84.2–96.3] | 99.9 [99.4–100] | 0.95  |
| RDT Pan                  | 60.7 [49.7–70.9]       | 99.8 [99.3–100]        | 96.4 [87.7–99.6] | 96.6 [95.2–97.6] | 0.73  |
| RDT Pf                   | 85.4 [76.3–92.0]       | 99.5 [98.8–99.8]       | 93.8 [86.2–98.0] | 98.7 [97.8–99.3] | 0.88  |
| Microscopy               | 49.4 [38.7–60.2]       | 99.3 [98.5–99.7]       | 86.3 [73.7–94.3] | 95.6 [94.1–96.8] | 0.60  |

**Abbreviations:** RDT Pan: rapid diagnostic test band to detect *Plasmodium* lactodehydrogenase; RDT Pf: band for detection of *P. falciparum*-specific Histidine Rich Protein II; PURE-LAMP: procedure for ultra-rapid extraction–loop-mediated isothermal amplification; Pan: Tubes for diagnosis of *Plasmodium* genus; Pf: *P. falciparum*-specific tubes.

Discussion
The results of the present study showed high sensitivity and specificity for a malaria diagnosis by the PURE-LAMP method on dried blood spots in a setting of low *P. falciparum* transmission. There was a decrease of 10 times in the rate of positivity between the two study periods, which could indicate the progress Haiti is making toward the goal of malaria elimination.

Our previous evaluation of the PURE-LAMP method with dried blood spots conducted with imported cases in a non-endemic setting showed a sensitivity over 96% and specificity of 100% [16]. A review of previously reported evaluations of the Loopamp™ MALARIA Pan/Pf Detection Kit with >35 samples per group in diverse settings showed sensitivity in a range from about 90% up to 100% and specificity from about 85–100% [11–16]. The results of the present study also lie within these ranges.

In the present study, all discrepancies between the nested PCR and the PURE-LAMP Pan results were negative by nested PCR and positive by PURE-LAMP Pan; they were also negative by microscopy and mostly negative by RDT (except for one RDT Pf-positive result). We think it is possible for some (or all) being true positives as the precision is lower for samples with parasitemia around PCR's limit of detection. Three samples became positive by nested PCR after repeating the test. The discrepant samples had *tt* values longer than the 15 min expected after the start of the reaction [26] (Table 3). This could indicate a very low amount of the parasites’ DNA on the template, which required longer amplification time to produce enough magnesium pyrophosphate to be detected by the turbidimeter. However, we did not find a significant correlation between *tt* values and parasitemia in this dataset. A stricter experimental approach regarding blood sample volume and cell distribution will be necessary to determine whether this parameter can be helpful to estimate parasitemia.
### Discrepancies between PURE-LAMP and nested-PCR

| Sample code | PURE-LAMP Pan (tt) | PURE-LAMP Pf (tt) |
|-------------|--------------------|-------------------|
| 111         | Positive (27.5)    | Negative          |
| 174         | Positive (16.2)    | Positive (19.3)   |
| 234         | Positive (21.5)    | Positive (23.3)   |
| 242         | Positive (22.5)    | Positive (23.5)   |
| 277         | Positive (19.1)    | Positive (17.2)   |
| 704         | Positive (18.3)    | Positive (21.5)   |
| 707#        | Positive (22.0)    | Positive (18.1)   |
| 713         | Positive (19.5)    | Positive (20.5)   |
| 1310        | Positive (20.2)    | Positive (21.5)   |
| 276*        | Positive (24.2)    | Negative          |

Abbreviations: PURE-LAMP: procedure for ultra-rapid extraction–loop-mediated isothermal amplification; PCR: polymerase chain reaction; tt: turbidity threshold time; Pan: tubes for diagnosis of *Plasmodium* genus; Pf: *P. falciparum*-specific tubes.

Average tt of PURE-LAMP Pan was 16.4 min (SD:1.8; range:13.1–24.2) among 82 concordant positives but 20.8 (SD:3.2) min among 9 samples with PURE-LAMP Pan (+) and nested PCR (-). All discrepant samples were negative by microscopy. # Denotes a sample positive by RDT Pf. * Denotes a sample positive by nested PCR and RDT. All other samples were negative by RDT and nested PCR.

The RDT Pf band had a sensitivity over 85% and also agreed almost perfectly with the nested PCR results (*kappa*: 0.88). Being among the three brands validated in the Haiti—the First Response Malaria Ag HRP2 (Premier Medical Corporation Ltd., Watchung, NJ, USA), CareStart Malaria HRP2 (Pf) (Access Bio, Inc., Monmouth Junction, NJ, USA) and SD Bioline [25]—the RDT Pf band of the latter test was confirmed here to be an efficient option for the diagnosis of febrile cases. However, only the PURE-LAMP and nested PCR allowed the detection of asymptomatic infections in this study.

Among the RDT-negative samples, two were observed with microscopic parasitemia of > 0.6%, although microscopy had the lowest sensitivity in the present study. We considered the possibility of having some RDT Pf false negatives due to Histidine Rich Protein 2 (*hrp2*) gene deletion, but *hrp2* and *3* could be amplified for those two samples and eight more that were negative by the RDT Pf band but positive by nested PCR and PURE-LAMP. Three more samples- RDT Pf (-), nested PCR and PURE-LAMP (+)- could not be amplified for *hrp2*, nor two among them for *hrp3*. Other genes such as *pfcrt* and *k13* could not be amplified for those three samples either, so this does not prove deletion. A recent report also did not find *hrp2/3* deletion in Haiti [29]. This result could have been caused by variation in antigen expression, blood concentration or a technical error.
In general, a decrease of malaria prevalence was observed between 2017 and 2018 in Haiti [4]. The impact of hurricane Matthew might also have influenced malaria transmission in 2017 whereas malaria control interventions might have had a better impact in 2018.

While the prevalence of infection was significantly decreasing in hospitals and was reported to the national control program, some malaria foci may require special intervention including a different method of testing. In Dame-Marie (Grand’Anse), absolutely no febrile patients were diagnosed as malaria positive during the weeks of hospital recruitment in 2018 (Fig. 1). However, when a single-day community recruitment effort was organized in a locality about 3 km away from the hospital, two positives were first detected by the RDT Pf band. The number of positives from that day increased to five after performing PURE-LAMP and nested PCR. The first two positives were subjects with histories of fever, whereas the other three had no recent history of fever. It is not known whether the febrile patients who participated in the community screening had planned to visit the hospital. The locality in question was known by the regional staff for having a higher risk of malaria infection and seemed to correlate well with a hotspot as defined by Bousema et al. [30]. Still, the present study showed that the RDT and microscopy methods were not exposing an accurate picture of malaria positivity.

Several studies have highlighted the importance of asymptomatic infections for transmission in low-transmission settings [5, 31–33]. It was estimated that submicroscopic carriers are the source of 20–50% of all human-to-mosquito transmissions when transmission reaches very low levels [5]. The present study provides data about an eligible method that can help detect those submicroscopic carriers that may be used for mass or reactive screening for downstream treatment.

The present findings are also important as they show how far the efforts to stop malaria have come in Haiti and the risk of a setback that was still there to be nullified. Despite the difference observed in positive rates between the periods studied, the challenge to achieve malaria elimination is enormous. Nevertheless, this small number of asymptomatic infections is important for the community, considering that submicroscopic infections may still be transmitted and that the possible worst effect will occur in non-immune subjects. In future molecular studies in Haiti, the distribution and significance of these asymptomatic infections need to be explored in a larger sample size.

**Conclusion**

The present study confirmed the high performance of the PURE-LAMP method for malaria testing with dried blood spots. It allowed the detection of symptomatic cases and asymptomatic infections missed by RDT and microscopy in a low-transmission setting. Malaria prevalence was found to be decreasing in Haiti; and a specific focus of infection appeared to require intervention with sensitive malaria diagnostic methods and treatment. We recommend the use of this diagnostic system in targeted mass screening and treatment to detect the remaining human infectious reservoirs for their clearance as a means to accelerate the elimination of malaria in Haiti.
Declarations

Ethics approval and consent to participate

The protocol of this study was reviewed and approved by the NCGM Ethics Committee (Ref: NCGM-G-002260-00) in Japan and the Haitian National Bioethics committee (Ref: 1617-48). Consent was obtained in person from participants over the age of majority and from a tutor in the case of minors.

Consent for publication

All the authors have consented to the publication of this article.

Availability of data and material

The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Competing interests

The kits used for this study were provided by Eiken Chemical Co. Ltd.

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Partial funding for field operations were provided by Eiken Chemical Co. Ltd.

Authors’ contribution

JPV, study design, data collection, experiments, data analysis, writing – original draft, writing – review; AVE, study design, ethical review in Haiti, data collection, experiments; KKY, ethical review in Japan, project administration, writing – review; JB, fieldworks supervision; SK, study design, funding acquisition, project administration, supervision, writing – review. All authors read and approved the final manuscript.

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**Figures**

**Figure 1**

Location of the study area. Haiti is located in the Americas. The three departments (administrative divisions) of the study are highlighted in grey, and the others are shown in white. The number of participants by department, year and nested PCR diagnosis, is shown based on recruitment setting, hospital or community.
Figure 2

Flowchart of the study subjects. Abbreviations: PURE-LAMP: procedure for ultra-rapid extraction–loop-mediated isothermal amplification; PCR: polymerase chain reaction; Pan: tubes for diagnosis of Plasmodium genus; Pf: P. falciparum-specific tubes; RDT: rapid diagnostic test.

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