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Plasmodium falciparum Parasitemia and Band Sensitivity of the SD Bioline Malaria Ag P.f/Pan Rapid Diagnostic Test in Madagascar

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Abstract. Current malaria rapid diagnostic tests (RDTs) contain antibodies against *Plasmodium falciparum*–specific histidine-rich protein 2 (PfHRP2), *Plasmodium* lactate dehydrogenase (pLDH), and aldolase in various combinations. Low or high parasite densities/target antigen concentrations may influence the accuracy and sensitivity of PfHRP2-detecting RDTs. We analyzed the SD Bioline Malaria Ag P.f/Pan RDT performance in relation to *P. falciparum* parasitemia in Madagascar, where clinical *Plasmodium vivax* malaria exists alongside *P. falciparum*. Nine hundred sixty-three samples from patients seeking care for suspected malaria infection were analyzed by RDT, microscopy, and *Plasmodium* species–specific, ligase detection reaction-fluorescent microsphere assay (LDR-FMA). *Plasmodium* infection positivity by these diagnostics was 47.9%, 46.9%, and 58%, respectively. *Plasmodium falciparum*–only infections were predominant (microscopy, 45.7%; LDR-FMA, 52.3%). In all, 16.3% of *P. falciparum*, 70% of *P. vivax*, and all of *Plasmodium malariae*, *Plasmodium ovale*, and mixed-species infections were submicroscopic. In 423 *P. falciparum* mono-infections, confirmed by microscopy and LDR-FMA, the parasitemia in those who were positive for both the PfHRP2 and pan-pLDH test bands was significantly higher than that in those who were positive only for the PfHRP2 band ($P < 0.0001$). *Plasmodium falciparum* parasitemia in those that were detected as *P. falciparum*–only infections by microscopy but *P. falciparum* mixed infections by LDR-FMA also showed similar outcome by the RDT band positivity. In addition, we used varying parasitemia (3–0.0001%) of the laboratory-maintained 3D7 strain to validate this observation. A positive pLDH band in high *P. falciparum*–parasitemic individuals may complicate diagnosis and treatment, particularly when the microscopy is inconclusive for *P. vivax*, and the two infections require different treatments.

INTRODUCTION

The currently available malaria rapid diagnostic tests (RDTs) come in various formats and contain antibodies targeted to three parasite antigens: *Plasmodium falciparum*–specific histidine-rich protein 2 (PfHRP2) and two enzymes in the plasmodial glycolytic pathway, lactate dehydrogenase (pLDH) and aldolase, in various combinations.[1,2] The monoclonal antibodies (mAbs) used against PfHRP2 can recognize the paralog gene product PfHRP3, which has a sequence homology of more than 75% in the tandem repeat region to PfHRP2.[3,4] In parasites having *pfhrp2* deletion, recognition of PfHRP3 could enhance the sensitivity of the PfHRP2-detecting RDTs.[5] The antibodies used against the two glycolytic enzymes can recognize *P. falciparum*–specific LDH (Pf-pLDH), *Plasmodium vivax*–specific LDH (Pv-pLDH), pan-pLDH, and pan-aldolase—the latter two are for all four major human *Plasmodium* species (*falciparum*, *vivax*, *malariae*, and *ovale*). *Plasmodium* LDH shares ≥ 90% amino acid identity among all species, and therefore, pan-pLDH RDTs using mAbs against common epitopes can detect all those four major human malaria parasites.[6] Aldolase in combination with PfHRP2 for malaria RDTs has been used for the diagnosis of *P. falciparum* and non–*P. falciparum* species, but with a poor performance for the latter group.[1] Depending on the antigen/antigens targeted, the available malaria RDTs can detect *P. falciparum* only, *P. vivax* only, all four major human *Plasmodium* species, or a combination of species. No currently available RDT can specifically identify *Plasmodium malariae* or *Plasmodium ovale* infections.[7]

In recent years, the role of malaria RDTs in surveillance has grown significantly, and they are now the main tools in clinical case management, particularly in resource-limited settings in sub-Saharan Africa.[8,9] In settings where all infections are managed in the same way (e.g., with artemisinin-based combination therapy), there is no apparent clinical advantage of having an RDT that can distinguish falciparum from non-falciparum infection; a pan-only test would suffice. If treatment of the two infections is different—notably in the context of *P. vivax* radical cure—distinguishing between falciparum and non-falciparum infection becomes a priority. Irrespective of immediate case management and treatment, epidemiological surveillance benefits from knowing the breakdown of RDT results to monitor trends in numbers of cases attributable to *P. falciparum* and/or *P. vivax*. Tests with a *P. falciparum*–specific line and pan-specific line (PfHRP2/pan-pLDH), however, cannot distinguish between *P. falciparum*–only infections and mixed infections with *P. falciparum*.

A quality control and evaluation program was established in 2008 between the WHO and the Foundation for Innovative New Diagnostics (WHO-FIND) to evaluate the performance of malaria RDT products. Currently, 11 malaria RDTs are WHO prequalified,[10] including the SD Bioline Malaria Ag P.f/Pan RDT (product code 05FK60/05FK63, Standard Diagnostics, Inc., Suwon City, Kyonggi Province, Republic of Korea) used in the present study. In our recent cross-sectional study of asymptomatic participants, the sensitivity and specificity of this RDT were 87% and 90%, respectively, when compared with a molecular *Plasmodium* species–specific, post–polymerase chain reaction/ligase detection reaction-fluorescent microsphere assay (PCR/LDR-FMA).[11]

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A number of host and parasite factors may influence the accuracy and sensitivity of PfHRP2-detecting RDTs. Among these are very low or high parasite densities/target antigen concentrations.[12,13] A recent WHO-FIND product testing study[13] analyzed 18 combination RDT products for PfHRP2 and pan band (17 pLDH and one aldolase) positivity against a panel of approximately 100 *P. falciparum* parasite samples, which were collected from patients with active, PCR-confirmed *P. falciparum* mono-infections and without a history of antimalarial therapy in the month preceding the analysis. Tests were performed using each sample diluted to 200 and 2,000 or 5,000 parasites/μL densities. At the higher parasite densities, 99.7% of the tests were positive for *P. falciparum* and 96.7% of those tests were positive on both the PfHRP2 and pan bands. At 200 parasites/μL, 92.3% of the tests were positive, but only 57.1% of those tests were positive on both bands. These results indicated that there was a difference in the sensitivity of the PfHRP2 and pLDH test bands in the detection of active infection. This finding was consistent with that previously reported for the SD Bioline Malaria Ag P.f/Pan RDT (05FK60), tested against samples collected from returned travelers, where the proportion of tests positive for both the PfHPRP2 and pLDH bands progressively increased with parasite density from 5.3% at < 100 parasites/μL to 98.5% at $> 1,000$ parasites/μL.[14] A PfHRP2/pLDH combination RDT may be useful in increasing the diagnostic specificity for falciparum malaria in certain settings,[15] but in settings where *P. falciparum* and *P. vivax* co-circulate, a positive pLDH band in high *P. falciparum*–parasitemic individuals may complicate diagnosis and treatment,[16] particularly when the microscopy is inconclusive for *P. vivax*, and the two infections require different treatments.[17]

The main objective of the present study was to analyze how the SD Bioline Malaria Ag P.f/Pan RDT performs in relation to *P. falciparum* parasitemia in Madagascar, where clinical *P. vivax* malaria exists alongside *P. falciparum*.[18,19]

MATERIALS AND METHODS

Study site, subjects, and protocol. Following on from a previously described cross-sectional survey,[20] longitudinal surveillance of patients seeking treatment for suspected malaria was established in three health centers of Ampasimpotsy, a rural area in the western foothills of Madagascar. During an 11-month period of this surveillance (September 2015–July 2016), 963 samples were analyzed by three diagnostic approaches: RDT, microscopy, and PCR/LDR-FMA. The study protocol was approved by the ethical review panels of University Hospitals Case Medical Center, Cleveland, OH; the National Institutes of Health; and the Ministry of Public Health, Madagascar.[20]

Malaria infection diagnostics. Patients with a suspected malaria infection seeking treatment at the study health centers were invited to enroll in the study. Participants were screened for malaria parasites by testing fingertip capillary blood using three diagnostic approaches. First, as per Madagascar National Malaria Control Program (NMCP) protocols, RDT diagnosis was performed at the time of consultation. Results were recorded as 100 (only control band positive [not infected]), 101 (control and PfHRP2 bands positive [*P. falciparum*–only infection]), 110 (control and pan-pLDH bands positive [non–*P. falciparum* infection]), and 111 (control, PfHRP2, and pan-pLDH bands positive [*P. falciparum*–only or *P. falciparum* mixed infection]). Light microscopy diagnosis was then performed by technicians who were blinded to the RDT results. Two microscopy reads for each slide were recorded. Where these were discordant, a third read by a WHO-certified microscopist was performed. Mean parasitemias were calculated from the concordant pairs of results. Finally, blood samples were spotted onto filter papers, dried, and processed for PCR/LDR-FMA molecular diagnosis of *Plasmodium* species. The dried blood spot protocol was used to extract genomic DNA using a QIAamp® 96 DNA Blood Kit (Qiagen, Valencia, CA). All methods for PCR amplification of small subunit rRNA target sequences and *Plasmodium* species–specific detection by LDR-FMA have been described in detail by McNamara et al.[21] Genomic DNA extracted from *P. falciparum*–, *P. vivax*–, *P. malariae*–, and *P. ovale*–infected blood samples, provided by the Malaria Research and Reference Reagent Resource Center and Dr. W. E. Collins (Centers for Disease Control and Prevention), served as positive controls. DNA was extracted from 50 to 100 μL of each sample using a QIAamp DNA Micro Kit (QIAGEN).

Treatment. Any RDT-positive cases were treated by the health facility physician in accordance with the Malagasy Ministry of Health guidelines.[22] Irrespective of the specific RDT bands, any positive cases were treated with an age-adjusted dose of artesunate/amodiaquine (AS-AQ). Radical cure for *P. vivax* was not available. In the absence of AS-AQ supplies, physicians reported prescribing quinine as an alternative.

In vitro cultivation of *P. falciparum* strain 3D7. The parasite strain was cultured in RPMI 1640 (Corning™ cellgro™, Manassas, VA), supplemented with 200 mM L-glutamine, 200 mM hypoxanthine, 50 mg/mL gentamicin sulfate, and 10% AlbuMAX, under conditions described previously.[11]

Statistical analysis. All data manipulations and analyses were performed in Microsoft Excel. A two-sample (or unpaired) *t*-test was performed to test whether the mean *P. falciparum* parasitemia in samples that were positive only for the PfHRP2 band (RDT 101) was different from that in those that were positive for both the PfHRP2 and pan-pLDH test bands (RDT 111). $P < 0.05$ was considered significant.

RESULTS

Malaria infection diagnostics. Full diagnostic results were available from a total of 963 patients seeking care for suspected malaria infection at the study health centers between September 2015 and July 2016. Of these, 461 individuals (47.9%) were RDT positive (101, $N = 78$; 110, $N = 11$; 111, $N = 372$) and 502 were RDT negative. Overall, 452 (46.9%) were microscopy positive (*P. falciparum*–only infections = 440, *P. vivax*–only infections = 12) and 511 were microscopy negative. Parasitemia ranged from 120 to 975,990 parasites/μL for *P. falciparum*–only infections and from 334 to 249,661 parasites/μL for *P. vivax*–only infections. No *P. malariae*, *P. ovale*, and mixed-species infections were detected by microscopy. A full comparison of the RDT results with the microscopy results is presented in Table 1. These results were highly concordant (98.9%) and, together, indicate the predominance of *P. falciparum* mono-infections. Even among the RDTs positive for both the PfHRP2 and pLDH test bands, 98.6% were from

Table 1
Comparison of RDT and microscopy

RDT Status	Microscopy positive: *Plasmodium falciparum* positive	Microscopy positive: *Plasmodium vivax* positive	Microscopy negative	Total
101	72	0	6	78
110	0	11	0	11
111	367	1	4	372
100	1	0	501	502
Total	440	12	511	963

RDT = rapid diagnostic test. RDT status codes, as described in Materials and Methods (Malaria infection diagnostics).

samples that were microscopy positive for *P. falciparum*–only infections.

Overall, 559 samples (58%) were *Plasmodium* species PCR positive and 404 samples were *Plasmodium* species PCR negative. *Plasmodium* species determination by LDR-FMA revealed that a total of 526 samples were positive for *P. falciparum*, 40 for *P. vivax*, 12 for *P. malariae*, and eight samples were positive for *P. ovale* infections. The distribution of single- and mixed-species infections was as follows: *P. falciparum*–only infections = 504, non–*P. falciparum* single-species infections = 31 (majority, *P. vivax* = 27), and mixed-species infections = 24 (majority, *P. falciparum*–*P. vivax* = 13) (Table 2). Comparison of these results with the microscopy results indicates that 16.3% of *P. falciparum*, 70% of *P. vivax*, 100% of *P. malariae*, and 100% of *P. ovale* infections were submicroscopic (SMI). Further comparison of these results with the RDT results is presented in Table 2. This analysis also shows that 15.9% of *P. falciparum*–only, 74.2% of non–*P. falciparum* single-species, and 37.5% of mixed-species infections were not detected by RDTs.

***Plasmodium falciparum* parasitemia and RDT test band positivity.** Of all 963 samples, 423 *P. falciparum* mono-infections were detected by microscopy and by LDR-FMA analysis. Assessment of these *P. falciparum* mono-infected patients by the SD Bioline Malaria Ag P.f/Pan RDT found 0.2% to be RDT negative, 15.1% to be positive only for the PfHRP2 band, and 84.6% to be positive for both the PfHRP2 and pan-pLDH test bands. No RDT was positive only for the pan-pLDH test band. The mean parasitemia in samples that were positive for both the PfHRP2 and pan-pLDH test bands (range: 200–975,990 parasites/μL) was significantly higher than that in those that were positive only for the PfHRP2 band (range: 120–51,388 parasites/μL) (unpaired *t*-test, $t = -10$, $P < 0.0001$) (Figure 1).

Because we did not detect any mixed-species infection by microscopy, we next considered *P. falciparum* parasitemia in those that were detected as *P. falciparum*–only infections by microscopy but *P. falciparum* mixed infections by LDR-FMA (Table 2). Similar to the aforementioned analysis, *P. falciparum* parasitemia in samples that were positive for both the PfHRP2 and pan-pLDH test bands ($N = 7$, range: 1,129–47,429 parasites/μL [one sample was microscopy positive for *P. vivax* only, 33,672 parasites/μL]) was noticeably higher than in those that were positive only for the PfHRP2 band ($N = 5$, range: 206–1,983 parasites/μL). Small sample sizes prevent further analysis of these results.

In addition to these field study–based observations, we used varying parasitemia of the laboratory-maintained 3D7 strain to show that, indeed, serial reduction of parasitemia (3–0.0001%) is associated with a clear-cut difference in the sensitivity of the test bands of this RDT (Figure 2). Band intensities were not recorded for our study samples. Nevertheless, a visual evaluation of the RDTs used for varying parasitemia of the 3D7 strain showed that, starting from 0.1% parasitemia, the pan-pLDH band was noticeably weaker than the PfHRP2 band (Figure 2).

DISCUSSION

The performance of different RDT types has been widely evaluated in different epidemiological and clinical settings, aiming to validate their use in routine care. Advancing efforts related to RDT evaluation have clearly contributed to overall malaria surveillance and individual treatment. In Madagascar, RDTs for malaria were first trialed in 2003.[23,24] A policy shift toward their routine use across the island began in 2007, and these now represent the only method of malaria diagnosis outside referral hospitals.[25] In this country of heterogeneous malaria epidemiology, *P. falciparum* predominates[25,26]; however, clinical *P. vivax* malaria exists alongside *P. falciparum*.[18,19] Given the country's current focus on malaria elimination,[27] correct interpretation of different RDT results, obtained with a PfHRP2/pan-pLDH combination RDT, is therefore important from both epidemiological surveillance and treatment standpoints.

The main epidemiological findings of this study are summarized as follows: the test positivity rates for *Plasmodium* infection in patients seeking treatment with suspected malaria diagnosed by RDT, microscopy, and molecular assay were 47.9%, 46.9%, and 58%, respectively. *P. falciparum*–only infections were predominant, as detected by both microscopy (45.7%) and LDR-FMA (52.3%). Whereas 16.3% of *P. falciparum* infections were SMI, 70% of *P. vivax* and all of

Table 2
Comparison of RDT and PCR/ligase detection reaction-fluorescent microsphere assay

RDT Status	PCR+: *Plasmodium falciparum* positive	PCR+: *Plasmodium vivax* positive	PCR+: *Plasmodium malariae* positive	PCR+: *Plasmodium ovale* positive	PCR+: Mixed	PCR–	Total
101	66	0	0	0	5*	7	78
110	0	8	0	0	2†	1	11
111	358	0	0	0	8‡	6	372
100	80	19	2	2	9§	390	502
Total	504	27	2	2	24	404	963

PCR– = polymerase chain reaction negative; PCR+ = polymerase chain reaction positive; RDT = rapid diagnostic test. RDT status codes, as described in Materials and Methods (Malaria infection diagnostics).
* *P. falciparum*–*P. vivax* = 1, *P. falciparum*–*P. malariae* = 2, *P. falciparum*–*P. ovale* = 1, *P. falciparum*–*P. malariae*–*P. ovale* = 1.
† *P. falciparum*–*P. vivax.*
‡ *P. falciparum*–*P. vivax* = 7, *P. falciparum*–*P. malariae* = 1.
§ *P. falciparum*–*P. vivax* = 3, *P. falciparum*–*P. malariae* = 2, *P. falciparum*–*P. malariae*–*P. ovale* = 2, *P. malariae*–*P. ovale* = 2.

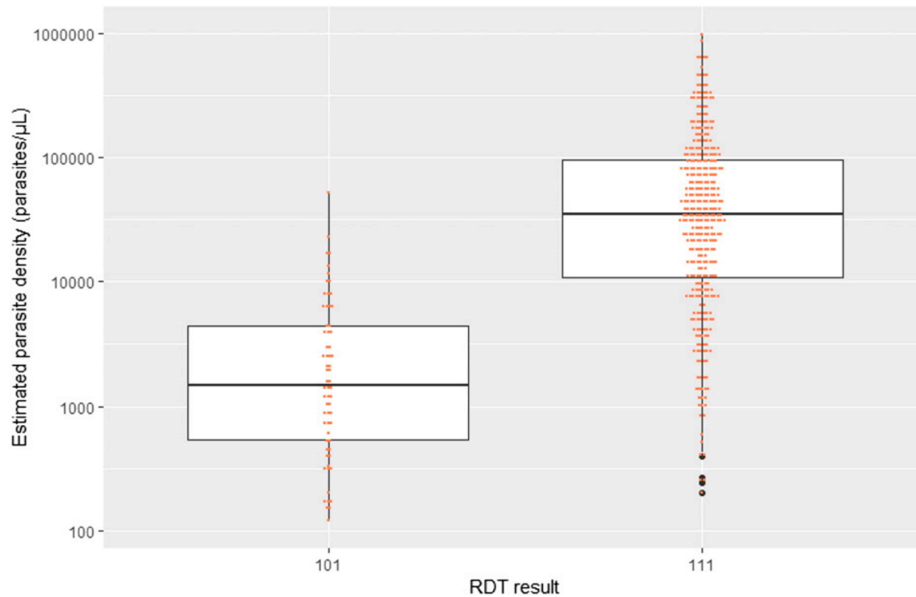

FIGURE 1. Box plots showing the distribution of *Plasmodium falciparum* parasitemia by rapid diagnostic test (RDT) band positivity in 422 monoinfections. Each dot represents a sample. RDT code 101 = control and *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) bands positive; 111 = control, *Plasmodium* lactate dehydrogenase (pan-pLDH, pan), and PfHRP2 bands positive. This figure appears in color at www.ajtmh.org.

P. malariae, *P. ovale*, and mixed-species infections were SMI. These results are generally in agreement with the results of a previous cross-sectional survey of this same study population, in which *P. malariae* and *P. ovale* were not detected by microscopy.[20] For the success of malaria control/elimination program in Madagascar, observation of high proportions of SMI infections, particularly the distribution of non–*P. falciparum* parasite species, should be further assessed. There was one RDT$^-$ microscopy$^+$ LDR-FMA$^+$ sample (*P. falciparum* parasitemia 774 parasites/μL), which could be considered false negative or may have *pfhrp2* deletion. The specimens collected between April 2014 and August 2015 as part of this same study, however, showed no evidence of *pfhrp2* deletion in parasites from this population.[11] Similarly, previous studies elsewhere in Madagascar have found no evidence of this gene deletion.[28,29]

In addition to these epidemiological findings, this study offers a field evaluation of the SD Bioline Malaria Ag P.f/Pan RDT, focusing on a comparison between *P. falciparum* parasite densities by RDT results. Using *P. falciparum* parasitemia data as continuous values, instead of discrete values[13] or ranges of values,[14] samples detected by both PfHRP2 and pan-pLDH test bands showed a significantly higher parasitemia than those detected

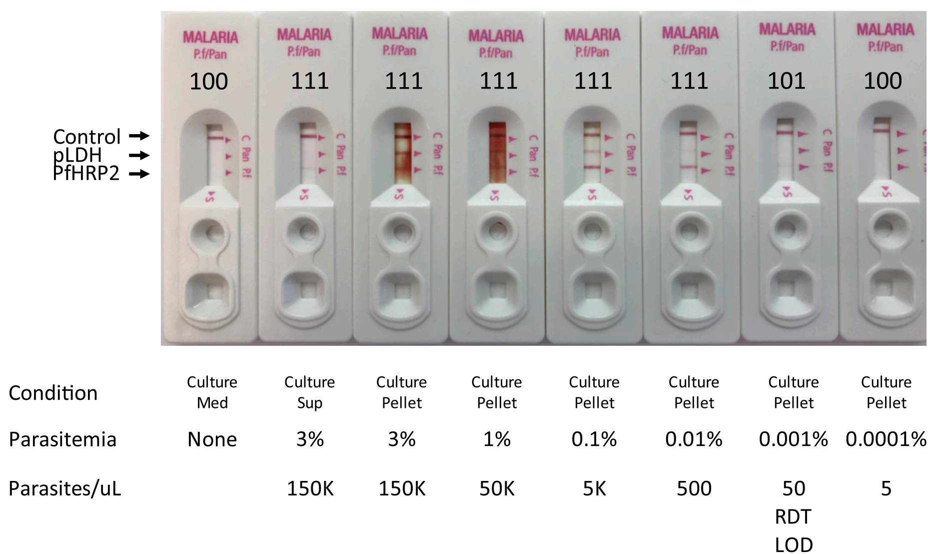

FIGURE 2. Rapid diagnostic tests (RDTs) showing band positivity in relation to varying parasitemia of the 3D7 culture. 100 = only control band positive, 101 = control and *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) bands positive, 111 = control, *Plasmodium* lactate dehydrogenase (pan-pLDH, pan), PfHRP2 bands positive. LOD = limit of detection, Med = medium, Sup = supernatant. The red background at 3% and 1% parasitemia could be because of the high red blood cells:buffer ratio, but did not obscure the test lines. This figure appears in color at www.ajtmh.org.

only by the PfHRP2 test band. Serial dilutions of *P. falciparum* strain 3D7 culture were also used to further validate that varying parasitemia is an important factor that influences the sensitivity of the different bands of this combination RDT. Together, these results are consistent with those previously reported using the same RDT.[13,14] Furthermore, similar patterns were also reported for other similar products, such as the CareStart Malaria HRP2/pLDH (Pf/pan) Combo RDT,[30] and for a different, *P. falciparum*–specific SD Malaria Ag P.f (05FK90) product, which targets PfHRP2 and Pf-pLDH.[31]

Among the samples that were diagnosed as *P. falciparum*–only infections by microscopy, there were *P. falciparum* mixed infections as detected by LDR-FMA; a number of them were *P. falciparum–P. vivax* mixed infections (Table 2). Although the prevalence of those mixed infections was low, there was a noticeable difference in their *P. falciparum* parasitemia based on their RDT status. It is likely that in the higher *P. falciparum*–parasitemic samples, the *P. vivax* infections were missed by microscopy. Although the RDTs targeting Pv-pLDH have undergone limited evaluation, rare but consistent false-positive Pv-pLDH test lines have been observed among *P. falciparum* samples, especially at high parasite densities.[16] Thus, depending on the type of RDT used, high *P. falciparum* parasitemia can lead to incorrect interpretation: either not mentioning the "true" possibility of mixed infection or mentioning the "false" possibility of mixed infection. In addition, even when both PfHRP2 and pan bands (pLDH or aldolase) were positive, the intensity of the pan band was lower than that of the PfHRP2 band.[13,14,30,31] Both band intensities (visually scored, five categories) were correlated with parasite densities, with considerable overlap between categories.[13,14,30] Faint bands are often difficult to see and may be missed by health workers in reduced lighting conditions or with reduced visual acuity.[13] This may inflate the proportion of RDTs reported as having a positive PfHRP2 band and negative pan band.

The detection limit of the SD Bioline Malaria Ag P.f/Pan RDT used in this study is 50 parasites/µL for the PfHRP2 antigen and 100 parasites/µL for the pan-pLDH antigen (manufacturer's note). The levels of pLDH activity seem to closely follow the levels of parasitemia in both initial diagnosis and while following treatment of patients with *P. falciparum* infection.[32,33] However, in regions with low parasite densities, pLDH-detecting RDTs appear to perform less well than PfHRP2-detecting RDTs.[1] On the other hand, there is only a very limited correlation between parasitemia and PfHRP2 level, at best, in clinical samples.[34–38] Because there are similar ranges of concentrations for PfHRP2 and pLDH antigens within *P. falciparum*–infected blood samples, with a significant positive correlation between the two, it may be that the difference in antibody-binding avidity between PfHRP2 (multiple binding epitopes) and pLDH (single epitope) is a reason for the observed differences in positivity and intensity of the respective test bands.[13] The PfHRP2 and pLDH antigen concentrations were not measured in our study, which may be a limitation to explain how high parasitemia influences the sensitivity of the different test bands.

The current Malagasy Ministry of Health guidelines indicate that RDT-positive participants be treated with a weight-adjusted course of AS-AQ, irrespective of the specific RDT results. In addition, the country's current national strategic plan (2018–2022) calls for dual applications of the antimalarial primaquine (PQ): 1) as a single low dose for blocking *P. falciparum* transmission in epidemic-prone zones and in low transmission areas targeting pre-elimination status and 2) in its 14-day formulation for *P. vivax* radical cure. However, neither application of PQ is widely deployed as yet, because of procurement and other logistical issues, and uncertainties relating to safety in glucose-6-phosphate dehydrogenase (G6PD)–deficient patients (NMCP, personal communication [A. C. R.]). Our recent survey of G6PD deficiency in the same population as described here found that it was relatively common among males (> 10%).[39] Whereas most of the phenotypically deficient cases (N = 37) that were genotyped for known *G6PD* single-nucleotide polymorphisms were associated with the A^{376G} or $A\text{-}^{202A/376G}$ variants (N = 25 of 26), one individual carried the severe $Mediterranean^{563T}$ variant.[39] It would be advisable to screen all *P. vivax* patients for G6PD enzyme activity before administering radical cure doses of PQ, given the limited access to emergency care in the event of an adverse event. In addition, to treat such patients correctly, it would be necessary to differentiate cases with both PfHRP2 and pan-pLDH bands positive into *P. falciparum*–only and *P. falciparum* mixed infections. As an alternative approach, it is recognized that better diagnostic tests are needed for *P. vivax*. A recent report presents and discusses the rationale for *P. vivax*–specific diagnostic target product profiles, contributing to the rational development of fit-for-purpose diagnostic tests suitable for the clinical management, control, and elimination of *P. vivax* malaria.[17]

Thus, although the current RDTs have enabled the paradigm shift from presumptive clinical diagnosis to parasite-based confirmation of malaria in areas where microscopy was unavailable, interpretation of the different RDT results is important to allow appropriate case management. As these RDTs are usually performed outside of laboratory settings, it is important for malaria control programs to develop quality control systems that ensure these front-line diagnostic tests are being performed, interpreted, and documented correctly.

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