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Short Communication

Performance of an immuno-rapid malaria *Pf/Pv* rapid diagnostic test for malaria diagnosis in the Western Brazilian Amazon

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Abstract

Introduction: Rapid diagnostic tests (RDTs) for detecting *Plasmodium* antigens have become increasingly common worldwide. We aimed to evaluate the accuracy of the Immuno-Rapid Malaria *Pf*/*Pv* RDT in detecting *Plasmodium vivax* infection compared to standard thick blood smear (TBS) under microscopy. **Methods:** Hundred and eighty-one febrile patients from the hospital's regular admissions were assessed using TBS and RDT in a blinded experiment. **Results:** RDT showed a sensitivity of 98.9%, specificity of 100%, and accuracy of 99.5% for *P. vivax* infection when compared to TBS. **Conclusions:** The RDT is highly accurate, making it a valuable diagnostic tool for *P. vivax* infection.

Keywords: Rapid diagnostic test. *Plasmodium vivax*. *Plasmodium falciparum*. Sensitivity. Specificity.

Malaria remains a prevalent and serious public health issue in developing countries. In 2015, the World Health Organization (WHO) reported 212 million malaria cases worldwide. On the American continent, Brazil has contributed to over 30% of these cases, the majority occurring in the Brazilian Amazon[1].

Malaria diagnosis is based on the microscopic examination of Giemsa-stained thick blood films collected by finger pricking. However, this diagnosis demands experienced and trained personnel to correctly identify the infection as well as a bright field microscope and reagents for staining. In the last decade, interest in the development of malaria rapid diagnostic test (RDT) kits for the detection of *Plasmodium* species has increased due to their stability, simple operation and storage, along with a better cost-effectiveness ratio compared to standard microscopy. RDTs detect malaria antigens, usually in 5-15 μL of whole blood, through an immunochromatographic assay containing monoclonal antibodies against specific parasite antigens such as *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2), plasmodial aldolase, and plasmodial lactate dehydrogenase (pLDH)[2-4].

The Brazilian Ministry of Health recommends the use of RDTs in non-endemic or isolated areas where microscopy is not readily available. In 2015, the National Program for Malaria Control (NPMC) suggested the SD-Bioline Malaria Ag *Pf*/*Pf*/*Pv*® RDT which relies on the detection of *Pf*HRP2 and pLDH as well as *P. vivax* pLDH[4].

The development and validation of such tests with high sensitivity that allow the specific detection of *P. vivax*, responsible for the highest number of malaria cases in the country, are of great importance for the improvement of RDT-

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based diagnosis. Thus, the aim of this study was to evaluate the diagnostic performance of the Immuno-Rapid Malaria *Pf*/*Pv* RDT (*Imuno-Rápido Malária Pf/Pv*) test for *P. vivax* malaria diagnosis in a tertiary health unit in the Western Brazilian Amazon.

This was a prospective study to assess the diagnostic performance of the Immuno-Rapid Malaria *Pf*/*Pv* RDT compared to that of light microscopy which is currently the gold standard for malaria diagnosis. Samples were collected between November 2016 and April 2017 from the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD) in Manaus, State of Amazonas, Brazil. This is a reference institution for malaria diagnosis, treatment, and research in the Western Brazilian Amazon and has received more than 5,000 malaria cases in the last 2 years, with almost a 100% of cases being *P. vivax* monoinfection[5]. Febrile patients were recruited consecutively based on the hospital's regular admissions, independent of sex or age. For this study, sex, age, and previous episodes of malaria were recorded on admission. After malaria diagnosis, patients were treated with the standard 3-day chloroquine and 7-day primaquine according to national guidelines[6]. All patients provided written informed consent. Approval for the study design was obtained from the FMT-HVD's ethics committee (CAAE number: 46481215.2.0000.0005).

Hospital laboratory staff performed both tests on admission; RDT and thick blood smear (TBS) analysis were blinded. For the TBS, fingerprick-collected blood samples were placed directly onto a clean slide and spread in a square-like form using the corner of another slide. After drying at room temperature, blood films were processed and colored using the Walker method[6] which uses a hypotonic solution of methylene blue for 2 seconds to lyse red blood cells and Giemsa for 10 minutes to stain the cells; each step is followed by gently rinsing with buffered water. Experienced microscopists then examined the slides under light microscopy and recorded the total parasite count per mm^3 of blood.

The Immuno-Rapid Malaria *Pf*/*Pv* RDT prototypes were provided by Wama Diagnóstica (São Paulo, Brazil) and stored according to the manufacturer's recommendations (2ºC-30ºC until the expiration date). This test uses monoclonal antibodies against pLDH and *Pf*HRP2 to diagnose *P. vivax* and/or *P. falciparum* infection, respectively. The test is composed of a ready-to-use test vial and diluent (buffered phosphate with 0.095% sodium azide). Briefly, 5 µL of fingerprick-collected blood was placed in the sample well. After the sample was absorbed, 2 drops of diluent were dispensed in the reagent well. Results were read exactly 20 minutes later and validated only if the control line was also shown.

The target sample size of 181 patients was used with an estimated power of 97.5%. We then compared the RDT results to those of the TBS, which is the gold standard. Positivity for *vivax* malaria by TBS was defined when any *P. vivax* blood-stage form was seen under light microscopy. For the RDT analysis, results were considered positive only when the control and *Pv* lines were both evident. Diagnostic parameters, such as sensitivity, specificity, positive and negative predictive values, and total accuracy with their respective 95% confidence intervals (CIs), were calculated for *P. vivax* malaria using the statistical package Stata version 13 (College Station, USA).

Of the total of 181 febrile patients who participated in the study, most were male (64.1%), and the mean age was 41.7 years. Most patients had up to 3 previous episodes of malaria (93.3%). Regarding test performance, 95 patients were positive for *P. vivax* (52.5%) and 3 for *P. falciparum* (1.6%) malaria via the TBS (**Figure 1**). One false-negative sample for *vivax* malaria by RDT had 60 parasites/mm^3 in TBS. Additionally, for *falciparum* malaria, 1 of the 3 infected patients presented a false-negative result although the sample contained 4,680 parasites/mm^3 of blood. The mean parasitemia detected by TBS for *P. vivax* malaria was 1,206.5 parasites per mm^3 of blood (**Table 1**). For *P. vivax* malaria, the RDT showed a sensitivity of 98.9% (95% CI 94.3-99.8), a specificity of 100% (95% CI 95.7-100.0), and a total accuracy of 99.5% (95% CI 96.9-99.9) when compared to TBS under light microscopy (**Table 2**).

It was shown that the Immuno-Rapid Malaria *Pf*/*Pv* RDT was effective in diagnosing *P. vivax* infection. RDTs have been used for malaria diagnosis worldwide, especially in areas where light microscopy examination cannot be carried out. In Brazil, the Ministry of Health initially suggested the use of RDTs such as ICT-*Pf*/*Pv*® and OptiMal® which allow the detection of *Plasmodium* spp. infection and differentiate *P. falciparum* from other species[6]. However, none of these tests are specific for *P. vivax*, the most prevalent species in Brazil and extra-African areas, which is often present in endemic regions that are difficult to access.

The performance of RDTs has been evaluated in the Brazilian Amazon. A study carried out in the State of Pará, which evaluated the performance of ICT-*Pf*/*Pv*® under various temperature conditions, showed 61.8% sensitivity and 100% specificity when compared to regular light microscopy, regardless of parasite density[7]. Another study also carried out in the Amazon region, evaluated the OptiMal-IT®. This test showed good sensitivity in all the conditions studied, only failing in 2 samples of *Plasmodium malariae*[8]. Similarly, a study performed at a primary health care unit in Manaus, Amazonas comparing OptiMal-IT® and ICT-*Pf*/*Pv*® rapid tests showed sensitivities of 72% and 78% and specificities of 92% and 100%, respectively, compared to light microscopy[9]. Therefore, it is essential that more sensitive diagnostic tools are validated to correctly identify infected patients.

In 2015, the NPMC of the Brazilian Ministry of Health indicated the use of the SD-Bioline Malaria Ag *Pf*/*Pf*/*Pv*® RDT for the diagnosis of malaria This test shows a 100% sensitivity for *Pf*HRP2, 99,7% for *P. falciparum* pLDH, 98.2% for *P. vivax,* and an overall specificity of 99.3%[4]. Detecting more than one species of *Plasmodium* in a single RDT could reduce the occurrence of false-negative results, thus improving diagnosis. The use of *P. vivax*-specific RDTs in endemic areas for surveillance, epidemiological studies, and active case search contributes to faster results and lower costs regarding infrastructure and human resources.

The Immuno-Rapid Malaria *Pf*/*Pv* test detects the main malaria-causing species in Brazil and is based on the detection

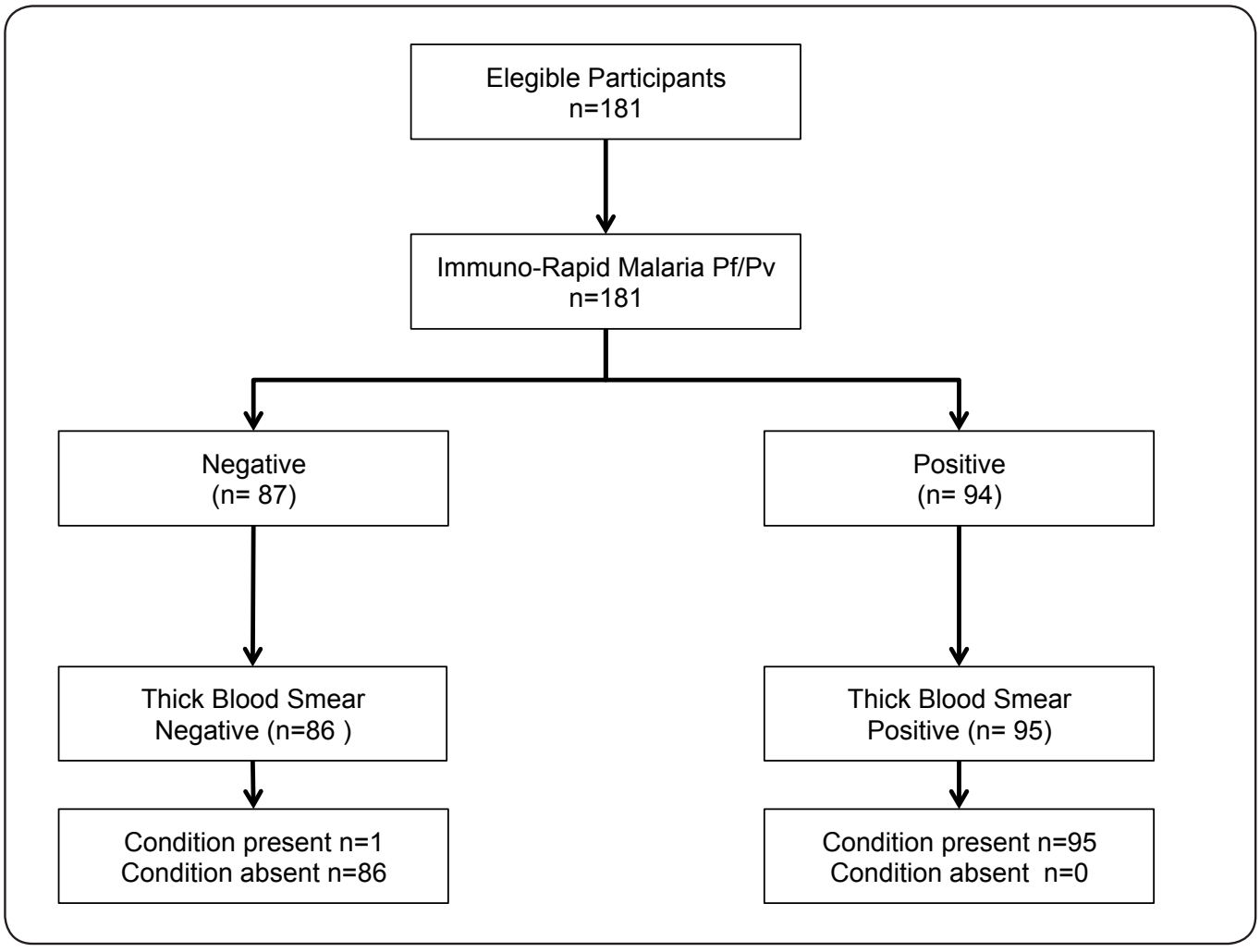

FIGURE 1: Flowchart of participants' inclusion for *P. vivax* malaria accuracy analysis.

of *Pf*HRP2 antigen and *P. vivax* pLDH. The focus of this study was the detection of *P. vivax* using the Immuno-Rapid Malaria *Pf*/*Pv* RDT test and performing an accuracy analysis using light microscopy as the gold standard. The test showed excellent sensitivity (98.9%) and specificity (100%), even higher than the tests suggested by the NPMC. Only 1 patient was misdiagnosed as negative for *vivax* malaria by RDT, and the sample had the lowest parasitemia among the samples analyzed (60 parasites/mm^3). A lower sensitivity of pLDH-based tests in samples with low parasitemia is described in the literature[10,3,11], and more tests, when available, should be performed in such cases to correctly identify patients. Despite this false-negative result, the Immuno-Rapid Malaria *Pf*/*Pv* RDT was effective in detecting parasitemia levels close to that observed in this sample. Therefore, the Immuno-Rapid Malaria *Pf*/*Pv* RDT may be a valuable and accurate tool, especially in isolated areas, since it does not require special training or lab equipment, ultimately enhancing *vivax* malaria diagnosis, treatment, and epidemiological surveillance.

In this study, one false-negative *P. falciparum* patient was observed. Recently, *Pf*HRP2/3 gene variations have challenged *P. falciparum* diagnosis when using RDTs. Several studies have reported deletions in genes encoding these proteins, thus leading

TABLE 1: Baseline demographic and laboratory data of 181 participants.

Variables	n (%)
Sex, n (%)	
M	116 (64.1)
F	65 (35.9)
Age [mean ± SD]	41.7 ± 14.4
Positivity for *P. vivax* by TBS (n, %)	95 (52.5)
Positivity for *P. vivax* by RDT (n, %)	94 (51.9)
Positivity for *P. falciparum* by TBS (n, %)	3 (1.6)
Positivity for *P. falciparum* by RDT (n, %)	2 (1.1)
P. vivax parasitemia by TBS [mean ± SD]	2706.5 ± 3763.7
Previous episodes of malaria (n, %)	
1	56 (30.9)
1-3	113 (62.4)
>4	12 (6.6)

SD: standard deviation; **RDT:** rapid diagnostic test; **TBS:** thick blood smear.

TABLE 2: Diagnostic performance of Immuno-Rapid Malaria *Pf/Pv* rapid diagnostic test compared to thick blood smear examination (gold standard) for detection of *P. vivax* malaria.

Accuracy parameters	% (n/N)	95% CI
Sensitivity	98.9 (94/95)	94.3 - 99.8
Specificity	100 (86/86)	95.7 - 100.0
Positive Predictive Value	100 (94/94)	96.1 - 100.0
Negative Predictive Value	98.8 (86/87)	93.7 - 99.8
Accuracy	99.5 (180/181)	96.9 - 99.9

CI: confidence interval.

to false-negative results[12-14]. In some areas of the Brazilian Amazon, the prevalence of *Pf*HRP2 and *Pf*HRP3 deletions is as high as 31% and 50%, respectively, as reported by Viana and colleagues[15]. Our study showed 1 false-negative patient by the RDT; however, molecular diagnosis could not be performed to assess gene deletions. In such cases, non-RDT confirmation should be performed, and more studies are needed to monitor *P. falciparum* gene deletions worldwide.

One limitation of this study was the insufficient number of samples of *P. falciparum* monoinfection or mixed infection (*vivax* + *falciparum*) cases during the study period to assess RDT performance in such situations. In addition, low parasitemia levels (mostly in asymptomatic individuals), kit quality (manufacturing conditions and reproducibility between batches), and storage conditions are factors that influence the RDT's performance. According to the WHO[3], the performance of RDTs is highly affected by environmental factors, such as elevated temperatures in tropical regions. Our results showed that despite testing in a health unit located in an area endemic for malaria in the Amazon region, it was possible to detect *P. vivax* infections with great success. However, more studies are needed to evaluate the performance of RDTs in real field conditions, which often involve wide temperature/humidity variations and harsh and usually long-lasting transportation to isolated areas. The use of RDTs in elimination settings requires steady efforts to maintain regular testing and quality case management. Furthermore, confirmation that *P. vivax* has been completely eliminated requires the detection of hypnozoites; however, available tests are still unable to detect these forms.

In conclusion, the Immuno-Rapid Malaria *Pf*/*Pv (Imuno-Rápido Malária Pf*/*Pv*) RDT showed considerable diagnostic performance in detecting *P. vivax* malaria. Care should be taken in situations of low parasitemia or submicroscopic infections and when molecular diagnostic tests are not promptly available since the RDT might yield false-negative results. Further studies are needed to access the reliability of *P. falciparum* diagnosis using this test in the field. The use of such diagnostic tools may represent a step towards malaria elimination in endemic areas.

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