Original Article

Low sensitivity of malaria rapid diagnostic tests stored at room temperature in the Brazilian Amazon Region

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Abstract
Introduction: In remote areas of the Amazon Region, diagnosis of malaria by microscopy is practically impossible. This study aimed to evaluate the performance of two rapid diagnostic tests (RDTs) targeting different malaria antigens stored at room temperature in the Brazilian Amazon Region.
Methodology: Performance of the OptiMal *Pf*/Pan test and ICT-Now *Pf*/Pan test was analyzed retrospectively in 1,627 and 1,602 blood samples, respectively. Tests were performed over a 15-month period. Kits were stored at room temperature in five community health centres located in the Brazilian Amazon Region. RDT results were compared with thick blood smear (TBS) results to determine sensitivity, specificity, and accuracy of the RDT.
Results: The sensitivities of the OptiMal *Pf*/Pan test were 79.7% for *Plasmodium falciparum* malaria diagnosis and 85.7% for non-*P. falciparum* infections. The results showed a crude agreement of 88.5% for *P. falciparum*, and 88.3% for non-*P. falciparum* infections (*Kappa* index = 0.74 and 0.75, respectively). For the ICT-Now *Pf*/Pan test (CI 95%), the sensitivities were 87.9% for *P. falciparum* malaria diagnosis and 72.5% for non-*P. falciparum* infection. Crude agreement between the ICT-Now Pf/Pan test and TBS was 91.4% for *P. falciparum* and 79.7% for non-*P. falciparum* infection. The *Kappa* index was 0.81 and 0.59 for the final diagnosis of *P. falciparum* and non-*P. falciparum*, respectively. Higher levels of parasitaemia were associated with higher crude agreement between RDT and TBS.
Conclusions: The sensitivities of RDTs stored at room temperature over a 15-month period and performed in field conditions were lower than those previously reported.

Key words: malaria; rapid diagnostic tests; sensitivity; specificity; field conditions; *Plasmodium falciparum*

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Introduction

In 2009, over 300,000 cases of malaria were reported in Brazil [1]; most of them were caused by *Plasmodium vivax*. Although less frequently, *Plasmodium falciparum* has also been responsible for severe cases, mainly when diagnosis and treatment have been delayed. Currently, early malaria diagnosis and treatment are the cornerstones of the Brazilian strategy for the disease control [1]. Microscopic detection of *Plasmodium spp.* using the stained blood smears is a simple, highly sensitive and low-cost method that is the gold standard for malaria diagnosis. However, this method is time-consuming and its interpretation requires considerable expertise, particularly at low levels of parasitaemia. Malaria rapid diagnostic tests (RDT) are a relatively new and evolving technology which can provide parasite-based diagnosis in remote areas where microscopy is difficult to support and there is limited control of test storage conditions and supervision of users [2].

RDTs are based on the antibody detection of antigens specific to the malaria parasite present in the blood of infected people, such as histidine rich protein-2 (HRP2), lactate dehydrogenase (pLDH), and aldolase [2,3]. In general, the sensitivity and specificity of RDTs are higher than 95% when they

are performed under good storage conditions and when parasitaemia levels are higher than 100 parasites/µL for *P. falciparum* or higher than 500 parasites/µL for *P. vivax* [4]. A higher the level of parasitaemia is associated with better the agreement between RDT and TBS [5,6,7].

The World Health Organization (WHO) recommends that RDT should be used only when its sensitivity and specificity are above 95% in patients with parasitaemia above 100 parasites/mL (WHO, 2000). In Brazil, the National Malaria Control Programme (NMCP) prescribes the use of RDT solely for remote, difficult to access, endemic areas in the Brazilian Amazon [8]. In these locations, the diagnosis of malaria through microscopic analysis is practically impossible because of harsh environmental conditions combined with limited access to electricity and refrigeration, which are necessary for optimal functioning of sensitive equipment. Moreover it is difficult to recruit trained microscopy professionals to work in these remote areas.

Most of the companies that manufacture RDT recommend that the kits be stored at temperatures ranging from 2ºC to 30ºC (35.6ºF to 86ºF). The expiration dates for these tests are usually established according to such conditions. It is well-known that the shelf life and sensitivity of RDT are reduced when the kits are stored in temperatures above those recommended [9]. It has already been demonstrated that increased temperatures lower the colour intensity of the chromatographic strips as well as the sensitivity of some RDTs for malaria antigens [10].

In the Brazilian Amazon the RDT kits are inevitably transported and stored at temperatures above 30°C (86° F) for prolonged periods of time. Therefore, it is important to monitor the quality and performance of the RDT when weather conditions are not ideal. The purpose of this study was to evaluate the performance of two RDT targeting different malaria antigens that were stored at room temperature in the Brazilian Amazon Region.

Methodology

The study was performed in five endemic municipalities for malaria in the Brazilian Amazon region: Colniza (MT), Porto Velho (RO), Manaus (AM), Macapá (AP) and Tucuruí (PA). Symptomatic patients with positive TBS for malaria attending health-care centres in the five selected cities were eligible to participate in this study. Signed, informed consent was obtained from all participants, or from a parent or legal guardian for subjects younger than 18 years old. All patients were treated and monitored by the research team using drugs recommended by the Brazilian NMCP. This study was approved by the Research Ethics Committee of the Federal University of Mato Grosso, as well as by the National Research Ethics Committee.

Patients participated in the study during a 15-month period from May 2005 to July 2006. To ensure availability of RDT kits throughout the period of the study, a similar number of tests was performed in each month. Approximately two tests per work day were performed in Colniza and in Porto Velho, and one test per work day was performed in Manaus, Tucuruí, and Macapá.

Two commercial test kits were used: the RDT OptiMal Pf/Pan malaria test kit (DiaMed, Cressier, Switzerland) and the RDT ICT-Now *Pf/*Pan malaria test kit (Binax, Inc., Scarborough, Maine, USA). The target antigens of the OptiMal *Pf*/Pan test are lactate dehydrogenase (LDH) specific for *P. falciparum (*pfLDH*)*, and LDH for all species of *Plasmodium (*panLDH*)*. The target antigens for the ICT-Now *Pf*/Pan test are histidine rich protein-2 *(*pfHRP2*)* to diagnose *P. falciparum* and pan-malarial aldolase (pan-aldolase). Both kits belonged to the same manufacturing lot and were stored under the same ambient conditions as the room in which the RDT were performed. In the field, the RDT were performed according to manufacturer's instructions by two trained and experienced professionals from the health-care centre.

All RDT kits were used within their expiry date. The same lot number for each RDT (46110.80.01 for the OptiMal *Pf/*Pan test and 017181 for the ICT-Now *Pf/*Pan test) were used over the course of the study. The RDT kits were transported in sealed 6 cm (2.4 inch) thick polystyrene foam boxes from the research centre to each health care centre, where the kits remained in the same boxes in which they were transported and placed in a ventilated, cool place. All centers were built with bricks and clay tile roofs, and remained open all day long. The foam box was opened in the morning and afternoon daily, and only for the time necessary to remove the kits that were going to be used. The temperature inside and outside the foam box was measured by a calibrated thermohygrometer (Incoterm, Porto Alegre-RS, Brazil; range 10°C to 50°C accuracy ± 1°C each time the box was opened.

Since the number of available kits at the beginning of the study was limited, only TBS-positive patients were included in this RDT analysis. A new TBS was performed to confirm the presence of *Plasmodium*

infection. The microscopic examination was performed by an experienced health professional from the health-care centre's staff. Parasite density was determined by applying a counting technique in which the number of parasites counted in 200 microscopic fields was converted to parasites per microlitre of blood, considering that 200 microscopic fields of the smear were equivalent to 0.4 μL of blood [11]. A different health professional who was not aware of the patient's TBS result conducted the RDT on the same blood sample.

The RDT results were compared with the TBS results to determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Results from patients showing mixed malaria infections after the microscopic review were excluded from the analysis. Since only TBS-positive patients were included in this study, samples from patients with TBS positive for *P. vivax* were used to determine the specificity of each RDT for the diagnosis of *P. falciparum* infection and samples from patients with TBS positive for *P. falciparum* were used to determine the specificity of each RDT for the diagnosis of non-*P. falciparum* infection. All slides with discordant TBS/RDT results were reviewed by an experienced malaria microscopist.

The agreement between RDT and TBS results was analysed by the *Kappa* index. Confidence intervals of 95% (CI 95%) were determined for all parameters analysed. Chi-square for linear trend were calculated in order to test the effect of the parasite density on the agreement between RDT and TBS. Analysis of variance was used to compare the mean temperature of the study sites, registered throughout the research period. Linear regression was used to test the relation between RDT accuracy and the amount of time the RDTs were stored under field conditions. Significance was set at $p < 0.05$. Analyses were performed using the Epidata Analysis package (version 2.2.1) (Epidata Association, Odense, Denmark).

Results

Of the 1,760 patients with acute malaria included in the study, 506 (28.8%), 477 (27.1%), 276 (15.7%), 207 (11.8%), and 294 (16.7%) were from Colniza, Porto Velho, Manaus, Macapá, and Tucuruí, respectively. Enrolled patients were predominantly male (71.4%) and the mean age was 27.2 (standard deviation [SD] 14.5) years, ranging from one to 79 years. Microscopic examination revealed that 1,158 (65.8%) of the subjects had *P. vivax*, 586 (33.3%) had *P. falciparum*, and 16 (0.9%) had mixed infections presenting with both *P. falciparum* and *P. vivax*. None of the patients were diagnosed with *Plasmodium malariae*. Of the 1,193 TBS performed, 153 (12.8%) revealed a parasitaemia level of 99 parasites/μL or less, 239 (20.0%) between 100 and 500 parasites/μL, 186 (15.6%) between 501 and 1000 parasites/μL, 469 (39.3%) between 1001 and 5000 and 146 (12.3%) with more than 5000 parasites/μL. The mean parasitaemia count was 2,302 (SD 3,420) parasites/μL.

During the 15 months of the study, the mean (SD) of the maximum room temperature at the five locations where the RDT were stored was 30.3°C (2.1°C), ranging from 20.2°C to 34.4°C. No significant difference in temperature was observed among the study sites. Inside the foam boxes where the kits were stored, the mean (SD) of the maximum temperature was 29.4°C (1.6°C), ranging from 22.7°C to 33°C.

The OptiMal *Pf/*Pan test was performed in 1,602 blood samples; negative results for *P. falciparum* were found in 1,098 (68.5%) patients, and positive results for P. *falciparum* were found in 504 (31.5%) patients. The ICT-Now *Pf/*Pan test was performed on 1,627 samples, and negative results for *P. falciparum* were found in 1066 (65.5%) patients, while positive results for P. *falciparum* were found in 561 (34.5%) patients (Table 1).

The OptiMal *Pf/*Pan test was positive in 428 of 537 individuals with *P. falciparum*-positive TBS and negative in 989 of the 1,065 individuals with *P. falciparum*-negative TBS. Thus sensitivity (CI 95%), specificity (CI 95%), PPV (CI 95%) and NPV (CI 95%) of the OptiMal *Pf/*Pan test were 79.7% (76.1%-82.9%), 92.9% (91.2%-94.3%), 84.9% (81.5%-87.8%) and 90.1% (88.2%-91.7%), respectively for *P. falciparum* malaria diagnosis. In 109 of the patients, the results of this RDT were false-negative; *i.e.*, 35 (6.5%) were positive for non-*P. falciparum* and 74 (13.8%) were negative for all species of *Plasmodium*. False-positive results were found in 76 (7.1%) patients with TBS negative for *P. falciparum* (Table 1).

Regarding the diagnosis of non-*P. falciparum* infections, the OptiMal *Pf/*Pan test showed true positive results in 913 of the 1,065 patients with *P. vivax* confirmed by TBS, and true negative reactions in 502 of the 537 patients with TBS negative for this species. The sensitivity (CI 95%), specificity (CI 95%), PPV (CI 95%) and NPV (CI 95%) of the OptiMal *Pf/*Pan test for non-*P. falciparum* was 85.7% (83.5%-87.7%) and 93.5% (91.1%-95.3%), 96.3% (94.9%-97.3%) and 76.8% (73.4%-79.8%), respectively.

Table 1. Performance characteristics of two RDT compared to microscopy in field conditions in the Amazon Region, Brazil

	RDT results	***Plasmodium falciparum*** infection			RDT results	***Plasmodium vivax*** infection		
		Positive	Negative	Total		Positive	Negative	Total
OptiMal *Pf*/Pan	*P. falciparum*	428	76	504	Non-*P. falciparum*	913	35	948
	Non-*P. falciparum*/Negative	109	989	1098	*P. falciparum*/Negative	152	502	654
	Total	537	1065	1602	Total	1065	537	1602
	Sensitivity (%; CI95%):	79.7; 76.1-82.9				85.7; 83.5-87.7		
	Specificity (%; CI95%):	92.9; 91.2-94.3				93.5; 91.1-95.3		
	Positive predictive value (%; CI95%):	84.9; 81.5-87.8				96.3; 94.9-97.3		
	Negative predictive value (%; CI95%):	90.1; 88.2-91.7				76.8; 73.4-79.8		
	Crude agreement (%; CI95%):	88.5; 86.8-89.9				88.3; 86.7-89.8		
	Kappa index (%; CI95%):	0.74; 0.69-0.79				0.75; 0.70-0.80		
ICT-Now *Pf*/Pan	*P. falciparum*	488	73	561	Non-*P. falciparum*	777	35	812
	Non-*P. falciparum*/Negative	67	999	1066	*P. falciparum*/Negative	295	520	815
	Total	555	1072	1627	Total	1072	555	1627
	Sensitivity (%; CI95%):	87.9; 85.0-90.4				72.5; 69.7-75.0		
	Specificity (%; CI95%):	93.2; 91.5-94.6				93.7; 91.4-95.4		
	Positive predictive value (%; CI95%):	87.0; 83.9-89.5				95.7; 94.1-96.9		
	Negative predictive value (%; CI95%):	93.7; 92.1-95.0				63.8; 60.5-67.0		
	Crude agreement (%; CI95%):	91.4; 89.9-92.7				79.7; 77.7-81.6		
	Kappa index (%; CI95%):	0.81; 0.76-0.86				0.59; 0.55-0.64		

OptiMal Pf/Pan: pfLDH and panLDH-based RDT.
ICT-Now Pf/Pan: HRP2 and aldolase-based RDT.

False-positive and false-negative results occurred in 35 (6.5%) and 152 (14.3%) patients, respectively.

The analysis of all the OptiMal *Pf*/Pan test results showed crude agreement (CI 95%) of 88.5% (86.8%-89.9%) for *P. falciparum*, and of 88.3% (86.7%-89.8%) for non-*P. falciparum* infections. The *Kappa* index (CI 95%) for these agreements were 0.74 (0.69-0.79) and 0.75 (0.70-0.80), respectively (Table 1).

The ICT-Now *Pf*/Pan test was positive in 488 of 555 individuals with *P. falciparum* positive TBS, with sensitivity (CI 95%) of 87.9% (85.0%-90.4%). Out of 1,072 individuals with TBS negative for *P. falciparum*, 999 were also negative by the ICT-Now *Pf*/Pan test, with specificity (CI 95%) of 93.2% (91.5%-94.6%). The PPV (CI 95%) and NPV (CI 95%) for *P. falciparum* infection were 87.0% (83.9%-89.5%) and 93.7% (92.1%-95.0%), respectively. For patients with TBS positive for *P. vivax,* sensitivity (CI 95%) of this RTD was 72.5% (69.7%-75.0%), specificity (CI 95%) was 93.7% (91.4%-95.4, PPV (CI 95%) was 95.7% (94.1%-96.9%), and NPV (CI 95%) was 63.8% (60.5%-60.7%). The crude agreement (CI 95%) between the ICT-Now *Pf/Pan* and TBS was 91.4% (89.9%-92.7%) for *P. falciparum* and 79.7% (77.7%-81.6%) for non-*P. falciparum*. The agreement adjusted by *Kappa* index (CI 95%) was 0.81 (0.76-0.86) and 0.59 (0.55-0.64) for the final diagnosis of *P. falciparum* and non-*P. falciparum*, respectively (Table 1).

The agreement between RDT and TBS results was negatively associated with the amount of time and room temperature in which the RDT was stored (Figure 1). The geometric mean of parasitaemia counts in patients with concordant results between the OptiMal *Pf*/Pan test and the TBS was 845.6 parasites/µL; in patients with discordant results the mean was 148.4 parasites/µL ($p < 0.0001$). For the ICT-Now *Pf*/Pan, the geometric mean of parasitaemia counts was 804.3 parasites/µL for concordant results and 403.4 parasites/µL for the discordant results ($p < 0.0001$).

To analyze the agreement between the results according to the antigens detected by the RDT, parasitaemia levels were stratified into less than 100, 100 to 500, 501 to 1,000, 1,001 to 5,000, and more than 5,000 parasites/µL. Agreement was positively correlated with the level of parasitaemia for both antigens detected for non-*P. falciparum*, and was statistically significant for aldolase ($p < 0.001$) and for LDH ($p < 0.001$). However, a negative correlation was observed between the agreement of the RDT targeting the HRP2 antigen for the diagnosis of *P. falciparum* and parasitaemia levels higher than 500 parasites/µL. A greater reduction in agreement occurred when the parasitaemia level was higher than 5,000 parasites/µL ($p < 0.001$). No significant negative correlation was observed for the RDT targeting the pfLDH antigen, even when the parasitaemia level was higher than 5,000 parasites/µL (Table 2).

Discussion

In general, studies evaluating the performance of RDT are conducted under adequate conditions in which tests are transported and stored at temperatures recommended by the manufacturer. Several studies have shown high sensitivity and specificity of the RDT, regardless of the antigens targeted by the RDT [6,12,13,14,15,16]. In this study, RDT were conducted exclusively under field conditions and were exposed to environmental conditions typical of the Brazilian Amazon region for a prolonged period of time. The sensitivities and accuracies of OptiMal *Pf*/Pan and ICT-Now *Pf*/Pan tests were lower than those reported in studies performed in Africa, Asia, South America and at some travel medicine clinics [6,12,13,14,15,16,17,18,19]. Low agreement between RDT and TBS results was found for both the diagnosis of *P. vivax* and *P. falciparum,* regardless of whether panLDH, pfLDH, aldolase, or HRP2 was targeted. Similar findings were also obtained by Ratsimbasoa *et al.,* who evaluated three RDT targeting *P. falciparum* pLDH and pan-malaria pLDH under storage conditions similar to those used in this study [20]. It is probable that the low agreement between test results found in this study was caused by RDT being stored at higher temperatures than those recommended. For the diagnosis of *P. falciparum* using pfLDH-based RDT, The sensitivity of pfLDH-based RDT in the diagnosis of *P. falciparum* was also found to be lower than that found in previous studies [6, 12], including those conducted under field conditions [20]. Exposure to high temperatures (with or without high humidity) has been suggested as a possible explanation for the poor performance of RDT in the tropics. RDT components can be affected by heat in several ways: heat-induced denaturation of antibodies in the test membrane can prevent their binding to the target antigen; damage to the nitrocellulose membrane forming the strip can change its flow characteristics or cause the antibody to detach from the membrane; and vulnerability to heat can vary with different RDT cassette designs [10].

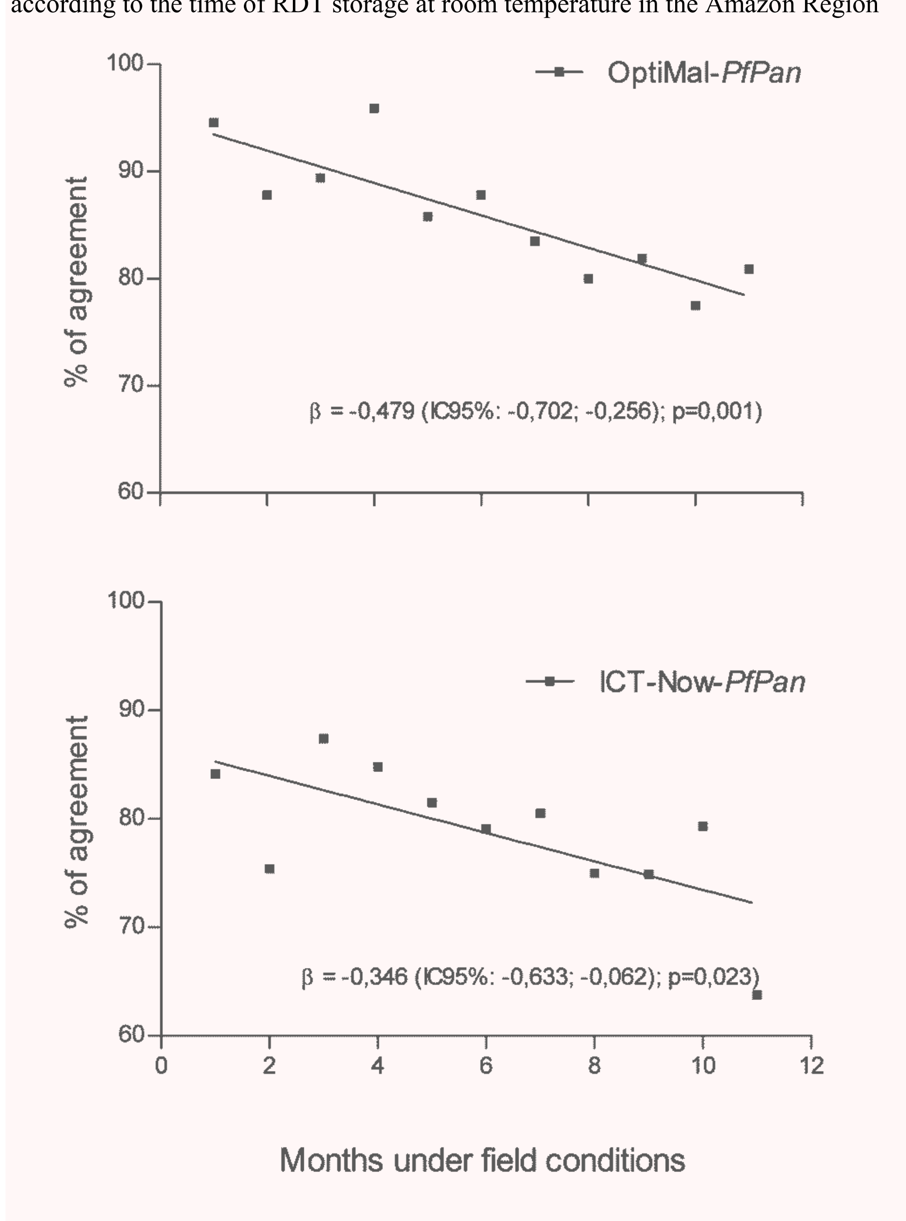
The sensitivity of the ICT-Now Pf/Pan test for non-*P. falciparum* diagnosis was lower than that reported in other studies [14,21]. In fact, low

Table 2. Agreement between the results of two RDT and microscopy, according to the level of parasitaemia and the RDT targeting malaria antigens

Parasites/ µL	OptiMal *Pf*/Pan							ICT-Now *Pf*/Pan						
	Overall results			**panLDH**		**pfLDH**		**Overall results**			**Aldolase**		**HRP2**	
	True (positive + negative)	False (positive + negative)	OR	n (%)	OR	n (%)	OR	True (positive + negative)	False (positive + negative)	OR	n (%)	OR	n (%)	OR
>5000	121 (85.8%)	20 (14.2%)	2.9	97 (92.4)	6.4	24 (66.7)	0.8	122 (85.9%)	20 (14.1%)	3.6	95 (92.2)	7.7	27 (69.2)	0.1
1001-5000	386 (91.7%)	35 (8.3%)	5.3	301 (94.7)	9.3	85 (82.5)	2.0	354 (87.2%)	52 (12.8%)	4.0	263 (88.0)	4.6	91 (85.0)	0.3
501-1000	162 (90.0%)	18 (10.0%)	4.3	106 (91.4)	5.6	56 (87.5)	2.9	137 (80.1%)	34 (19.9%)	2.4	77 (72.0)	1.6	60 (93.8)	1.1
100 - 500	154 (75.5%)	50 (24.5%)	1.5	90 (80.4)	2.2	64 (69.6)	0.9	154 (66.4%)	78 (33.6%)	1.2	60 (45.5)	0.5	94 (94.0)	2.2
< 100	98 (67.6%)	47 (32.4%)	1.0	59 (65.6)	1.0	39 (70.9)	1.0	94 (63.1%)	55 (36.9%)	1.0	44 (47.8)	1.0	50 (87.7)	1.0
	$p < 0.001$*			$p<0.001$*		$p=0.27$*		$p < 0.001$*			$p<0.001$*		$p<0.001$*	

*: *Chi-square for linear trends.*

Figure 1. Agreement between the results of two RDT and microscopy for the malaria diagnosis according to the time of RDT storage at room temperature in the Amazon Region



sensitivity for this species has been previously described for the *Plasmodium* aldolase-based RDT, even when these tests were conducted under laboratory conditions [18,22]. This observation suggests that aldolase is an antigen with low sensitivity for the diagnosis of *P. vivax*, regardless of the environmental conditions under which this test is performed. Another explanation for the low performance of the aldolase-detecting RDT would be the low concentration of the parasite´s aldolase due to the presence of a polymorphism of the gene responsible for the production of aldolase. This finding was already been reported for some *Plasmodium sp* isolates of various origins, including some from Brazil [23].

On the other hand, the ICT-Now *Pf/*Pan test was observed to be a highly sensitive test for the diagnosis of *P. falciparum*, probably because of the presence of anti-HRP2 in the RDT. A meta-analysis of the performance of several RDTs showed that the HRP2-detecting RDT for the diagnosis of *P. falciparum* demonstrate a higher degree of agreement with the

TBS method [24]. However, the agreement observed in this study was lower than that found in similar studies performed in malaria endemic areas of Africa [13,16,17], Asia [15], South-America [5], in travel medicine clinics in Europe [18] and the United States [14], and even in a group of patients with high levels of parasitaemia. Although exposure to high temperatures can explain the low sensitivity of the ICT-Now *Pf*/Pan test for the detection of *P. falciparum* [10], other factors must also be considered. For example, the absence of the target antigen in the parasite due to polymorphism or deletion of the gene that codes for HRP2 found in strains isolated in several malaria endemic regions is a possibility [23,25,26]. However, the occurrence of these polymorphisms was described in just one strain of *P. falciparum* isolated in Brazil [27].

Previous studies have demonstrated that parasitaemia is a decisive factor in the performance of different RDTs, regardless of the antigen targeted by the test [19,24,28,29]. Curiously, parasitaemia levels of the patients evaluated in this study were not positively associated with the RDT performance for the diagnosis of *P. falciparum*. On the contrary, a higher frequency of false negative results in the ICT-Now *Pf/*Pan test occurred in patients with parasitaemia levels higher than 5,000 parasites/µL. These findings have been previously described for tests targeting HRP2 performed in blood with parasitaemia levels higher than those described here, and have been attributed to the prozone effect, in which false-negative or false-low results in immunological tests are caused by an excess of either antigens or antibodies in the blood [30,31].

Of the numerous studies published on the performance of RDT, only two of them state that the tests were stored at room temperature [20,32]. In most of the studies performed in endemic regions the tests were kept under ideal storage conditions [5,15,17,25]. In this study, the intentional exposure of the RDT to a wider temperature range (20.2°C to 34.4°C) for a prolonged period of time can be considered the main factor responsible for the low sensitivity observed. In fact, other investigators have observed a significant reduction in the levels of agreement between the results of the RDT and the TBS under simulated high temperatures ranging from 35°C to 60°C [10,33]. In these cases, a lower level of agreement occurred in tests based on panLDH or aldolase enzymes, even when associated with HRP2.

In Brazil, the use of RDT is recommended in locations that do not have the infrastructure for the installation of cooling equipment. The temperature in such places is usually high during most of the year, and this can affect the performance of the RDT. Also noteworthy is the observation that RDT showed low sensitivity for the diagnosis of *P. vivax*, the most common species in the Brazilian Amazon. The WHO recommends that RDT should be applied in patients with parasitaemia levels above 100 parasites/ µL only when its sensitivity and specificity are above 95% [3]; thus the results of this study demonstrate the need for not only meticulous selection of the RDT to be used in programmes to control malaria, but also strict adjustment of its distribution and storage routine.

Some limitations of this study should be mentioned. It is possible that cross-reactions between *falciparum* and non-*falciparum* species may have underestimated the specificity of the RDTs since the RDT were performed only in *Plasmodium* positive samples. However, it is known that the rate of cross reactivity among HRP-2, pLDH and aldolase is very low [2]. Moreover, the specificities detected in this study for both RDTs were not different from those reported in studies which performed the tests under ideal conditions [4,34]. Another limitation of this study involves the quality of microscopic species differentiation; TBS were not read twice on an independent basis, which may have underestimated both the specificity and sensitivity of the tests. Unfortunately, the microscopy was not corroborated by PCR in this study. However, as all slides with discordant TBS/RDT results were reviewed and verified by an experienced malaria microscopist, the likelihood of bias was low. In addition, it is possible that the accuracy of both RDTs may have been overestimated as the reader of RDT was not blinded in this study and only TBS positive patients were included. This could have overestimated the accuracy of both RDT.

Other factors could explain the lower performance of the RDT found in this study, in comparison with the results seen in similar studies, including level of parasitaemias and field constraints (*e.g.*, light for the RDT result reading, expertise of the technicians, field studies facilities in comparison to reference, *etc*.). In fact, the average parasitaemia patients included in this study was lower than that reported in other studies [5,6] and should have led to false negative results of RDT, as already demonstrated [19].

Conclusions

The sensitivities of the two RDTs studied (the OptiMal *Pf/*Pan test and the ICT-Now *Pf/*Pan test)

stored at room temperature over a 15-month period and performed in field conditions was lower than those previously reported. The OptiMal *Pf/Pan* test showed better sensitivity and agreement with TBS than the ICT-Now *Pf/Pan* test for the diagnosis of *P. vivax*. In addition, the RDT based on the detection of HRP2 showed better sensitivity and agreement with the TBS than that based on the detection of *pf*LDH for the diagnosis of *P. falciparum.* For the diagnosis of *P. vivax*, higher levels of parasitaemia corresponded with better the agreement between RDT and TBS results. However, parasitaemia levels higher than 1 000 parasites/µL were negatively associated with the agreement between the RDT and TBS results for the diagnosis of *P. falciparum*, regardless of the RDT evaluated.

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