A Novel Immunochromatographic Test Strip Versus A Commercial Rapid Diagnostic Test for the Detection of Imported *Plasmodium* Species in Guangxi Province, China: A Comparative Evaluation

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Abstract

Background: China has made significant progress towards malaria elimination by achieving zero reports of indigenous malaria cases over two consecutive years. The diagnosis of malaria, which is based on microscopic examination, still remains a challenge, due to a lack of sensitivity for detecting low-level parasitaemia. This study aimed to evaluate the diagnostic value of a novel immunochromatographic test strip for imported malaria.

Methods: After obtaining informed consent, blood samples were collected from migrants returning home from Africa to Shanglin County in Guangxi, PR China, in 2018-2019, and were tested with the novel test strips. The test results were compared with those of microscopic examination, commercial malaria rapid diagnostic test (RDT), and polymerase chain reaction (PCR) assay.

Results: A total of 535 samples were tested. Both microscopy and PCR test results showed a total of 162 (30.28%) malaria-positive samples, of which 99 were positive for *Plasmodium falciparum* (*P. falciparum*), three for *P. vivax*, five for *P. malariae*, and 50 for *P. ovale*, whereas five had mixed infections. The sensitivity, specificity, and efficiency of the novel RDT were 74.1%, 93.0%, and 87.3%, respectively, whereas its sensitivity of detecting *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* was 91.9%, 100%, 20.0%, and 44.0%, respectively. Furthermore, the sensitivity, specificity, and efficiency of the commercial RDT were 86.0%, 89.3%, and 88.2%, respectively, whereas its sensitivity of detecting *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* was 96.0%, 100%, 40.0%, and 70.0% respectively. The differential detection of *Plasmodium* species using the new RDT was significantly better compared to that of the commercial RDT (c^2 = 14.73; *P* = 0.0001), which can detect only *P. falciparum*. This was consistent with Kappa analysis (Kappa = 0.881). A significant difference (c*2* = 24.50; *P* < 0.0001) in the detection rate of other *Plasmodium* species was observed with an average consistency using Kappa analysis (Kappa = 0.716).

Conclusion: The novel malaria RDT test strip had high sensitivity and specificity in detecting *P. falciparum* and *P. vivax*. Improvement in *P. ovale* and *P. malariae* detection efficiency may lead to the scale-up of this RDT in the future.

Background

Malaria is one of the parasitic diseases that endanger human health with an estimated 228 million recorded malaria infection cases, and 405,000 deaths, worldwide, in 2018 [1]. Since the launch of the *National Malaria Elimination Action Plan* (2010–2020) in 2010, China has made significant achievements in malaria elimination [2], with zero reports of local indigenous cases for two consecutive years (2017 and 2018) [3, 4]. However, with frequent international trade and Chinese foreign investments, there has been an increase in imported malaria infections. Between 2011 and 2016, nearly 3,000 cases of imported malaria cases were reported each year [5]. This put the entire country at risk for malaria infections, especially in areas which still harbour mosquito *Anopheles* vectors. Without a timely diagnosis and effective treatment, the appearance of new cases of severe malaria, mainly caused by *Plasmodium falciparum* (*P. falciparum*), could be catastrophic [6], presenting a challenge in the malaria elimination efforts in China [7]. Early and accurate detection of malaria infection is important in reducing its associated morbidity and mortality. The gold standard for the detection of malaria infection is microscopic examination, while the most reliable method is genetic examination using polymerase chain reaction (PCR) or deoxyribonucleic acid (DNA) sequencing [2]. However, rapid diagnostic tests (RDTs) are an alternative to diagnosis based on clinical grounds or microscopy, particularly where good quality microscopy services cannot be readily provided. RDTs detect different target antigens for *P. falciparum* identification, such as histidine rich protein 2 (HRP2) [3–5] or *P. falciparum*-specific lactate dehydrogenase (PfLDH) [5–7], whereas *P. vivax*-specific lactate dehydrogenase (Pv-pLDH) [8], and aldolase [3, 5, 6] or *Plasmodium* lactate dehydrogenase (pLDH)[5, 7, 9, 10] are detected for *P. vivax*, *P. ovale* and *P. malariae* identification. Although the usefulness of RDTs for *P. falciparum* identification is well recognised [11, 12], few studies have described the performance of RDTs in the identification of imported *Plasmodium* species, particularly that of *P. ovale* and *P. malariae*.

The purpose of this article was to determine the diagnostic performance of a recently developed immunochromatographic test strip by comparing the test results with those of microscopic examination, PCR, and commercial malaria RDT, using blood samples collected from Chinese migrants returning from Africa to Shanglin County in Guangxi during 2018–2019.

Methods

Study area and population

Shanglin County is located in the southeast of Guangxi. It is home to the largest population of migrant workers from Africa and Southeast Asia, where the imported malaria cases account for 80.0% of the total number of cases in Guangxi each year. The study was conducted in Shanglin County in Guangxi, China between 2018 and 2019. Chinese migrants returning from Africa, with or without fever, willing to sign an informed consent form were included in the study. Migrants who failed to sign the consent form or were unable to provide a blood sample were excluded from the study. A total of 535 participants were enrolled in the study.

Malaria diagnosis using microscopy

Three blood smears were prepared for each study participant. Briefly, three Giblet-stained blood smears were prepared for each participant, including thick and thin smears, and then examined under a microscope by two experienced microscopists, according to the microscopic examination of blood smear for *Plasmodium* detection guide, (WS/T 569–2017) [10]. When no *Plasmodium* parasites were found in at least 200 fields, or the entire blood smear, the test result was declared negative. Any observation of *Plasmodium* parasite was considered positive, and was followed by the determination of *Plasmodium* speciation. When there was a discrepancy in the interpretation of the microscopy results, a third microscopist with a level 1 World Health Organization (WHO) external competency assessment certificate evaluated the controversial results and made the final decision. The RDTs were performed and the results were recorded immediately. The malaria diagnosis reference laboratory in the Guangxi Zhuang Autonomous Region used microscopy and PCR to check all the positive cases, and randomly screened 30% of the negative blood samples for blind check. When either the microscopy or PCR test result was positive, a

malaria diagnosis was provided. The workers returning from Africa with negative test results were allocated to the control group. Furthermore, 100 blood samples were collected randomly from examinees at Guangxi Centre Disease Control and Prevention and used as negative controls.

Malaria diagnosis using nested PCR

The iAUTOMAG automatic nucleic acid extraction instrument AU1001 (BioTeke Corporation), C1000 PCR instrument (Bio-Rad), CX23 optical microscope (Olympus), QIAamp DNA Mini Kit (Qiagen), PCR Premix TaqDNA polymerase (Takara), RDT reagent strip (commercial RDT with production batch number of W05480105), and our newly developed malaria diagnostic immunochromatographic test strip capable of differential detection of *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*., were used.

Extraction of DNA using a DNA extraction kit (Qiagen, Hilden, Germany) was conducted according to the manufacturer's instructions. The 18SSU r RNA gene was chosen as the target for malaria detection. Primers used for the study. Nested PCR reactions in a total volume of 25 μL for each reaction were performed. In the first round of PCR amplification the following components were included in the samples: template DNA 2 μL, upstream and downstream primers 0.5 μL, Premix Taq enzyme 12.5 μL, ddH_2O 8.5 μL. The reaction conditions were as follows: 94 ℃ 3 min, 94 ℃ 30 sec, 58 ℃ 30 sec, 72 ℃ for 1 min, 35 cycles; and finally 72 ℃ for 5 min. In the second round of PCR amplification the following components were included in the samples: template DNA 2 μL, upstream and downstream primers 0.5 μL, Premix Taq enzyme 12.5 μL, ddH_2O 8.5 μL. The reaction conditions were as follows: 94 ℃ 3 min, 94 ℃ 30 sec, 58 ℃ 30 sec, 72 ℃ for 1 min, 35 cycles, and finally 72 ℃ for 5 min. The PCR products from the second round were then analysed by 2.5% agarose gel electrophoresis and observed with a gel imager.

Malaria diagnosis using RDTs

Two types of RDTs were used to test the blood samples. The commercial RDT was used according to the manufacturer's instructions (Table 1). The novel malaria RDT is an immunochromatographic test strip that uses antibodies to detect malaria. Briefly, 5 μL of whole blood sample was first added to a reaction which was then stopped by adding four drops of buffer solution. The results were interpreted within 15 minutes. For the novel RDT test strip, first, 5 μL of whole blood sample was added, followed by four drops of cell dissociation buffer solution. The results were read within 15 minutes. The novel malaria RDT test strip has a control line (C), a detection line "1" indicating a *P. falciparum* infection (T1), a detection line "2" indicating a *P. vivax* infection (T2), and a detection line "3", indicating an infection by other species of *Plasmodium*.

Table 1
Interpretation of the results of the novel malaria rapid diagnosis test (RDT)

Positive test		**Negative test**		**Invalid test**	
Line	Interpretation	Line	Interpretation	Line	Interpretation
C plus T1	Positive for pf only	C only	Negative	No line	Invalid test Repeat test with a new kit
C plus T2	Positive for pv or pm or po or a mix of these. Differentiation of the species is not possible.			T1 or T2 only	Invalid test Repeat test with a new kit
C plus T1 and T2	Positive for pf,it may also represent a mixed infection of pf with pv,pm or po. Differentiation between a pf only infection and a mixed infection containing pf and another malaria species is not possible with this test.			T1 or T2 only	Invalid test Repeat test with a new kit
1. Source: Commercial RDT reagent insert 2018 page 6.					

2.Pf, *P. falciparum*; Pv, *P. vivax*; Pm, *P. malariae*; Po, *P. ovale*.

Data analysis

The data were recorded using the Microsoft Excel 16.0 software. The descriptive analysis of the test results was performed using the SPSS 17.0 software. The microscopy and PCR results were set as the gold standard. The sensitivity, specificity, and accountability (Kappa data) of the novel malaria RDT test strip were evaluated in comparison with those of the commercial RDT test kit.

Results

From a total of 535 blood samples collected from Chinese migrants, 162 (30.28%) tested positive for malaria infections, of which 99 (61.11%) were positive for *P. falciparum*, three (1.85%) were positive for *P. vivax*, five (3.09%) for *P. malariae*, 50 (30.86%) for *P. ovale*, while five had mixed infections. The median, maximum, and minimum age of the study participants were 40.87, 64 and 18, respectively. More than 98.32% of the study participants were male. The microscopy and PCR malaria positivity rates, used as the reference standards, were 30.28%, while the malaria positivity rates using the novel RDT and the commercial RDT were 22.43% and 25.98%, respectively. The PCR malaria positivity rate, used as a reference standard, was 30.28%.

Visual comparison of the results of the novel and commercial RDT in the Plasmodium **blood sample test**

The novel malaria RDT could differentiate *P. falciparum* (colour in T1) and *P. vivax* (colour in T2, no colour in T1). A positive test for *P. malariae* or *P. ovale* infection was indicated by colour in T3 (no colour in T1 and T2). However, it was challenging distinguishing between *P. ovale* and *P. malariae* infections. The commercial RDT test could differentiate *P. falciparum* (T1 displaying colour) from other *Plasmodium* species (*P. vivax, P. malariae*, or *P.ovale*). The presence of these three *Plasmodium* was indicated by colour in T3 (no colour in T1 and T2). However, this test could not distinguish between these three species. (Fig. 1)

The detection of different Plasmodium **species using the two RDTs**

Using the microscopy and PCR results as the reference standards (a case was regarded positive when either test result was positive), the novel malaria RDT had a total sensitivity of 74.1%, specificity of 93.0%, and test efficiency of 87.3%, Kappa = 0.690 ($P < 0.0001$). The sensitivity of detecting *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* was 91.9%, 100%, 20.0%, and 44.0%, respectively. The commercial RDT had a total sensitivity of 86.0%, specificity of 89.3%, and test efficiency of 88.2%, Kappa = 0.729 ($P < 0.0001$). The sensitivity of detecting *P. falciparum, P. vivax, P. malariae*, and *P. ovale* was 96.0%, 100%, 40.0%, and 70.0%, respectively (Tables 2 and 3).

Table 2
Test results of the two RDTs in imported cases

Microscopy results	Number of cases	Number of positive cases				
		Commercial RDT		Novel malaria RDT		
		Pf	Pan	Pf	Pv	Pan
Pf	99	95	/	91	/	/
Pv	3	0	3	0	3	/
Pm	5	0	2	0	0	1
Po	50	0	35	0	0	22
Mix	5	2	2	2	1	0
Total	162	139		120		
(-)	373	40		26		

(Among the five mix infection cases, two were Po and Pf, one was Po and Pv, and two were Po and Pm). Pf, *P. falciparum*; Pv, *P. vivax*;; Pm, *P. malariae*; Po, *P.ovale*.

Table 3
Diagnostic performance of the novel and commercial RDTs in the detection of malaria

Diagnostic parameter	Total		Pf		Pv		Pm		Po	
	Commercial	Novel	Commercial	Novel	Commercial	Novel	Commercial	Novel	Commercial	Novel
True positive	139	120	95	91	3	3	2	1	35	22
True negative	333	347	333	347	333	347	333	347	333	347
False positive	40	26	40	26	40	26	40	26	40	26
False negative	23	42	4	8	0	0	3	4	15	28
Sensitivity (%)	85.8	74.1	96.0	91.9	100	100	40.0	20.0	70.0	44.0
(95% CI)	79.25–90.60	66.49–80.49	89.39–98.70	84.24–96.19	31.00-100	31.00-100	7.26–82.96	1.05–70.12	55.22–81.71	30.27-58.65
Specificity (%)	89.3	93.0	89.3	93.0	89.3	93.0	89.3	93.0	89.3	93.0
(95% CI)	85.58–92.14	89.83–95.31	85.58–92.14	89.83–95.31	85.58–92.14	89.83–95.31	85.58–92.14	89.83–95.31	85.58–92.14	89.83-95.31
Diagnostic efficiency (%)	88.2	87.3	90.7	92.8	89.4	93.1	88.6	92.1	87.0	87.2

Differential analysis of P. falciparum **and other** Plasmodium **species**

The commercial RDT had a specificity of 89.3%, with a *P. falciparum*-detection sensitivity of 96.0% (Kappa = 0.752; $P < 0.0001$), while its detection sensitivity for other *Plasmodium* species was 69.0% (Kappa = 0.502; $P < 0.0001$). The novel malaria RDT had a specificity of 93.0%, with a *P. falciparum*-detection *s*ensitivity of 91.9% (Kappa = 0.796;$P < 0.0001$) and a detection sensitivity of 44.8% for other *Plasmodium* species (Kappa = 0.396; P < 0.0001) (Table 4).

Table 4
Comparison of test results between *P. falciparum* and other species

Microscopy results	Sample number	Commercial RDT			Novel malaria RDT		
		Positive	Negative	Detection rate (%)	Positive	Negative	Detection rate (%)
Pf	99	95	4	96.0	91	8	91.9
Other *Plasmodium* species	58	40	18	69.0	26	32	44.8
Mix.	5	4	1	80.0	3	2	60.0
(-)	373	40	333	10.7	26	347	7.0

A differential visual comparison between the commercial and novel malaria RDTs showed a statistically significant difference ($\chi 2 = 14.73$; $P = 0.0001$) in the *P. falciparum* detection rate, with a good consistency according to Kappa analysis. Furthermore, a statistically significant difference ($\chi 2 = 24.50$; $P < 0.0001$) regarding the detection rate of other *Plasmodium* species was observed with Kappa analysis-based average consistency (Kappa = 0.716).

Discussion

With the acceleration in the progress towards national malaria elimination in 2020 [11], China needs to ensure timely detection and treatment of active malaria cases to prevent reintroduction. However, as China continues its efforts in the pursuit of malaria elimination and sees no indigenous cases nationwide for many years, plus frequent transfer of the microscopists, it is challenging to maintain the capacity of microscopy [13]. Moreover, the low density of *Plasmodium* and protozoan morphology within asymptomatic carriers in endemic areas also contribute to difficulty in sustaining the capacity of microscopy [14].

In recent years, China has seen a fair number of imported *P. vivax* and *P. ovale* malaria cases. These two species are difficult to be detected using the traditional microscopy technique, hindering the accomplishment of malaria control [15]. RDTs based on immunochromatographic analysis technology have developed rapidly recently, with nearly one hundred commercial kits on the domestic and foreign markets. RDTs are simple and convenient to operate and time-saving, as the test results are straightforward and easy to interpret, and no other instruments are needed. After training, healthcare professionals can skilfully use RDTs. Following continuous improvement, RDTs now show good sensitivity and specificity [16].

RDTs have been recommended by the WHO as a convenient visual way of diagnosing malaria [17]. A study in Sudan by Osman et al., [18] showed that the RDTs they used produced a relatively high number of false positive samples. The RDT was compared to PCR and an agreement of only 81.2 and k = 0.48 (sensitivity 69%, specificity 84%) was reported. Herrera et al., [16] in a study in endemic areas of Colombia, showed that the percentage concordance between visual and device interpretation of RDT was 98.5% and 99.0% for *P. vivax* and *P. falciparum*, respectively. Megnekou et al. [19] used RDTs to test individuals at the Marie Reine Health Centre in Etoudi, a peri-urban area of Yaoundé, Cameroon, from August 2013 to January 2015. The specificity of these RDTs was 96.49% and 100%. Studies have shown differences in the ability of different RDT products to detect malaria. In recent years, it has also become a common way for routine or active malaria surveillance of four *Plasmodium* species differentiation among migrants [20, 21]. To maximise the test outcomes, it is imperative to further study and optimise the RDT products.

A total of 162 malaria positive cases were detected using the novel RDT product in Guangxi, China. The results showed that the novel RDT had a general sensitivity of 74.1%, a specificity of 93.0%, and a test efficiency of 87.3%, (Kappa = 0.690; $P < 0.0001$), among which the specificity and sensitivity for *P. falciparum* detection were 93.0% and 91.9%, respectively. Collectively, our data showed that the two RDTs had a similar capacity for *P. falciparum* detection, specificity, and test efficiency. Both test strips could detect the three samples of *P. vivax*, but statistical significance was not observed due to the low sample number.

The on-site test result showed that the novel malaria RDT products had potential advantages over the commercial RDT products. For example, the novel RDT could distinguish *P. falciparum* from *P. vivax*. This could help local medical institutions adopt a timely and appropriate treatment. Furthermore, due to the wide distribution of *Anopheles sinensis* in most endemic provinces of China, an accurate and quick identification of *P. vivax* will help the local Centres for Disease Control and Prevention carry out a timely foci response during the transmission season [22]. However, the efficiency of this novel test for *P. malariae* and *P. ovale* detection needs to be further improved for a large-scale application.

One limitation of this study is that the development of the novel RDT test is still in a preliminary stage. Therefore, the experimental design requires further improvements. As the monoclonal antibody (Mab) used in this novel RDT was still the pan antibody obtained from immunisation with *P. falciparum* lactate dehydrogenase and was not sensitive enough, the detection rates of *P.vivax*, *P. malariae*, and *P. ovale* were relatively low. In the future, further studies are required using Mabs with a better sensitivity and specificity for *P. vivax*, *P. malariae*, and *P. ovale*.

Conclusion

The newly developed malaria diagnostic immunochromatographic test strip had high sensitivity and specificity in detecting *P. falciparum* and *P. vivax*. However, an improvement of the test efficiency for *P. ovale* and *P. malariae* detection may be important for scaling up its application.

Declarations

Declarations

Ethics approval and consent to participate

Written informed consent for participation and blood specimen collection was obtained from all the participants in the study.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available, due to the fact that they are part of research project that is still ongoing. They are available from the corresponding author, however, on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Shi-Zhu Li, Duo-Quan Wang, Xiao-Nong Zhou and Kang-Ming Lin designed the study and developed the protocol. Kassegne Kokouvi, Faraji Abilahi, Michael Mihayo, Fan Yang analyzed and Shen-Ning Lu interpreted the data. Ying-Jun Qian, Jun-Yun Wang, Feng Shi and Ying-Jun Qian organized and supervised the study in field. Jian Qin, Jian-Feng Chen, An-Xiang Ma, Jun Li and Yi-Chao Yang conducted the study in field, and entered the data. Kang-Ming Lin wrote the first draft of the paper. All authors read and approved the final manuscript.

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Figures

Figure 1

Malaria blood sample test results using two different rapid diagnosis tests (RDTs) 1, 3, 5, 7, 9: novel malaria RDT test strips; 2, 4, 6, 8, 10: commercial RDT test strips; 1, 2: P. falciparum species-specific infection; 3, 4: P. vivax species; 5, 6: P. malariae species-specific infection; 7, 8: P. ovale species-specific infection; 5, 6: P. malariae species-specific infection; 7, 8: P. ovale species-specific infection; and 9, 10: negative control.