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## Original article

# Urine-based rapid diagnostic test for diagnosis of *Plasmodium falciparum* malaria

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**ABSTRACT**

**Background:** Malaria is a severe disease, so delay in treatment could increase morbidity and mortality. World Health Organization recommends confirmation of the diagnosis by laboratory test before initiation of therapy. Smear microscopy and polymerase chain reaction are methods approved by WHO for diagnosing malaria, but they are time-consuming, operator-dependent, and require laboratory staff training. WHO has recommended the Blood-based Rapid Diagnostic Test (RDT) as an acceptable method for diagnosing malaria. It provides rapid results and can be performed with limited resources. **Methods:** Four hundred and fourteen febrile cases admitted to Khartoum North General Hospital, Khartoum, Sudan during January 2021 - June 2021 with clinical suspicion of malaria were examined for the presence of asexual forms of *Plasmodium falciparum* in peripheral blood smears. Blood smear microscopy-positive patients who matched the acceptance criteria were included, while negative patients were selected as control cases. Blood and urine samples were examined with the same RDT kits designed for blood. **Results:** Fifty-eight blood smear-positive cases and 50 febrile blood smear-negative cases were enrolled in the study. The sensitivity and specificity of urine-based RDT were 82.76% and 92.00%, respectively, while blood-based RDT was 96.55% and 96.00%. **Conclusion:** This study aimed to evaluate the performance of urine-based RDT through comparison with blood-based RDT and the use of blood smear microscopy as the reference method. Although the results showed acceptable performance of both tests, more extensive sample size studies should be conducted to consider urine samples as an alternative sample for diagnosing *Plasmodium falciparum* malaria

## Introduction

Malaria is a severe, sometimes fatal, parasitic disease caused by the genus *Plasmodium* (*P*), which affects humans and animals. The infected female Anopheles mosquito is the primary vector that transmits the disease when it bites humans. Four main *Plasmodium* species can cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae. P. knowlesi* recently noticed causes malaria in humans, but it affects apes (zoonotic). *Plasmodium falciparum* is the most dangerous one, responsible for most of the world's malaria mortality [1].

Malaria is widely distributed in many countries of the tropical and subtropical world. African countries have the highest proportion of global malaria cases; they represented 94% of malaria cases in 2019. The estimated number of global malaria deaths was about 409 000 in 2019. (WHO Report, 2020). *Plasmodium falciparum* was the major malaria parasite causing the disease in

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most estimated cases in many WHO regions in 2018, which represented 99.7% of cases in the WHO African Region, 71% of cases in the Eastern Mediterranean Region, 65% in the Western Pacific Region, and 50% of cases in the WHO South-East Asia Region [2].

Under-diagnosis of malaria leads to delay in treatment which increases the morbidity and mortality of the disease. In contrast, over-diagnosis of malaria results in the irrational use of antimalarial drugs, increasing resistance to treatment, and exposing patients to drug side effects, regardless of the high cost of treatment. Despite the availability of many diagnostic approaches for malaria, none yet meet the requirements of an ideal test for disease management and control in endemic areas [3].

Thick smear microscopy is the standard golden method for diagnosing malaria, but it is time-consuming and may induce errors at many levels [4]. It is operator-dependent and requires laboratory staff training to obtain high-quality results inaccessible in developing countries endemic to the disease. Despite its high specificity (99%), to some extent, it has low sensitivity (57%) [5].

The real-time polymerase chain reaction (RT-PCR) is the most sensitive method for the diagnosis of malaria, but it is impracticable for clinical use in a large field because of its expensive cost, high-level training needs, and unavailability in almost all diagnostic laboratories in malaria-endemic countries [6].

A rapid diagnostic test (RDT) kit provides a suitable alternative method for diagnosing malaria; it uses immune-chromatographic material impregnated with monoclonal antibodies against plasmodium antigens in an infected person's blood. The WHO has recommended Blood-based RDT as an acceptable method for diagnosing malaria [7]. The commonly targeted antigen is the histidine-rich protein 2 (HRP-2) produced by *P. falciparum* during asexual forms and early gametocytic stages. It is a water-soluble protein present in the *P. falciparum* cytoplasm and serum of the infected patient. It is released early during infection and persists after treatment. Unfortunately, blood-based RDT doesn't differentiate between recent and previous infections. Moreover, it is an invasive procedure with aseptic needs for blood collection [7].

The HRP-2 is a water-soluble protein and could be detected in many body fluids such as urine and saliva. As urine is an ultra-filtrate of blood, urine can be used rather than blood for diagnosing *P. falciparum* malaria. Urine-based RDT for malaria has many advantages such as:

- Urine samples are ease to access than blood samples.
- It is a non-invasive procedure that eliminates the risk of infection (blood-borne infections) when obtaining a blood sample from a patient accidentally to a health worker or between patients, especially in rural areas with limited infection control measures. Also, prevent the risk of local infection at the blood access site.
- It is a fast and easy method to be performed and doesn't require electricity, specific equipment, or high training. RDT is less cost than other malaria investigations.

This study aimed to evaluate the performance of urine-based RDT through comparison with blood-based RDT and the use of blood smear microscopy as a reference method.

## Material and Methods

### Study site

The study was carried out at Khartoum North General Hospital, Khartoum North locality, Khartoum state, Sudan. Khartoum state has a population of about 8 million distributed in 7 localities. Khartoum North is an industrial and agricultural city with a population of approximately 2,000,000. Khartoum North General Hospital is a 425 -bedded secondary care hospital with a large catchment area beyond Khartoum North, including neighboring localities. Although transmission occurs throughout the year, high transmission happens during autumn and winter (July to December). Overall, the prevalence of malaria parasite in Sudan is around 5.9% [2].

### Study design and enrolment criteria

The current study is a prospective observational case-control study was conducted during January - June 2021, after getting ethical approval from the Ethics Committee of Ain Shams University (FMASU MS 494/2019), Ethics Committee of the Ministry of Health and Population in Egypt (4-2020/10) and Ethics Committee of Federal Ministry of Health in Sudan (4-8-20).

All febrile patients with clinical suspicion of malaria who were admitted to Khartoum North General Hospital of both genders ≥ 18 years during January

- June 2021 were screened for the presence of asexual forms of *P. falciparum* by blood smear microscopy. Conditions like proteinuria, hematuria, or rheumatoid arthritis could affect the test's performance [7]. Patients with these conditions were excluded from the study. Smear-positive febrile patients (58) who met the eligibility criteria were enrolled, whereas 50 smear-negative febrile patients were enrolled as controls.

**Study procedure**

All cases and controls were subjected to blood and urine collection according to standard operating procedures.

**Blood smear procedure**

Blood was obtained from all febrile patients (cases and controls) that have been enrolled in the study through venipuncture. 2 drops of blood were placed onto two glass slides. The first blood drop was used for the preparation of the thick film. The blood drop was swirled with the corner of a slide making a circle of about 1 centimeter in diameter, then allowed to dry without fixative. After drying, the spot was stained with diluted Giemsa (3%) for 30-45 min and washed by keeping the slide in a buffered water jar for 3 min. The slide was left to dry by air vertically. The second blood drop was used to prepare the thin film; it was prepared by immediately placing the smooth edge of a spreader slide in the blood drop with a 45° angle between slide and spreader, then smearing the blood with a rapid and steady spread along the surface. The film was left to air-dry and then fixed with absolute methanol. After drying, the sample was stained with diluted Giemsa (3%) for 30-45 min and washed by briefly dipping the slide in and out of a jar of buffered water. The slide was then allowed to air-dry in a vertical position [8].

The thick and thin blood smears had been microscopically examined by oily lenses (100 x) for the presence of an asexual form of *P. falciparum*. The blood smear was considered positive if asexual parasite forms had been seen. In contrast, it was deemed negative if 100 thick film fields had been examined without detecting an asexual form of *P. falciparum* [9].

**Rapid Diagnosis Test procedure**

The principle of blood-based RDT for diagnosing *P. falciparum* malaria is the detection of the HRP-2 antigen in the blood of an infected person. Tow ml of venous blood in an EDTA tube and random midstream urine in a sterile additives-free 100 ml screw cup had been collected from all individuals enrolled in the study. RDTs were performed using both urine and blood samples of the same patients and controls using commercially available kits (Standard Q Malaria Test kit, SD Biosensor, Korea). The procedure was done as per manufacturer instructions blindly without knowledge of the smear microscopy results.

A sample of 5 μL of blood or urine was added to the sample well and allowed to flow along with the test cassette. In the case of the blood sample, 1-2 drops of buffer were added. The test was considered positive if both control and test bands had appeared, and the appearance of only the control band was interpreted as negative. If the test band appeared only, the result was discarded as invalid, and the test was repeated.

**Statistical analysis**

**Sample size justification**

Depending on the last estimated population (2018), Khartoum North city's population is around 2,000,000. The expected frequency of outcome factor (P. falciparum malaria) in the population, as per WHO Report, 2019, was (5.9% ± 0.5). The confidence limit is as % of 100 (absolute +/–%). The calculated acceptable margin of error was 5%, and the design effect for cluster survey difference was 1. The calculated sample size for the 95% confidence level was equal to 85. So, our sample size should be equal to or more than 85 (calculated by Open Epi Info, version 7.2.4.0 (2020), free statistical software developed by the Centers for Disease Control and Prevention, USA).

**Data analysis**

Data were analyzed using Statistical Package for the Social Sciences version 23. The performance (the sensitivity and specificity) of RDT kits for both blood and urine were calculated by comparing their results with blood smear microscopy (the reference method). Furthermore, the kappa value had been calculated to reveal agreement while the Probability level (*p*-value) was calculated to express significance. Accuracy also had been calculated.

**Results**

Four hundred and fourteen febrile patients suspected clinically of malaria were screened through peripheral blood smear microscopy. Out of these, 58 (14%) cases were *P. falciparum* positive **(Table 1)**. Fifty subjects of blood smear-negative patients were enrolled as controls.

Out of the 58 blood films for *P. falciparum* malaria positive cases, 56 (96.55%) were blood-based RDT positive for *P. falciparum*, while from the 50 blood films for *P. falciparum* malaria negative controls, 2 (4%) were blood-based RDT positive for *P. falciparum* **(Table 2)**. Compared to blood smear microscopy, the sensitivity and specificity of blood-based RDT for *P. falciparum* in the current study were 96.55% and 96.0%, respectively, while the positive and negative predictive values were 96.55% and 96%, respectively. Regards to the Probability level (*p*-value > 0.001) is highly significant with almost perfect agreement (Kapa = 0.928191). Overall accuracy was 96%.

Out of the 58 blood films for *P. falciparum* malaria positive cases, 48 (82.76%) were blood-based RDT Positive for *P. falciparum*, while out of the 50 blood films for *P. falciparum* malaria negative controls, 4 (8%) were blood-based RDT positive for *P. falciparum* (**Table 3**). Compared to blood smear microscopy, the sensitivity and specificity of blood-based RDT for *P. falciparum* in the current study were 82.76% and 92.0%, respectively, while the positive and negative predictive values were 92.31% and 82.14%, respectively. Regards to the Probability level (*p*-value > 0.001) is highly significant with substantial agreement (Kapa = 0.770213). Overall accuracy was 87%.

**Table 1.** Results of blood film for *Plasmodium falciparum* malaria (BFFPfM).

| BFFPfM | Frequency | Percent |
|---|---|---|
| **Positive** | 58 | 14.0% |
| **Negative** | 356 | 86.0% |
| **Total** | 414 | 100.0% |

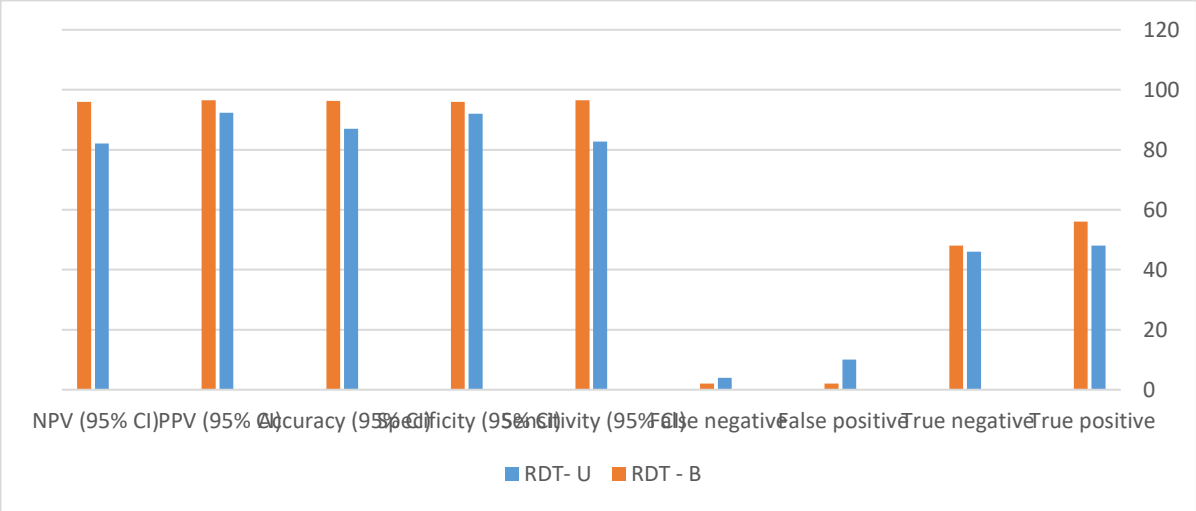
**Table 2.** Blood-based RDT results regarding blood smear microscopy for *Plasmodium falciparum* malaria (BFFPfM) as a reference method.

| | Blood-based RDT | | Total |
|---|---|---|---|
| | Positive | Negative | |
| **Positive** | 56 | 2 | 58 |
| **Negative** | 2 | 48 | 50 |
| **Total** | 58 | 50 | 108 |

**Table 3.** Urine-based RDT results regarding blood smear microscopy for *Plasmodium falciparum* malaria (BFFPfM) as a reference method.

| | Urine-based RDT | | Total |
|---|---|---|---|
| | Positive | Negative | |
| **Positive** | 48 | 4 | 52 |
| **Negative** | 10 | 46 | 56 |
| **Total** | 58 | 50 | 108 |

**Figure 1.** Diagnostic performance of blood-based RDT and urine-based RDT for detection of *Plasmodium falciparum* regarding blood film for *Plasmodium falciparum* malaria as a reference method.




## Discussion

Malaria is a life-threatening disease that requires urgent management. Misdiagnosis of malaria increases morbidity and mortality of the disease; on the other hand, treatment of malaria without laboratory confirmation may lead to antimalarial drug resistance [10].

World Health Organization (WHO) recommended the initiation of malaria treatment after confirming the diagnosis by laboratory test [11]. Smear microscopy is the standard golden method for the diagnosis of malaria. Still, it is time-consuming and operator-dependent and requires laboratory staff training to obtain high-quality results. At the same time, PCR is the most sensitive method for diagnosing malaria. Still, it is impracticable for clinical use in a large field because of its expensive cost and high-level training needs [7].

The blood-based point of care rapid diagnostic tests had been recommended by the WHO in 2010 as an acceptable method for diagnosing malaria. It was approved by the United States Food and Drug Administration (FDA) in 2007 [12]. Furthermore, it was accepted by European Union's conformity (CE). It provides rapid results and can be performed anywhere with limited resources. It is an invasive procedure with the need for blood collection, which may be followed by infection [13].

Detection of malaria parasite antigens or DNA in other body fluids rather than blood such as saliva and urine has been established, so these body fluids can be used to diagnose malaria. WHO has not yet approved RDT using urine or saliva as a method for diagnosing malaria [14].

Urine sample collection is a non-invasive procedure, easier to access, and less infectious than blood sample collection. To limit blood withdrawal, a urine sample was suggested as an alternative sample for *P. falciparum* malaria diagnosis [7].

**Oyibo et al.** in 2017 evaluated Urine Malaria Test (UMT) for *P. falciparum* malaria diagnosis in 1800 febrile patients in korodu and Somolu, Lagos State, Nigeria [11]. They had used RDT dipstick designed especially for urine, developed by Fyodor Biotechnologies, USA. They found the sensitivity and specificity of the test were 85% and 84%, respectively. Their sensitivity and specificity were different from our results (higher sensitivity and lower specificity in their study) may be attributed to the use of a specific urine dipstick that allows the screening of a large urine volume.

**Samal et al.** evaluated RDT using urine samples for diagnosis of *P. falciparum* malaria in 381 febrile patients in Rourkela, Odisha, India, and found the sensitivity and specificity of the test were 86.67 % and 94.12%, respectively [7]. Their results come in agreement with ours.

In 2018 **Mohamed et al.** evaluated Urine Malaria Test (UMT) for *P. falciparum* malaria diagnosis in 120 febrile patients (52 smear-positive cases) admitted to Abbasia Fever Hospital, Cairo, Egypt [15]. Their test sensitivity and specificity were 55.56% and 71.43%, respectively. The difference in their results from others may be due to

different detection methods used or the presence of antibodies against the HRP-2 antigen in the blood of enrolled patients (most of them had come from malaria-endemic countries).

In 2020 **Aninagyei et al.** evaluated RDT using urine and saliva samples for malaria diagnosis in 864 suspected malaria patients in Accra, Ghana, and found the sensitivity of urine and saliva were 35.2% and 57.0%, respectively [14]. Lower diagnostic performance might be attributed to study not designed specifically for *P. falciparum* malaria antigens but other malaria parasites antigens were included or because the study not excluding patients with other comorbidities that could affect the detection of malaria antigens in saliva or urine. Many factors have been suspected to affect the performance point care urine-based RDT such as the level of parasitemia, HRP-2 antigen production by the parasite (some types of *P. falciparum* have HRP-2 gene mutations which result in lowering HRP-2 antigen production), rate of HRP-2 antigen filtration (there is a variation of HRP-2 antigen production during the day), presence of antibodies to HRP-2 antigen in the patient blood (previous infection) and the RDT manufacturing. Parasite sequestration in tissues may reduce the HRP-2 antigen level in the blood, diminishing ultrafiltration of this antigen in the urine. Ultimately, proteolytic cleavage of excreted proteins in urine might affect the performance of RDT formulated for the detection of the intact antigen in the blood [16].

In the current study, the four false-positive cases among controls detected by urine-based RDT were patients with upper respiratory infection who had previous malaria infection (1), gastroenteritis (1), and urinary tract infection (2). The first case might be due to the presence of residual HRP-2 antigen from the previous malaria infection. In gastroenteritis, there is an increase in acute-phase protein, which might hand out this false-positive result. Factors such as increased acute-phase protein and proteinuria associated with urinary tract infection might have contributed to these false-positive results.

The specificity of both blood-based RDT and urine-based RDT in this study was comparable, while the sensitivity of the blood-based test is higher than the urine-based one. However, the results showed acceptable performance of both tests, indicating that the kit used in this study could be of value for detecting *P. falciparum* malaria in urine and using urine as an acceptable source for diagnosing *P. falciparum* malaria.

Non-involvement of some cases in this study, such as children, patients with hematuria, proteinuria, or rheumatoid arthritis, prevents the generalization of these results to the whole community.

## Conclusion

This study aimed to evaluate the performance of urine-based RDT through comparison with blood-based RDT and the use of blood smear microscopy as a reference method. The specificity of both blood-based RDT and urine-based RDT in this study was comparable, while the sensitivity of the blood-based test is higher than the urine-based test. Despite that, the results showed acceptable performance on both tests. Thus, urine samples in point-of-care testing can be used as a simple, easy to obtain, non-invasive, and less infectious screening method.

More extensive sample size studies including all excluded subjects should be conducted to seek WHO approval for considering urine samples as an alternative sample for diagnosing *P. falciparum* malaria.

## Conflict of interest

We declare we have no conflicts of interest to disclose.

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## Abbreviations

**BFFPfM** Blood Film for *P. f falciparum* Malaria.

**CDC** Centers for Disease Control and Prevention.

**CE** European Union's conformity.

**DNA** Deoxyribonucleic acid.

**EDTA** Ethylene diamine tetraacetic acid.

**FDA** Food and Drug Administration.

**FMASU** Faculty of Medicine Ain Shams University.
**HRP-2** Histidine-rich protein 2.
***P*** *Plasmodium*.
**PCR** Polymerase chain reaction.
**RDT** Rapid diagnostic Test.
**USA** United States of America.
**UMT** Urine Malaria Test.
**WHO** World Health Organization.

**Declaration**

We declare that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

**Availability of data**

The BFFM, Blood-based RDT, and Urine based RDT results data used to support the findings of this study are available upon request.

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