

## Research Article

# Detection of malarial parasite in urine of malaria patients: a future diagnostic approach

Vyankatesh T. Anchinmane\*, Rakesh T. Shedge

Department of Pathology, Topiwala National Medical College & BYL Nair Ch Hospital, Mumbai Central, Mumbai-08, India

**Received:** 20 April 2016

**Accepted:** 25 April 2016

### \*Correspondence:

Dr. Vyankatesh T. Anchinmane,  
E-mail: [a.vyankatesh@rediffmail.com](mailto:a.vyankatesh@rediffmail.com)

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## ABSTRACT

**Background:** Current definitive screening for active malaria infection necessitates drawing blood. The non-invasive, cost-effective malaria tests that minimize the need for blood collection are the need of time. QDx Malaria PAN/Pf rapid malaria card test (QDx rapid malaria test) is an immunochromatographic test that detects the presence of malarial antigens (pLDH and HRP-2) in the blood sample for the diagnosis of malaria. These malarial antigens are also released in the urine. The study was conducted to determine the sensitivity, specificity and accuracy of the QDx Rapid Malaria test for diagnosis of malaria in blood and urine.

**Methods:** Blood and urine specimens were obtained from 75 malaria blood smear positive cases (test samples) and 25 malarial negative cases (controls). Then urine and blood specimen of each case were evaluated for QDx rapid malaria test. Using microscopy as gold standard, the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the QDx rapid malaria test for urine and blood were calculated.

**Results:** The accuracy of QDx rapid malaria test for malarial parasite detection was 97% for blood and 38 % for urine.

**Conclusions:** Rapid malaria test processed with urine may be a useful non-invasive and cost-effective malaria diagnostic technique in future.

**Keywords:** Rapid malaria test, Malarial antigens, Urine

## INTRODUCTION

Current detection or screening for malaria infection necessitates drawing blood by finger prick or venipuncture. The need to draw blood causes difficulties in certain communities with blood taboos and poses limitations for repeated measurement.<sup>1</sup> There are numerous malaria rapid diagnostic tests that are commercially available which contains specific anti-malaria antibodies to detect malaria antigen (pLDH, HRP-2, p-aldolase) in the blood.<sup>2,3</sup>

QDx Malaria PAN/ Pf Rapid Malaria card test, Nicholas Piramal India Limited, is one of the malaria rapid diagnostic tests that detect - pLDH and HRP-II malarial

antigens in the blood by immunochromatographic assay. As these malarial antigens have been also detected in the urine, we assessed the efficiency of the QDx rapid malaria test in the blood and in the urine using microscopy as gold standard.<sup>4-6</sup>

## METHODS

This study was conducted from July to December 2015 in the tertiary hospital in Mumbai. 100 specimens of blood and urine from cases of fever formed the study material. Out of these 100 cases, the 75 cases were clinically suspected to be suffering from malaria and were positive for malaria on blood smear. The 25 specimens from cases

of urinary tract infection, viral fever, respiratory tract infection etc. formed the control.

### Immunochromatographic test

All the blood samples were processed for QDx rapid malaria test. It is an immunochromatographic test that detects the presence of pan malaria specific antigen (pLDH) for the detection of all non-falciparum malarial parasites whereas the detection of *P. falciparum* utilises recognition of specific histidine rich protein-2 (HRP-2). The monoclonal anti HRP-2 antibody and anti-PAN specific antibody are coated on the membrane of the test kit. When the blood sample malarial antigens combines with these malarial antibodies then pink-purple colored bands are formed which confirms test results are positive. The 5 µl of anti-coagulated blood sample or finger pricked blood sample take into sample well, 'S'. Then six drops of the clearing buffer taken into reagents well, 'R'. The test results are ready at the end of 15 minutes. When only one pink purple band appears in the control window 'C' then the blood sample is negative for the malarial infection. When in addition to control band, two pink purple bands appears at the 'Pf' and 'PAN' region in the test window then the blood sample is positive for the falciparum or mixed malarial infection. When in addition to control band, one pink purple band appears only at 'PAN' region in the test window then the blood sample is positive for the non-falciparum species.

### Microscopy

All the blood samples were processed for the Leishman stained thin blood smear. Two pathologists independently examined the slides and were also blinded to each other's interpretations and also to the results of the urine and blood QDx rapid malaria test. Slides were considered positive for malaria when asexual forms and/or gametocytes were found. Slides were considered negative if no parasites were seen after observing 100 high-powered fields. The 'parasitic density' calculated as the number of parasites counted on smear was multiplied by the patient's white blood cell (WBC) count, and the resulting value divided by the total number of WBC's counted during the microscopy examination. Parasite densities were classified into three groups, <500, 501–5,000 and >5,000 parasites/microlitre (µl). For each slide, parasites were counted against 200 WBC's. Fully automated blood cell counter did the WBC count. Conventional control measures were applied to hematology analyzers used in the study.

### Urine

All the urine samples were processed for urine microscopy and Benzidine tests to rule out the presence of red blood cells (RBC's). The benzidine test was performed by adding equal volume of urine sample to mixed reagent. The mixed reagent was prepared by adding equal volumes of saturated solution of benzidine

in glacial acetic acid to hydrogen peroxide. The development of blue colour indicates presence of blood or occult blood in the urine sample. Then all the urine samples (100 cases) were processed by QDx rapid malaria test with the same methodology that was followed for the blood sample.

### Data analysis

The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the QDx rapid malaria test for urine and blood were estimated using microscopy as gold standard. The variables measured were the number of true positives (TP), number of true negatives (TN), number of false positives (FP), and the number of false negatives (FN).<sup>7</sup>

## RESULTS

Of the 100 blood samples collected, 75 (test samples) were positive for malaria by smear examination. The majority were *P. falciparum* cases (40 cases-53.3%). All 25 control blood samples were negative for on smear. Out of 75 malarial smear positive cases, the 72 (96%) blood samples and 13 (17.3%) urine samples were positive for QDx rapid malaria test (Table 1).

**Table 1: Performance of QDx malaria rapid test- blood and urine relative to microscopy**

Malarial parasite	Microscopy +ve	QDx blood +ve	QDx urine +ve
<i>P. Falciparum</i>	40 (53.3%)	40 (53.3%)	07 (9.3%)
<i>P. Vivax</i>	19 (25.3%)	16 (21.3%)	03 (4%)
Mixed infection (V + F)	16 (21.3%)	16 (21.3%)	03 (4%)
Total	75 (100%)	72 (96%)	13 (17.3%)

(V = *P. Vivax*, F= *P. Falciparum*)

All 25 controls were negative by QDx rapid malaria test for urine and blood samples. The benzidine tests and microscopy examination performed on all urine samples were negative for RBC's.

With microscopy as the gold standard, the QDx rapid malaria test processed by blood giving a sensitivity of 96%, specificity of 100%, accuracy of 97%, PPV of 100% and NPV of 89.2% (Table 2).

And when microscopy was compared with QDx Rapid Malaria test processed by urine, the sensitivity, specificity, accuracy, PPV, NPV was 17.3%, 100%, 38%, 100% and 28.7% respectively (Table 3).

**Table 2: Comparison of performance QDx malaria rapid test-blood with microscopy.**

QDx blood result	Microscopy results			Sen.	Speci.	Accu.	PPV	NPV
	Pos.	Neg.	Total					
Positive	72	00	72	96%	100%	97%	100%	89.2%
Negative	03	25	28					
Total	75	25	100					

(Sen. = Sensitivity, Speci. = Specificity, Accu. = Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value, Pos. =Positive, Neg. = Negative)

**Table 3: Comparison of performance QDx malaria rapid test-urine with microscopy.**

QDx blood result	Microscopy results			Sen.	Speci.	Accu.	PPV	NPV
	Pos.	Neg.	Total					
Positive	13	00	13	17.3%	100%	38%	100%	28%
Negative	62	25	87					
Total	75	25	100					

(Sen. = Sensitivity, Speci. = Specificity, Accu. = Accuracy, PPV= Positive Predictive Value, NPV= Negative Predictive Value, Pos. =Positive, Neg. = Negative)

## DISCUSSION

To determine presence or absence of parasites could be valuable for communities with blood taboos and reduce compliance problems associated with collection of blood.<sup>1</sup> For these reasons, many researchers had experimented with the body fluids like urine, saliva etc. for detection of malaria. Alejandro M. Katzin et al showed that Western blotting technique could detect malarial antigens in urine.<sup>8</sup> Sungano Mharakurwa et al showed that *P. falciparum* infection could be detected in urine and saliva by PCR technique.<sup>1</sup>

Diagnosis is currently done by microscopy, which requires good training and simple laboratory facilities. On the contrary, the rapid immunochromatographic tests do not require a laboratory, electricity, or any special equipment. They target parasite antigens histidine-rich protein 2 (HRP2) of *P. falciparum* and pan-malarial parasite specific lactate dehydrogenase or Plasmodium aldolase using either monoclonal or polyclonal antibodies. Histidine-rich protein 2 of *P. falciparum* (PfHRP2) is a water soluble protein that is produced by the asexual stages and gametocytes of *P. falciparum*, expressed on the red cell membrane surface. Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the all four live human malaria species parasites and it is present in and released from the parasite infected erythrocytes.<sup>2</sup>

QDx rapid malaria test detects malarial antigens pLDH and Pf HRP2 in the blood of malarial patients by immunochromatographic principle. As these malarial antigens are also released in the urine<sup>4-6</sup> so in the present study, we processed blood and urine samples of malarial patients on QDx Rapid Malaria test as test sample. The

collection of urine is non-invasive, simple, safe, stress free, painless, and can be done by individuals with limited training, including patients. No special equipment is needed for collection and it allows for multiple or serial collections outside of the hospital.

The QDx rapid malaria test processed by blood gives a sensitivity of 96 %, specificity of 100%, accuracy of 97% (Table 2). All the five *P. vivax* cases that were positive by microscopy and negative by QDx rapid malaria test-blood had parasite densities less than 500 parasites/ $\mu$ L. The explanation for this phenomenon could be that the quantity of lactate dehydrogenase enzyme and HRP-2, the antigen detected by rapid malaria test, is in direct proportion to the number of parasites in the blood.<sup>9</sup>

When compared with microscopy as the gold standard, 13 out of the 75 microscopic malarial positive cases were positive by the QDx rapid malaria test processed by urine samples giving a sensitivity of 17.3 %, specificity of 100%, and accuracy of 38 %, PPV of 100% and NPV of 28.7% (Table 3).

All the urine samples were negative for RBC's by microscopy and by benzidine tests. So, in this study, it is proved that the 20 urine positive QDx rapid malaria test were positive due to water-soluble p LDH and HRP-2 antigens, which were released into urine of malarial patients and not because of RBC's in the urine.<sup>2</sup>

The only researcher, Genton B et al, did the similar type of study in the past. He showed that when using microscopy and PCR as reference, the ParaSight (R)-F test applied to blood had 84% sensitivity and 77% specificity and when the same test kit performed on urine had 81% sensitivity but only 26% specificity.<sup>4</sup>

In the present study, the QDx rapid malaria test performed on urine had only 38% accuracy. Because of the lack of accuracy, the QDx rapid malaria test performed on urine cannot be recommended.

## CONCLUSION

Detecting parasite antigens in urine will be affordable, rapid, non-invasive approach and safe for patients and technicians in resource-poor environments. The present study showed that malarial antigens could be detected in urine. More studies are necessary to determine its cost-effectiveness and result differences between the existing different brands of rapid malaria Immunochromatographic test with malaria patient urine sample. Now there is need of time to research for the newer Rapid Malaria test that can detect malarial antigens in urine with better accuracy.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: Not required*

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**Cite this article as:** Anchinmane VT, Shedge RT. Detection of malarial parasite in urine of malaria patients: a future diagnostic approach. *Int J Res Med Sci* 2016;4:1702-5.