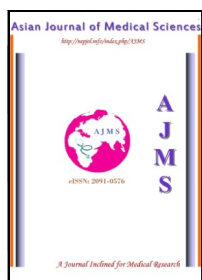


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A Review of Malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test

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Abstract

Objective: Microscopic detection of parasites has been the reference standard for malaria diagnosis for decades. However, difficulty in maintaining required technical skills and infrastructure has spurred the development of non-microscopic malaria rapid diagnostic devices based on the detection of malaria parasite antigen. We evaluated the QDx MALARIA PAN/Pf rapid immunochromatographic card test by comparing it with the conventional standard blood smear method for the detection of malaria.

Material & Methods: A total of 325 specimens of blood from cases of fever (falciparum malaria 88, vivax malaria 168, mixed infection of falciparum and vivax malaria 44, controls 25) were investigated by QDx Malaria Rapid test and blood smear method.

Results: The QDx Malaria Rapid test showed 96.6% sensitivity, 100% specificity and 96.9 % accuracy. However, these parameters were lower when the parasitaemia was less than 500 parasites / μ L.

Conclusion: QDx Malaria Rapid test was found to have enormous advantages over smear examination due to its high degree of sensitivity, specificity, speed and ease of performance.

Key Words: Malarial parasite; QDx MALARIA PAN / Pf rapid immunochromatographic card test; blood smear

1. Introduction

Malaria causes more than three hundred million acute infections and it kills at least one million people every year.¹ Microscopic analysis of appropriately stained thick and thin blood smears has been the standard diagnostic technique for identifying malaria infections for more than a century. The classical chosen method for detection of the malaria parasite is the examination of Giemsa or Leishman stained thick and thin blood films. This method allows estimating parasitaemia, to distinguish between parasite growth stages and to identify the four different Plasmodium species. The problems associated with implementing and sustaining a level of skilled microscopy appropriate for clinical diagnosis, particularly in the field setting, have prompted the development of a variety of malaria rapid diagnostic

devices. These are based on antibody capture of circulating antigens from Plasmodium species demonstrating them to be fast and reliable.²

There are numerous malaria rapid diagnostic tests that are commercially available, all of which detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies. The tests fall into a few basic types depending on which antigen is targeted. Most tests that detect *P. falciparum* are based on the histidine-rich protein-2 (HRP-2), which is specific to that species. Other tests detect the parasite enzyme lactate dehydrogenase (LDH), using either monoclonal antibodies that react with LDH of all species including *P. falciparum* (so-called PAN or pLDH), or antibodies specific for *P. falciparum* LDH.^{3,4}

The QDx MALARIA PAN/Pf Rapid card test-Piramal Healthcare, India (QDx Malaria Rapid test) is an immunochromatographic assay that detects and differentiates of *P. falciparum* and other malarial species

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in human blood. This test can be performed in less than 20 minutes and does not require highly trained personnel. The early detection and differentiation is of paramount importance due to incidence of cerebral malaria and drug resistance associated falciparum malaria and due to the morbidity associated with the other malarial forms. Our objective was to evaluate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP) and accuracy of the QDx Malaria Rapid test in comparison with standard microscopy for diagnosis of malaria.

2. Materials and Methods

The study was carried out in monsoon from June to September 2009 i.e. for the period of four months in Tertiary Hospital in Mumbai. 325 specimens of blood from cases of fever formed the study material. Out of these, 300 were from cases strongly suspected to be suffering from malaria. 25 specimens from cases of urinary tract infection, viral fever, respiratory tract infection etc. formed the control. Specimens of blood from all cases were processed for detection of malaria parasite by thin, stained blood smear and by QDx Malaria Rapid test.

2.1. Microscopy

The thin blood smears were stained with Leishman stain and read at $\times 100$ (oil immersion objective) of the microscope. Two skilled pathologists independently examined one slide for each patient. Both the pathologists were blinded to each other's interpretations as well as to the results of the QDx Malaria Rapid 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 white blood cell (WBC) count was done by fully automated Cell Counter AGD Erma PCE 210. Conventional control measures were applied to semiautomated hematology analyzers used in the study.

To calculate 'parasitic density', for all positive smears, the number of parasites counted was multiplied by the patient's WBC count and the resulting value divided by the total number of WBC's counted during the microscopic examination. The final data endpoint was a calculated parasitemia expressed as the number of stage parasites per microliter of whole blood. For each positive slide, parasites were counted against 200 leucocytes and expressed as parasites/microlitre (μl) of blood. Parasite densities were classified into <500 , $501-5,000$ and $>5,000$ for low, moderate and high parasitaemia respectively.^{5,6}

2.2. Immunochromatographic test

QDx Malaria Rapid test 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* utilizes 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 malaria 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 taken 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.

2.3. Data analysis

Once all the samples had been tested, sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the QDx Malaria Rapid test 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).

Sensitivity was calculated as $TP / (TP + FN)$, specificity was calculated as $TN / (TN + FP)$, the positive predictive value (PPV) was calculated as $TP / (TP + FP)$, and the negative predictive value (NPV) was calculated as $TN / (FN + TN)$. Test accuracy, the proportion of all tests that gave a correct result, were defined as $(TP + TN) / \text{number of all tests}$.¹

3. Results

A total of 325 patients were included for the study. Of the 325 blood samples collected, 300 were found to be positive for malaria by microscopy. Of these, 88 (29.3%) of these were positive for *P. falciparum*, 168 (56%) were positive for *P. vivax* species while 44 (14.6%) were positive for a combination of both *P. falciparum* and *P. vivax* species. The 25 patients suffering from non-malarial fever formed the control (Table 1).

Table-1: Performance characteristics of QDx Malaria Rapid test relative to microscopy of different Plasmodium species

Malarial Parasite	Microscopy + ve (%)	QDx Rapid + ve (%)
<i>P. falciparum</i>	88 (29.3%)	85 (28.3%)
<i>P. Vivax</i>	168 (56%)	165 (55%)
Mixed infection (V + F)	44 (14.6%)	40 (13.3%)
Total	300	290 (96.6%)

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

Using the QDx Malaria Rapid test, 290 (96.6%) of the 300 blood samples were positive for malaria. 85 (28.3%) of these were positive for *P. falciparum*, 165 (55%) were positive for *P. vivax* species and 40 (13.3 %) were positive for *P. falciparum* and *P. vivax*. All 25 control samples were negative by QDx Malaria Rapid test (Table 1).

Table-2: Comparison of performance of QDx Malaria Rapid test with microscopy at different parasite densities in detecting the different Plasmodium species

Parasitic density	Microscopy + ve	Rapid test + ve	Sensitivity (%)	Specificity (%)	Accuracy (%)
< 500	82	73	89	100	91.5
501- 5000	134	133	99.2	100	99.3
> 5000	84	84	100	100	100

Generally, parasites were detected in a greater proportion of samples as the parasite density increased in the three categories of *P. falciparum*, non-falciparum species and mixed infections by using the rapid test. At parasite densities of more than 5000 parasites/ μ L, the rapid test recorded the highest sensitivity (100%) and accuracy (100%) while performance characteristics dropped (sensitivity 89.0% and accuracy 91.5%) as parasite densities decreased to less than 500 parasites/ μ L (Table-2).

Overall, when compared with microscopy as the gold standard, 290 out of the 300 samples positive by microscopy were positive by the rapid test giving a sensitivity of 96.6%. All 25 control samples were negative for the Rapid Malaria tests which were also negative by microscopy giving a specificity of 100%. The 10 samples out of 300 samples those were negative by the rapid test were positive by microscopy. Out of these 10 cases, one case was showing only gametocytes and two were showing only schizonts. The remaining 6 cases had parasitic density less than 500 parasites/ μ L and one case had parasitic density in between 500 to 5000 parasites/ μ L. The percentage of all true positive and all true negative rapid test result gave an accuracy of 96.9%. (Table-3)

4. Discussion

Microscopic examination of properly prepared and stained

blood smears by well-trained microscopist is accurate and reproducible and allows for the identification of the plasmodium species, differentiating between stages and density of infection. However, it is not always feasible to sustain accurate microscopy especially in the remote areas.

The recent development of rapid and accurate tests for the detection of malaria is highly commendable. One of the major goals of developing such rapid tests was that these rapid tests should be handled with ease and accurately by relatively unskilled staff for prompt diagnosis of malaria especially in areas where microscopy may not be available or available⁷ and pose problems because of its limitations especially in some parts of the endemic areas where there is no electricity.

Now a days majority of microscopes works on electricity only so if there is no electricity, the microscope does not

Table-3: Comparison of overall performance QDx Malaria Rapid test with microscopy for the detection of Plasmodium species

Microscopy \ Rapid Test	Microscopy +ve		Microscopy -ve		Total
	+	-	+	-	
+	290	10	00	25	300
-	00	25	00	25	35
Total	290	10	00	25	325
Sensitivity		96.6%		96.6%	
Specificity		100%		100%	
Accuracy		96.9%		96.9%	
PPV		100%		100%	
NPV		71.4%		71.4%	

works. Therefore indirectly, electricity also plays important role in diagnosis of malaria by microscopic method.⁵

This study compared the diagnosis of malaria parasites by QDx Malaria Rapid test and traditional microscopy and found that the two methods yielded comparable results. As per our knowledge, this is a first study for the comparison of QDx Malaria Rapid test and traditional microscopy. A total of 325 symptomatic fever patients, 300 patients were suffering malaria. The blood smear examination identified all 300 symptomatic malarial cases were positive on smear. In our study, QDx Malaria Rapid test showed 96.6% sensitivity,

100% specificity, 96.9% accuracy, 100% positive predictive values and 71.4% negative predictive value. We found that the QDx Malaria Rapid test sensitivity was associated with the level of parasitemia; the sensitivity was 100% when the parasitemia was higher than 5,000 parasites/ μ L, but decreased to 89% with lower parasitemia levels of less than 500 parasites/ μ 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.⁸ This problem must be considered when this test is used in the field. Another limitation of the Rapid Malaria test is that it cannot identify patients with mixed infections of *P. falciparum* and *P. vivax*. The reason is that one of detected antigens is *P. falciparum* specific, but the other is not specific, because is a panmalaric antigen; therefore in mixed infections, the test will show a *P. falciparum* infection.³ The similar problem is also encountered with QDx Malaria Rapid test. It can be a therapeutic problem in endemic areas where more than one Plasmodium species coexists. The other limitation of the QDx Malaria Rapid test is that it detects only live parasites producing pLDH and pHRP-2. In our study we found 5 numbers of cases where only gametocytes (1 cases) and schizonts (2 cases) were found on the smear. All these 3 cases were negative by QDx Malaria Rapid test.

The other well-known companies for Rapid Malaria Test are OptiMAL and Tulip (Parascreen Pan/Pf). The approximate price for one test kit of QDx Rapid malaria Test is Indian Rupees 102/- which is much lower than the other companies.

Considering all the facts, QDx Malaria Rapid test is highly recommendable because it can detect all the species of plasmodium with good sensitivities and specificities at different parasite densities, thereby giving this tool a greater potential for use as an epidemiological tool for the control of malaria.

This study does not suggest that malaria antigen based tests should replace microscopy as a diagnostic tool at present, but can be used as an alternative or complimentary to microscopy where appropriate. Unlike microscopy, which requires substantial training and experience, this rapid rest was performed well with minimal training. The savings in technician time, including time to prepare stained blood smears and microscopically examination of the slides, the lack of the need to purchase and maintain expensive equipment, and the ability to work in remote areas where electricity and other infrastructure is lacking, make the use of antigen detection test methods tremendously appealing. These Malaria Rapid Tests are found to be useful for to decrease malaria over-diagnosis and the consequent overuse of valuable anti-malarials and under-diagnosis of alternative causes of fever.^{9,10}

5. Conclusion

In conclusion, the results obtained from this study demonstrate that the QDx Malaria Rapid test is suitable for use in mass surveillance programmes for the management and control of malaria. It has advantages over microscopic examination in speed of results (less than 20 minutes), ease of performance, as well as less need of laboratory facilities and health workers instruction. But it is important to consider that it has diagnostic difficulties in patients with low parasitemia levels and it cannot detect mixed infections, unlike microscopic examination. However, the cost is one of the most important disadvantages for widespread use in developing countries. More studies are necessary to determine its cost-effectiveness, compared with microscopic examination, and to detect the differences between the different brands, and compared to other Rapid Malaria Diagnosis Devices. Due to the inaccuracy encountered microscopic diagnosis of malaria in endemic remote areas and with the development of rapid diagnostic tests, they may, in the long run, be a realistic alternative to microscopy for the prompt diagnosis of malaria.

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