

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.801.122>

Comparison of Peripheral Blood Films Stained by Giemsa Stain, Rapid Diagnostic Tests for Antigen and Antibody for Detection of *Plasmodium vivax* and *Plasmodium falciparum* in Clinically Suspected Cases of Malaria

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ABSTRACT

Malaria is a disease of human which causes high morbidity and mortality. Malaria is one of the major public health challenges and syndromic approach is unreliable because of non-specific and overlapping symptoms with other febrile diseases. Various diagnostic techniques available include: Peripheral blood film examination, rapid diagnostic tests for antigen and one step pf/pv antibody detection. Materials and Methods: This study was conducted in Department of Microbiology, Dhiraj General Hospital, Dist., Vadodara. The blood samples collected from 100 clinically suspected cases of malaria were stained by Giemsa staining, tested by Rapid Diagnostic Test (RDT) for antigen as well as one step pf/pv antibody detection test as per standard procedures. Results: In the present study, out of total 100 cases tested, 66%, 63% and 58% were positive by Giemsa stain, RDT for antigen and antibody respectively. Conclusion: Thus on comparing all the three methods for diagnosis of malaria, PSMP by Giemsa staining, which is the gold standard, still remains the best method as it demonstrates the direct evidence but it needs expertise whereas rapid diagnostic tests for antigen detection gives quite similar results and is also accurate, saves time, can be used in fields and does not need expertise to interpret the results for *P. vivax* and *P. falciparum* separately. While using only malarial antibody test a number of acute malaria will be missed. Malarial antibody test is useful for seroepidemiological surveys and gives information regarding exposure to malaria but does not differentiate between present and past infection and thus should not be used as a single test for diagnosis of acute malaria.

Keywords

Giemsa staining, Malaria, Rapid Diagnostic Tests (RDTs)

Article Info

Accepted:

10 December 2018

Available Online:

10 January 2019

Introduction

Malaria is an infection caused by *Plasmodium* parasites. The parasites are spread to people through the bites of infected female *Anopheles* mosquitoes, called "malaria vectors." *P. falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for severe clinical manifestations

and most malaria-related deaths globally. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa in WHO Malaria key facts, 11 June 2018. The WHO African Region continues to account for about 90% of malaria cases and deaths worldwide. Fifteen countries – all but one in sub-Saharan Africa – carry 80% of the global malaria burden in the *World malaria report*

2017. Malaria is one of the major public health challenges and syndromic approach is unreliable because of non-specific and overlapping symptoms with other febrile diseases (Prakriti *et al.*, 2014). The disease is a protozoan infection of red blood cells transmitted by the bite of a blood-feeding female Anopheline mosquito (Gordon *et al.*, 2009, 22nd ed.)

Four species of malaria parasites were capable of infecting humans: *P.falciparum*, *P.vivax*, *P.ovale* and *P.malariae*; however recently a fifth *P.knowlesi* has been recognized as a significant human pathogen (Alan *et al.*, 2012, 9thed). Various diagnostic techniques available are including: Peripheral blood film examination, rapid diagnostic tests for antigen and one step Pf/Pv antibody detection.

Present study was conducted to find out sensitivity and specificity of serodiagnostic tests, that is commercially available to correlate Giemsa based microscopy, pf HRP-2 antigen test, pan malaria pLDH and malaria antibody test.

The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for patients with non malarial fevers in endemic areas (Mwangi *et al.*, 2005, Reyburn *et al.*, 2004, McMorro *et al.*, 2008). The accuracy of malaria diagnosis can be greatly enhanced by combining clinical and parasite based findings (Kyabayinze *et al.*, 2008). The standard method of malaria diagnosis is by the microscopic examination of Giemsa-stained blood smears (Rosenthal *et al.*, 2012). Expert microscopy gives information about parasite stage and parasitaemia. Rapid diagnostic tests (RDTs) that detect malaria parasite proteins by immunochromatography have been used as complementary detection methods for malaria

diagnosis (Bell *et al.*, 2006). RDTs detect a variety of proteins, including *P. falciparum* histidine-rich protein 2 (PfHRP2) and *P. falciparum* lactate dehydrogenase (PfLDH), both specific to *P. falciparum*, and also *Plasmodium* LDH (pLDH) and aldolase, enzymes shared by the 5 human pathogenic *Plasmodium* species (Moody *et al.*, 2002). RDTs for malaria are based on the detection of either histidine-rich protein 2, produced only by *Plasmodium falciparum*, or parasite specific lactate dehydrogenase produced by all four species (Baker *et al.*, 2005).

This test is simple, rapid, self-performing, qualitative, two site sandwich immunoassay, and sensitive for on the spot diagnosis of malaria (Malascan, Tulip Diagnostic kit literature). Malaria antibody detection gives information regarding exposure to malaria but do not differentiate between present and past infection (Hitendra Singh *et al.*, 2000). Hence it is not useful for diagnosis in acute case of malaria.

RDTs specific for detecting antibodies against *Plasmodium*-derived antigens also exist. Antibodies against parasite antigens appear within days to weeks after infection and persist for months or even years. Therefore, malaria antibody tests can detect parasite infection even at low parasitemia (< 50parasites/ μ l). Malaria antibody test, thereby, is useful in malaria endemic areas, in which light infection and/or re-infection of parasites are common. They are also helpful in testing patients who have recently been treated for malaria but their diagnosis is questioned as the microscopic examination of this case can result in a false-negative. Owing to these characteristics of malaria antibody tests, they are used for the screening of donated bloods in blood banks to prevent transfusion-transmitted malaria (Drakeley *et al.*, 2005; Vinetz *et al.*, 1998).

Materials and Methods

This study was conducted in Department of Microbiology, Dhiraj General Hospital, during 6 months period (from July 2016 to December 2016) at SBKS MI and RC, Piparia, Dist-Vadodara.

Samples

A total of 100 clinically suspected cases of malaria were included in the study. 5 ml of whole blood was collected into the collection tube containing EDTA by venepuncture from patients presenting clinically with fever with chills and rigor and other suggestive symptoms of malaria (Thomas *et al.*, 2012). Confirmation of diagnosis of malaria was done using three available techniques, the criteria for confirmation of positivity was positive by any of the three following methods.

Thick and thin blood smears

The peripheral smears stained with Giemsa stain For preparation of blood films, thoroughly cleaned 26 mm × 76 mm glass slides. Thick and thin blood smears were prepared as per the standard method. The smears were stained with Giemsa stain. Thick smears were reported negative after examination of 200-300 oil immersion fields with no parasites observed; a thin smear was given negative when no parasites were observed in 200 oil immersion fields (David *et al.*, 2002: 4thed).

Detection of antigen: Samples of all these 100 were also tested for Malarial Antigen detection by Rapid Diagnostic Test (RDT) using Malascan Pan/pf kit. The malaria pLDH /HRP2 test kit contains a membrane strip, which is precoated with two antibodies as two separate lines across a test strip. One monoclonal antibody is pan specific to pLDH

of the *Plasmodium vivax* and the other line consists of a monoclonal antibody specific to the HRP2 of the *Plasmodium falciparum* species. 5µl of whole blood was added into the sample well. Then two drops of assay buffer were added into the buffer well. The result was read in 20 min.

Detection of antibody: Also these samples were tested for detection of antibodies against malarial parasite using One step malaria pf/pv antibody test kit (Accucare One step Malaria Pf/Pv antibody test) as per manufacturer's instruction. The Malaria Antibody test contains a membrane strip, which is precoated with two monoclonal antibodies as two separate lines across a test strip. One monoclonal antibody (test line 1) is specific to the *P.falciparum* MSP and another monoclonal antibody (test line 2) is CSP of *Plasmodium* species (*P.falciparum*, *P.vivax*, *P.malariae*, *P.ovale*) The conjugate pad is dispensed with monoclonal antibodies conjugated to the colloidal gold, which are specific to *P.falciparum* MSP and pan specific to the CSP of other *Plasmodium* species.

Statistical analysis

Data was statistically analyzed using Medcalc -sensitivity and specificity, positive and negative predictive value testing.

Results and Discussion

A total of 66 samples were found to be positive out of these male patients were 44 and female patients were 22, using any of the three methods (Giemsa stain, RDT for antigen and antibody test). In the present study out of total 100 samples tested, 66, 63 and 58 were positive by Giemsa staining, RDT for antigen and One step Malaria Antibody test respectively (Table 1).

On examination of positive samples; Giemsa staining, RDT for antigen and one step malaria antibody test could confirm the diagnosis of *P. vivax* in 33 (50%), 30 (48%), 17(29.3%) and that of *P. falciparum* in 26 (39.3%), 17 (27%), 15(26%) and mixed infection (*P. falciparum*+ *P. vivax*) in 7(10.6%), 16(25.3%), 26(45%) respectively.

While sensitivity of Giemsa staining, RDT for antigen and one step malaria antibody test for detection of *P. vivax* was 100%, 90.9%,51.5% and specificity is 100%,100%, 100% and for

P. falciparum sensitivity of Giemsa staining; RDT for antigen and One step Malaria Antibody test was 100%, 65.3%, 57.6% and specificity of 100%,100%, 100%, respectively (Table 2 and 3). Thus the sensitivity of Giemsa staining, RDT for antigen and One step Malaria Antibody test for detection of *Plasmodium spp* was 100%, 95.4%, 87.8% and specificity was 100% for all the three. The RDT for antigen had a sensitivity and negative predictive value higher than the malaria antibody test.

Table.1 Comparison of various methods for detection of different species of malaria parasite (N=100)

S. No	Method	Malarial Parasite Species							
		None		<i>P. vivax</i>		<i>P. falciparum</i>		<i>P. vivax</i> + <i>P. falciparum</i>	
		n	%	n	%	n	%	n	%
1	Giemsa (N=100)	34	34%	33	33%	26	26%	07	7%
2	RDT Mal Ag(N=100)	37	37%	30	30%	17	17%	16	16%
3	One step Antibody(N=100)	32	32%	17	17%	15	15%	26	26%

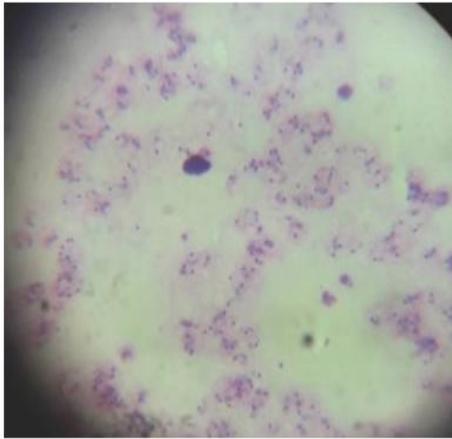
Table.2 Diagnostic efficacy of different methods for malarial parasite species

Method	Diagnostic Efficacy				
	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
For <i>P. vivax</i>					
Giemsa stain	100%	100%	100%	100%	100%
RDT	90.9%	100%	100%	95.71%	97.0%
One step antibody	51.52%	100%	100%	80.72%	84.0%
For <i>P. falciparum</i>					
Giemsa stain	100%	100%	100%	100%	100%
RDT	65.38%	100%	100%	89.1%	91.0%
One step antibody	57.69%	100%	100%	87.0%	89.0%
For <i>P. vivax</i>+ <i>P. falciparum</i>					
Giemsa stain	100%	100%	100%	100%	100%
RDT	100%	90.3%	43.7%	100%	91.0%
One step antibody	100%	79.5%	26.9%	100%	81.0%

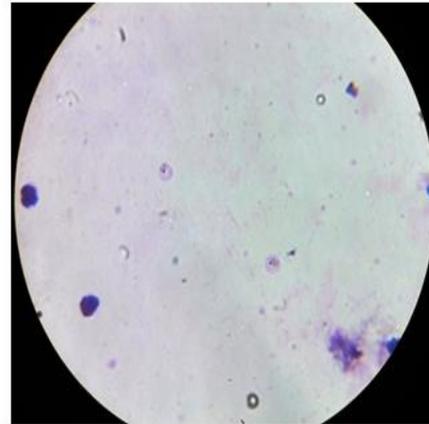
Table.3 Diagnostic efficacy of different methods for malarial parasite species

Method	Diagnostic Efficacy				
	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
RDT	95.4%	100%	100%	91.8%	97.0%
One step antibody	87.8%	100%	100%	80.9%	92.0%

Giemsa stain:Thick smear:*P.falciparum* ring and gamete



Giemsa stain:Thick smear:*P.vivax* late trophozoite

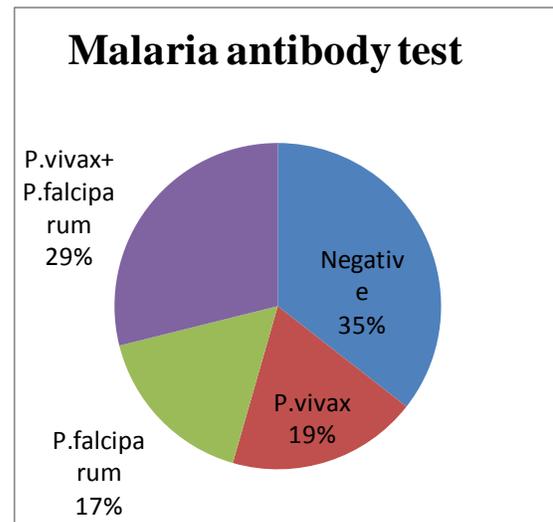
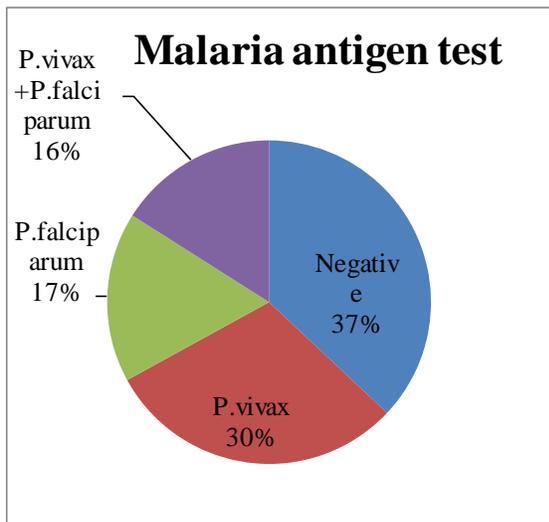
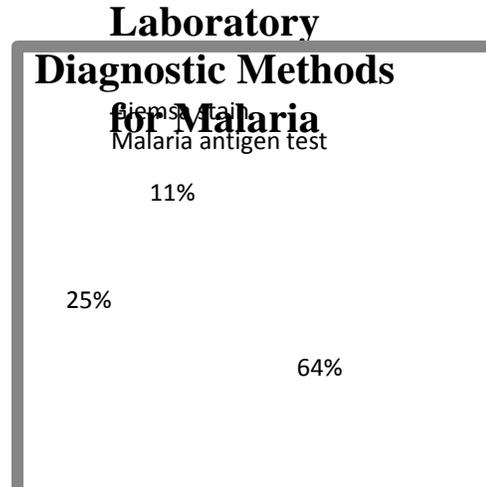
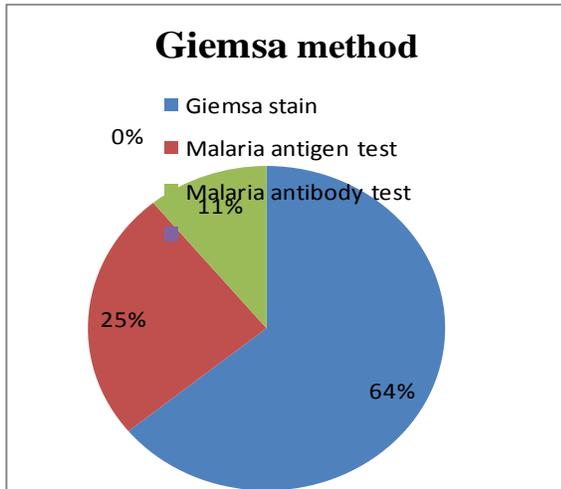


II: Rapid Malaria antigen test
1)Negative 2) *P.vivax* 3)Pf or mixed infection



III: Malaria Antibody Detection Test
1)Negative 2)Pf 3)Pv 4)Pf+Pv





In the present study, a total of 100 clinically suspected malaria patients were included. PSMP by Giemsa stain was positive in 66 patients and 34 pts were negative. Of the total 66 malaria positive, *P. falciparum* was in 39.4%, *P.vivax* in 50% and mixed infection in 10.6%. In similar study malaria positive *P. falciparum* was in 14% and *P. vivax* in 72% detected (Prakriti Vohra *et al.*, 2014),

In present study sensitivity and specificity of Giemsa stain is 100% each. In other studies Giemsa to be 72.9%, 93.1% sensitive and 100%, >95% specific for diagnosis of malaria respectively (Gay *et al.*, 1996 and

Craig *et al.*, 1997). Malarial antigen test was positive in 63 pts and 37 pts were negative. Of the total 63 malaria positive, *P. falciparum* was in 27%, *P. vivax* in 48% and mixed infection 25.3%. Also 3 pts out of 37 malarial antigen negative were positive by PSMP examination. Stauffer *et al.*, 2014, found the RDT for Malaria P.f./P.v. test to be 100% sensitive and 99% specific for *P. falciparum*, and 86% sensitive and 99% specific for *P. vivax*. Comparison with other studies (Aslan *et al.*, 2001), (Malik *et al.*, 2006) as well as a similar study done in our department (Sucheta *et al.*, 2013) shows the similar findings.

Some malaria infections detected by blood film were not detected by the Accucare Malarial Antigen test. This may be explained by the fact that increased awareness of malaria among the general public as led to a rampant misuse of antimalarial drugs in inadequate doses empirically for any fever and since malascan detects pLDH which is produced only by living parasites, the blood samples tested negative by malascan may have been due to dead parasites and not yet cleared from the host or this could be due to insufficient enzyme production which occurs during early malarial infection or the patient blood samples contained parasites at concentrations below the malascan test detection level (Beatriz *et al.*, 2012, Kakkilaya *et al.*, 2003).

Malaria Antibody test was positive in 58 pts and 42pts were negative. Of the total 58 malaria positive, *P. falciparum* was in 25.8%, *P. vivax* in 29.3% and mixed infection in 44.8% respectively. Also 34 pts out of 42pts negative for malaria antibody test were found to be negative for malarial Ag and PSMP examination; 5pts out of 42pts were positive for malarial Ag as well as PSMP examination and 3pts out of 42pts were positive by PSMP examination but negative by malaria Ag. Moreover, one patient out of 58 patients who were positive for malarial antibody, tested negative for PSMP examination and malaria antigen test. This patient was sickle test positive and the patient responded to antimalarial drug.

Microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films for screening and thin blood films for species' confirmation remain the gold standard for laboratory diagnosis (Bharti *et al.*, 2007).

In conclusion giemsa staining of peripheral smear, which is the Gold standard, as compared to existing techniques available for

diagnosis of malarial parasite, still remains the best method as it demonstrates the direct evidence. But then it needs expertise whereas rapid diagnostic test for antigen detection gives quite similar results and is also accurate, saves time, can be used in fields and does not need expertise to interpret the results for *P. vivax* and for *P. falciparum* separately. For *P. vivax* and *P. falciparum*, Giemsa stain is most sensitive and has better diagnostic accuracy. For *P. vivax*, RDT is most sensitive and has better diagnostic accuracy than *P.falciparum*. Half of the world's population is at risk of malaria, Rapid Diagnostic Tests (RDTs) is recommended for all patients with suspected malaria before treatment is started. While using only malarial antibody test, 15% of patients with acute malaria will be missed. Malarial antibody test is useful for seroepidemiological surveys and gives information regarding exposure to malaria but does not differentiate between present and past infection and thus should not be used as a single test for diagnosis of acute malaria.

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How to cite this article:

Sangita Vasava, Sucheta Lakhani, Jitendra Lakhani and Radhika Khara. 2019. Comparison of Peripheral Blood Films Stained by Giemsa Stain, Rapid Diagnostic Tests for Antigen and Antibody for Detection of *Plasmodium vivax* and *Plasmodium falciparum* in Clinically Suspected Cases of Malaria. *Int.J.Curr.Microbiol.App.Sci*. 8(01): 1163-1171.
doi: <https://doi.org/10.20546/ijcmas.2019.801.122>