# Malaria Diagnostic Tests: Microscopy And Rapid Diagnostic Test (RDT) Revisited

**Dr. Jain Subhash C[*], Dr. Gupta Deepak[**], Dr. Jain Madhu K[***]**

[*] Associate Professor Medicine, [**] Professor Medicine, [***] Senior Medical Officer and Deputy Controller,
Jhalawar Hospital and Medical College Society, Jhalawar

**Abstract:** In the prevention and control of malaria, Prompt and accurate diagnosis is the key to effective disease management. Giemsa microscopy and rapid diagnostic tests (RDTs) are the diagnostic tests each with characteristic strengths and limitations is the best way for accurate diagnosis has a key role for malaria control successfully. Reduction in morbidity and drug resistance intensity of malaria require a parasite based diagnostic methods. A parallel commitment is needed in production of antimalarial drug or malaria vaccine along with improvement in diagnostic tests and their availability to people in endemic areas. [Jain S NJIRM 2014; 5(4) :82-87]

**Key Words:** Microscopy, Malaria, Rapiddiagnostic test, Malaria diagnosis, Parasetaemia, Plasmodium, Antimalarial drug.

**Author for correspondence**: Dr. Subhash Chand Jain, Associate Professor Medicine, Jhalawar Medical College, C-35 Indira Colony, Opp SRG Hospital, Jhalawar, Rajasthan – 326001. Email: subhash.nopra@gmail.com

**Introduction:** Malaria is caused by a parasite called *Plasmodium*, which is transmitted through the bites of infected mosquitoes. In the human body, the parasites multiply in the liver, from where they spread to infect red blood cells. Five *Plasmodium* species have shown to infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi*.

According to the malaria report 2010 of the World Health Organization (WHO), malaria is currently endemic in over 100 countries with 3 billion people at risk of infection and around 225 million cases in 2009, leading to approximately 781,000 deaths.[2] *P. falciparum* was among the leading causes of death worldwide from a single infectious agent. *P. falciparum* is mostly confined to tropical climates; *P. vivax* also occurs in subtropical areas but is rare in Central-Africa, where *P. ovale* is prevalent. *P. malariae* is rare and *P. knowlesi*, the most recently described "zoonotic" species, is restricted to South-East Asia.

*Plasmodium* has a sexual cycle inside the mosquito and an asexual cycle inside men. During the bite of an infected mosquito, *Plasmodium* sporozoites are injected in the blood and will move to liver cells where they will develop into liver schizonts. After the rupture of the schizonts, thousands of merozoites are released into the bloodstream and each of them will invade a red blood cell (RBC). There they will develop into trophozoites which will mature into schizonts that on their turn will divide into new merozoites which will invade new RBCs. After one or two weeks, gametocytes will be produced. These will continue the sexual cycle when taken up by a mosquito during a next blood meal. As to the asexual cycle, liver schizonts of *P. vivax* and *P. ovale* may persist for months in the liver before releasing merozoites into the blood. The persistent ("dormant") forms are called hypnozoites; the delayed symptoms are called relapses.

Small children and pregnant women are particularly vulnerable to malaria. Likewise, also non-exposed travelers are vulnerable and each year, 10,000 malaria cases are reported among returned international travelers, although their real number is estimated at 30,000.[3]

Symptoms of malaria may vary and include fever, headache, aches and pains elsewhere in the body and occasionally abdominal pain, diarrhea and vomiting. If not treated, malaria can quickly become life-threatening. This is especially the case for *P. falciparum* which unlike *P. vivax/P. ovale* and *P. malariae* is not restricted to young or old RBCs and can cause elevated parasite densities. In addition, infected RBCs stick to the vascular endothelium, leading to obstructed blood flow in the organ capillaries and lethal complications such as cerebral malaria, pulmonary oedema and impairment of liver and renal functions. Prompt

diagnosis is essential for the treatment and outcome of *P. falciparum*. *P. vivax* accounts for almost half of the malaria infections worldwide and is no longer considered as a mild infection: complicated infections have been demonstrated in both endemic countries and in returned travelers[4, 5] and the species is more difficult to eradicate than *P. falciparum*.[6] Any attempt to estimate the number of malaria cases globally is likely to become subject to argument. [7, 8]

Clinical diagnosis is imprecise but remains the basis of therapeutic care for the majority of febrile patients in malaria endemic areas, where laboratory support is often out of reach. Malaria diagnosis is the most neglected area of malaria research accounting for less than 0.25% ($70000) of the US $323 million investment in research and development in 2004.[9]

Rationale therapy of malaria is necessary to delay the development of resistance, to avoid non target effects and cost effective alternative drugs. There for an accurate diagnosis is the only way of rationale therapy. Recently, confirmatory diagnosis regained attention before start of treatment because of more drug resistance and more costly drugs unaffordable to resource poor countries.[10]
Prompt treatment of malaria requires accurate and prompt diagnosis. Diagnosis based on clinical symptoms is notoriously non-specific and WHO now recommends parasite- based diagnosis for all patients.[11]This review addresses on microscopy and rapid diagnostic tests (RDTs), the two malariadiagnostics having the largest control.

**Microscopy:** In 1904,Gustav Giemsa introduced a mixture of methylene blue and eosin stains.[12] Microscopic examination of Giemsa – stained blood amear has subsequently become the gold standard of malaria diagnosis. Giemsa microscopy is regarded as the most suitable diagnosis instrument for malaria control because it is in expensive to perform, able to differentiate malaria species and quantify parasites. In the age of high quality light emitting diode (LED) illumination and solar battery chargers, microscopy has become more feasible in remote areas. However, microscopy requires well trained, competent microscopists, rigorous maintenance of functional infrastructures plus effective quality control (QC) and quality assurance (QA). Because of emergence of drug resistance, Artemisinin – based combination therapy (ACT) is already or will soon be the first line medication for P. falciparun treatment in most affected countries. Therefore it is essential to make an accurate diagnosis before writing ACT, which is more costly and less tolerated. The detection threshold in Giemsa – stained thick blood film has been estimated to be 4-20 parasite / mCL.[13, 14, 15]Under field conditions a threshold of about 50-100 parasite / mCL blood is more realistic.[16, 17]The variability along with the risk of untreated malaria in the light of safe, expensive therapy in the past led clinicians to treat febrile patients without regard to the laboratory results. [18,19,20]Even in developed countries, expert malaria microscopists are scarce and impaired microscopy-based diagnosis in hospital laboratories is common.[21,22,23,24]

The cornerstone of malaria diagnosis in the laboratory is microscopy. *Plasmodium* parasites are diagnosed by microscopic examination of a thick blood film, which consists of a superposition of several layers of blood cells. The red blood cells are lysed during the staining process. Thin blood films represent a monolayer of blood, allowing observation and assessment of the red blood cells' shape and inclusions and clear distinction of the different parasites, allowing species identification. Characteristics of RBC (dimensions, shape, inclusions and numbers infected) and *Plasmodium* parasites (size, stages, and dimensions) allow distinction between the different *Plasmodium* parasites. The differential diagnosis between *P. ovale* and *P. vivax* is notoriously difficult.[25]The parasite density of *Plasmodium* represents the count of the asexual parasites and is expressed as a number per µL blood or as a percentage of red blood cells infected. According to standard practice at the clinical laboratory (CLKB) from the Institute of Tropical Medicine (ITM), both thick and thin blood films are prepared, stained with Giemsa (pH 8.0) and examined by light microscopy using a × 500 magnification. An examination of 15 minutes

for a thick film with a minimum of 200 fields read is performed before a thick blood film is reported negative. Parasite densities are estimated by counting asexual parasites against 200 white blood cells (WBC) in thick blood films and converting this number to parasites/µl using the actual WBC count or, when this is not available, the standard 8,000 WBC/µl value [26,27].

Apart from not missing the diagnosis of malaria, differentiation of *P. falciparum* from the non-*falciparum* species is important, in particular not missing the diagnosis of *P. falciparum* because of the life-threatening potential of this species. Accurate identification of parasite density appears to be difficult in non-endemic settings[28], but high parasite densities exceeding 2% of red blood cells infected should berecognized, as this criterion constitutes an alert sign[11]. Expert microscopy should also recognize *P. falciparum* stages and hemozoin pigment in WBC: schizonts of *P. falciparum* in the peripheral circulation as well as hemozoin in the WBC in case of *P. falciparum* infection are indicators of a serious infection, whereas the exclusive presence of *P. falciparum* gametocytes after treatment is a normal finding [11,29].

**Rapid Diagnostic Test (RDT)**: Rapid diagnostic test is a device that detects malaria antigen in a small amount of blood, usually 5-15 µL, by immunochromatographic assay with monoclonal antibodies directed against the target parasite antigen and impregnated on a test strip. The result, usually a colored testline, is obtained in 5-20 minutes. RDTsrequire no capital investment or electricity, are simple to perform and are easy to interpret. Most commonly RDTs only detect P. falciparum; however, RDT'S that distinguish P. falciparum from the three non-falciparum species are available.

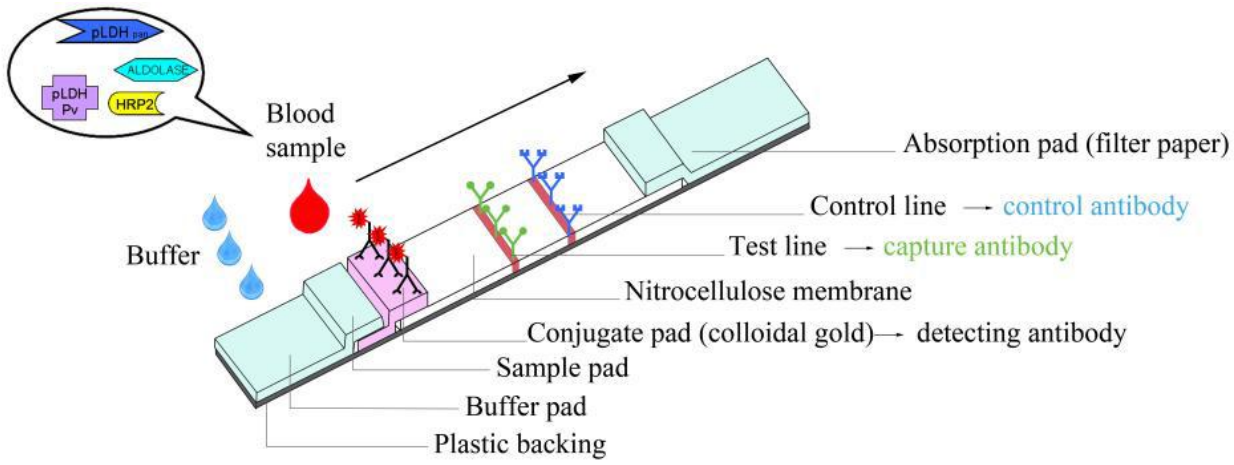


**Figure 1: Schematic drawing of the malaria RDT lateral-flow strip.**

To be a useful diagnostic, RDTs must achieve greater than 95% sensitivity [(30)] Most RDTs today have achieved this goal for P. falciparum, but not for non P. falciparum. In spite of more than 100 published RDT trialreports, comparative assessment is difficult because (1) trials do not share common guide lines (2) Clinical and epidemiologic characteristics of the study population vary (especially parasetaemia level) (3) reference standards are different (4) products of different lots may differ in quality or be damaged by extreme temperature or humidity during transportation and storages. Recent studies suggest that the tests were less sensitive for non-P. falciparum than for P. falciparum.[31, 32, 33]Overall RDT specificity is commonly above 85%, approaching 100% when used in some groups of returning non-immune travelers.[34, 35, 36, 37, 38, 39]In developed countries, RDTs can be useful in screening febrile returnees from endemic

areas[34]Self use by travelers produce variable out comes[42, 43] In developing countries RDTs make obsolete the sole dependence on clinical diagnosis for malaria in remote areas, where good microscopy has failed or never reached , RDTs are also recommended in situation exceeding microscopy capability, such as in an outbreak or in occupationally exposed groups.[44]As RDTs improve, including in sensitivity for P. Vivax and in ability to measure parasetaemia levels, the scope of RDTs will expand. Current RDTs are not intended to replace microscopy.

In light of rising cost of effective antimalarial therapy, over diagnosis can quickly impair medicine budgets. Prompt and accurate diagnosis will not only improve malaria treatment but will reduce mortality due to other febrile illnesses. Therefore, RDTs should be considered as tools for the complete management of febrile illnesses. RDTs become more cost effective as the price of antimalarials go up.

**Conclusion:** RDT tests have shown good sensitivity and specificity and the results are comparable with microscopy. Thus, RDT is useful for malaria diagnosis in both, endemic and non-endemic areas. RDT is an important tool in malaria diagnosis particularly in resource limited settings where microscopic diagnosis is not feasible. RDT also minimizes exclusively clinical diagnosis of malaria and in combination with microscopy, RDT can be used to improve malaria diagnosis.

**References:**


1. Malaria diagnosis and treatment guidelines for health workers in Ethiopia 2nd edition. 2004.
2. World Health Organization: World malaria report 2010. [http://whqlibdoc.who.int /publications/2010/9789241564106_eng.pdf ]
3. World Health Organization: International travel and health. 2010 [http://www.who.int/entity/ith/ITH2010.pdf ]
4. Habib AG, Singh KS: Respiratory distress in nonimmune adults with imorted malaria. *Infection* 2004, 32: 356-359.
5. Rogerson SJ, Carter R: Severe vivax malaria: newly recognised or rediscovered. *PLoS Med* 2008, 5: e136.
6. Phimpraphi W, Paul R, Witoonpanich B, Turbpaiboon C, Peerapittayamongkol C, Louicharoen C, Casademont I, Tungpradabkul
7. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI, 2005. Theglobal distribution of clinical episodes of Plasmodium falci-parum malaria.Nature 434:214217.
8. Bell DR, Jorgensen P, Christophel EM, Palmer KL, 2005. Malaria risk: estimation of the malaria burden.Nature 437:E3
9. Malaria R&D Alliance, 2005. Malaria Research and Development: An Assessment of Global Investment, November 2005. www.MalariaAlliance.org.
10. Barnish G, Bates I, Iboro J, 2004. Newer drug combinations formalaria.BMJ 328:1511–1512.
11. World Health Organization: Guidelines for the treatment of malaria, second edition, 2010. [http://whqlibdoc.who.int/publications/2010/ 9789241547925_eng.pdf ]
12. Fleischer B, 2004. Editorial: 100 years ago: Giemsa's solution forstaining of plasmodia.Trop Med Int Health 9:755–756.
13. Bruce-Chwatt LJ, 1984. DNA probes for malaria diagnosis. Lancet 1:795
14. Payne D, 1988. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. Bull World Health Organ 66:621–626.
15. Dowling MA, Shute GT, 1966. A comparative study of thick andthin blood films in the diagnosis of scanty malaria parasitaemia.Bull World Health Organ 34:249–267.
16. World Health Organization, 1988. Malaria diagnosis: memoran-dum from a WHO meeting. Bull World Health Organ 66:575–594.
17. Milne LM, Kyi MS, Chiodini PL, Warhurst DC, 1994. Accuracyof routine laboratory diagnosis of malaria in the United Kingdom.J Clin Pathol 47:740–742.
18. Othnigue N, Wyss K, Tanner M, Genton B, 2006. Urban malariain the Sahel: prevalence and seasonality of presumptive ma-laria and parasitaemia at primary care level in Chad.Trop MedInt Health 11:204–210

19. Lalloo D, Naraqi S, 1992. The diagnosis of malaria: traditionaland contemporary approaches. P N G Med J 35:243–248
20. Zurovac D, Midia B, Ochola SA, English M, Snow RW, 2006.Microscopy and outpatient malaria case management amongolder children and adults in Kenya. Trop Med Int Health 11:432–440.
21. Milne LM, Kyi MS, Chiodini PL, Warhurst DC, 1994. Accuracyof routine laboratory diagnosis of malaria in the United King-dom. J Clin athol 47:740–742.
22. Thomson S, Lohmann RC, Crawford L, Dubash R, RichardsonH, 2000. External quality assessment in the examination of blood films for malarial parasite within Ontario, Canada. ArchPathol Lab Med 124:57–60
23. Anonymous, 1997. The laboratory diagnosis of malaria. TheMalaria Working Party of The General Haematology TaskForce of the British Committee for Standards in Haematology. Clin Lab Haematol 19:165–170
24. Johnston SP, Pieniazek NJ, Xayavong MV, Slemenda SB,Wilkins PP, da Silva AJ, 2006. PCR as a confirmatory tech-nique for laboratory diagnosis of malaria.J Clin Microbiol 44:1087–1089
25. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, ernsdorfer WH: A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg 2007, 77: 119-127
26. World Health Organization: Basic Malaria Microscopy - Part II.Tutor´s Guide 2nd edition, 2010. [www.searo.who.int/LinkFiles/Malaria_malaria_microscopy_Tutors_guide2010.pdf]
27. World Health Organization: Basic Malaria Microscopy - Part I. Learner´s Guide 2nd edition, 2010. [www.searo.who.int/LinkFiles/Malaria_malaria_microscopy_Learners_guide2010.pdf]
28. Kettelhut MM, Chiodini PL, Edwards H, Moody A: External quality assessment schemes raise standards: evidence from the UKNEQAS parasitology subschemes. J Clin Pathol 2003, 56: 927-932
29. Grobusch MP, Hanscheid T, Gobels K, Slevogt H, Zoller T, Rogler G, Teichmann D: Comparison of three antigen detection tests for diagnosis and follow-up of falciparum malaria in travellers returning to Berlin, Germany. Parasitol Res 2003, 89: 354-357
30. World Health Organization, 2000. Malaria Diagnosis New Per-WONGSRICHANALAI AND OTHERS124spectives. Report of a Joint WHO/USAID Informal Consultation, October 25-27, 2000. Geneva: WHO.
31. Pattanasin S, Proux S, Chompasuk D, Luwiradaj K, Jacquier P,Looareesuwan S, Nosten F, 2003. Evaluation of a new Plas-modium lactate dehydrogenase assay (OptiMAL-IT) for the detection of malaria. Trans R Soc Trop Med Hyg 97:672–674.
32. Iqbal J, Muneer A, Khalid N, Ahmed MA, 2003. Performance ofthe OptiMAL test for malaria diagnosis among suspected malaria patients at the rural health centers. Am J Trop Med Hyg68:624–628
33. Playford EG, Walker J, 2002. Evaluation of the ICT malariaP.f/P.v and the OptiMal rapid diagnostic tests for malaria infebrile returned travellers. J Clin Microbiol 40:4166–4171
34. Moody A, Hunt-Cooke A, Gabbett E, Chiodini P, 2000. Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital forTropical Diseases, London Br J Haematol 109:891–894
35. Farcas GA, Zhong KJ, Lovegrove FE, Graham CM, Kain KC,2003. Evaluation of the Binax NOW ICT test versus polymerase chain reaction and microscopy for the detection ofmalaria in returned travelers. Am J Trop Med Hyg 69:589–592
36. Grobusch MP, Hanscheid T, Gobels K, Slevogt H, Zoller T, Rogler G, Teichmann D, 2003. Comparison of three antigendetection tests for diagnosis and follow-up of falciparum malaria in travelers returning to Berlin, Germany. Parasitol Res89:354–357
37. Palmer CJ, Bonilla JA, Bruckner DA, Barnett ED, Miller NS, Haseeb MA, Masci JR, Stauffer WM, 2003. Multicenter studyto evaluate the

OptiMAL test for rapid diagnosis of malaria inU.S. hospitals. J Clin Microbiol 41:5178–5182
38. Richardson DC, Ciach M, Zhong KJ, Crandall I, Kain KC, 2002.Evaluation of the Makromed dipstick assay versus PCR fordiagnosis ofPlasmodium falciparummalaria in returned trav-elers. J Clin Microbiol 40:4528–4530.
39. Hernandez E, De Pina JJ, Fabre R, Garrabe E, Raphenon G,Cavallo JD, 2001. Evaluation of the OptiMAL test in the di-agnosis of imported malarial outbreak. Med Trop (Mars) 61:153–157
40. Marx A, Pewsner D, Egger M, Nuesch R, Bucher HC, GentonB, Hatz C, Juni P, 2005. Meta-analysis: accuracy of rapid testsfor malaria in travelers returning from endemic areas. AnnIntern Med 142:836–846
41. Jelinek T, Grobusch MP, Harms G, 2001. Evaluation of a dip-stick test for the rapid diagnosis of imported malaria amongpatients resenting within the network TropNetEurop.ScandJ Infect Dis 33:752–754
42. Jelinek T, Amsler L, Grobusch MP, Nothdurft HD, 1999. Self-use of rapid tests for malaria diagnosis by tourists. Lancet 354:1609.
43. Trachsler M, Schlagenhauf P, Steffen R, 1999. Feasibility of arapid dipstick antigen-capture assay for self-testing of travelers'malaria. Trop Med Int Health 4:442–447
44. World Health Organization, 2004.The use of malaria diagnostictests.Manila: WHO Regional Office for the Western Pacific (WPRO).

| |
|---|
| Conflict of interest: None |
| Funding: None |