Laboratory Diagnosis Of Malaria-Various Method And It's Comparision

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Abstract : This is a comparative review of various techniques used for the detection of various malarial species, it's specificity, sensitivity and availability at tertiary care center with its cost effectiveness. [Mehta D et al NJIRM 2013; 4(3) : 138-143]

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Introduction: Malaria, the 'King of Diseases', continues to haunt and taunt mankind.[1] More than a century after identification of the causative parasites, and more than half a century after finding effective drugs and insecticides, the disease as old as humanity itself, affects more than 500 million and kills more than 3 million people every year [1]. The dreaded disease is difficult to eradicate and its control is possible ONLY with coordinated efforts of the general public, healthcare personnel and government agencies. And with global warming threatening to increase mosquito density and the spread of other mosquito borne infections like Dengue and Chikungunya, time has come for all of us to wake up. So early diagnosis of malaria is very much important. Various methods has been recommended by texts and literature. An afford has been made to classify the tests on the bases of their specificity, sensitivity ,cost effectiveness and easily availability to tertiary care center for accurate early diagnosis of malaria.

Following are various type of methods mentioned in various texts. Classification of method for diagnosis of malaria[1]

Microscopic Method
(A) Peripheral smear examination
(B) Quantitative Buffy Coat(QBC) test

Non-Microscopic Method
(A) Immuno chromatography test
(B) Polymerase Chain Reaction
(C) Detection of antibody
(D) Mass spectrometry
(E) Flowcytometry

1. Microscopic Methods : A. Peripheral Smear Examination for Malarial Parasite Peripheral smear examination for malarial parasite is the gold-standard in confirming the diagnosis of malaria. Thick and thin smears prepared from the peripheral blood are used for the purpose. The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia with a sensitivity of 5 to 10 parasites/µL of blood for an experienced laboratory professional. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear. The test takes about 60 to 120 minutes depending on the proximity of the laboratory and other factors mentioned above.

Drawback: (1) The existing needs of the blood smear examination are often not met in certain remote and poor parts of the world. (2)Detection of low levels of parasitemia, sequestered parasites of P. falciparum and past infections in aspiring blood donors is not possible (3) ascertaining viability of the detected parasites(4) difficulties in maintaining the required technical skills and resultant misdiagnosis due to poor familiarity and problems in accessing and activating the facility in emergencies are some of the deficiencies with the blood smear examination.

Material and Method: Thick smear: The thick smear of correct thickness is the one through which newsprint is barely visible. It is dried for 30 minutes and not fixed with methanol. This allows

the red blood cells to be hemolyzed and leukocytes and any malaria parasites present will be the only detectable elements. However, due to the hemolysis and slow drying, the plasmodia morphology can get distorted, making differentiation of species difficult. Thick smears are therefore used to detect infection, and to estimate parasite concentration.[2]

Thin smear: Air dry the thin smear for 10 minutes. After drying, the thin smear should be fixed in methanol. This can be done by either dipping the thin smear into methanol for 5 seconds or by dabbing the thin smear with a methanol-soaked cotton ball. While fixing the thin smear, all care should be taken to avoid exposure of the thick smear to methanol.Thin smear are used for identify the species.[2]

Staining: A number of Romanowsky stains like Field's, Giemsa's, Wright's and Leishman's are suitable for staining the smears. Thick films are ideally stained by the rapid Field's technique or Giemsa's stain for screening of parasites. The sensitivity of a thick blood film is 5-10 parasites/µl. Thin blood films stained by Giemsa's or Leishman's stain are useful for specification of parasites and for the stippling of infected red cells and have a sensitivity of 200 parasites/µl. The optimal pH of the stain is 7.2.Slides should be clean and dry. It is better to use neutral distilled water. 200 fields should be examined before declaring thick film negative.[2]

B. Quantitative Buffy Coat (QBC) Test [1,3]

The QBC Test is a new method for identifying the malarial parasite in the peripheral blood. It involves staining of the centrifuged and compressed red cell layer with acridine orange and its examination under UV light source. It is fast, easy and claimed to be more sensitive than the traditional thick smear examination.

Comparison between peripheral smear and QBC test for detecting malaria[3,4,5,6,7]

	Peripheral smear	QBC
Method	Cumbersome	Easy
Time	Longer, 60 - 120 minutes	Faster, 15 - 30 minutes
Sensitivity	5 parasites/µl in thick film and 200 / µl in thin film	Claimed to be more sensitive, at least as good as a thick film
Specificity	Gold standard	? False positives, artifacts may be reported as positive by not-so-well-trained technicians
Species identification	Accurate, gold standard	Difficult to impossible
Cost	Inexpensive	Costly equipment and consumables
Acceptability	100%	Not so
Availability	Everywhere	Limited
Other	--	Accidentally can detect filarial worms

Therefore, whenever in doubt, ask for a peripheral smear study, particularly for species identification.

2. Non-Microscopic Methods

A.Immunochromatographic Tests for Malaria Antigens [1] Although the peripheral blood smear examination that provides the most comprehensive information on a single test format has been the "gold standard" for the diagnosis of malaria, the immunochromatographic tests for the detection of malaria antigens, developed in the past decade, have opened a new and exciting avenue in malaria diagnosis. However, their role in the management and control of malaria appears to be limited at present.

Immunochromatographic tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets. Currently, immunochromatographic tests can target the histidine-rich protein 2 of P. falciparum, a pan-malarial Plasmodium aldolase, and the parasite specific lactate dehydrogenase. These RDTs(Rapid Detection Tests) do not require a laboratory, electricity, or any special equipment.

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, and shown to remain in the blood for at least 28 days after the initiation of antimalarial therapy. Several RDTs targeting PfHRP2 have been developed.

(2)Plasmodium aldolase is an enzyme of the parasite glycolytic pathway expressed by the blood stages of P. falciparum as well as the non-fa1ciparum malaria parasites. Monoclonal antibodies against Plasmodium aldolase are pan-specific in their reaction and have been used in a combined 'P.f/P.v' immunochromatographic test that targets the pan malarial antigen (PMA) along with PfHRP2.

(3)Parasite lactate dehydrogenase (pLDH) is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites and it is present in and released from the parasite infected erythrocytes. It has been found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immunocapture assay, a qualitative immunochromatographic dipstick assay using monoclonal antibodies, an immunodot assay, and a dipstick assay using polyclonal antibodies have been developed.

Comparison of Rapid Diagnostic Tests for Malaria Antigens			
	PfHRP2 tests	PfHRP2 and PMA test	pLDH test
Target antigen	Histidine rich protein 2 of P. falciparum, water soluble protein expressed on RBC membrane	Pan-specific Plasmodium aldolase. parasite glycolytic enzyme produced by all species and PfHRP2	Parasite lactate dehydrogenase. parasite glycolytic enzyme produced by all species
General test format	2 lines	3 lines	3 lines
Capability	Detects P. falciparum only	Can detect all 4 species	Can detect all 4 species
Non-falciparum species	Not detected	Detected; differentiation between the 3 not possible	Detected; differentiation between the 3 not possible
Mixed infections of P. falciparum with non-falciparum species	Appear as P. falciparum; differentiation not possible	Appear as P. falciparum; differentiation not possible	Appear as P. falciparum; differentiation not possible
Detection limit	>40-100 parasites/µL	Higher for P. vivax and other non-falciparum species	> 100-200 parasites/µL for P. falciparum and P. vivax; may be higher for P. malariae and P. ovale
Post-treatment persistence of antigens	Reported up to 31 days	Reported; longer for pan specific antigenemia than for PfHRP2	Reported up to 1 -3 weeks
Cross-reactivity between malarial species	Reported	Reported	Reported
Cross-reactivity with auto antibodies	Reported, high (up to 83% with rheumatoid factor)	Not known	Reported. low (3.3% with rheumatoid factor)
Indication of viability of parasites	No	No	Positive test indicates presence of viable parasitemia

Comparison of Peripheral Blood Smear Examination and RDTs for Malaria		
	Peripheral Smear	Rapid Diagnostic Tests
Format	Slides with blood smear	Test strip
Equipment	Microscope	Kit only
Training	Trained microscopist	'Anyone with a little training'
Test duration	20-60 minutes or more	5-30 minutes
Test result	Direct visualization of the parasites	Color changes on antibody coated lines
Capability	Detects and differentiates all plasmodia at different stages	Detects malaria antigens (PfHRP2/ PMA/pLDH) from asexual and/or sexual forms of the parasite
Detection threshold	5-10 parasites/µL of blood	1 00-500/µL for P. falciparum, higher for non-falciparum
Species differentiation	Possible	Cannot differentiate among non-falciparum species; mixed infections of P. falciparum and non-falciparum appear as P. falciparum
Quantification	Possible	Not possible
Differentiation between sexual and asexual stages	Possible	Not possible
Disadvantages	Availability of equipment and skilled microscopists, particularly at remote areas and odd hours	Unpredictable efficiency at low and very high parasitemia; cross reactions among plasmodial species and with auto-antibodies; persistence of antigens

B. Polymerase Chain Reaction (PCR)[1]: Using the non-isotopically labelled probe following PCR amplification, it is possible to detect malaria parasites. In travelers returning to developed countries, studies based on PCR have been found to be highly sensitive and specific for detecting all 4 species of malaria, particularly in cases of low level parasitemia and mixed infections. The PCR test is reportedly 10-fold more sensitive than microscopy, with one study reporting a sensitivity to detect 1.35 to 0.38 parasites/µL for P. falciparum and 0.12 parasites/µL for P. vivax. The PCR test has also been found useful in unraveling the diagnosis of malaria in cases of undiagnosed fever.

C.Detection Of Antimalarial Antibodies[1]: Antibodies to the asexual blood stages appear a few days after malarial infection, increase in titer over the next few weeks, and persist for months or years in semi-immune patients in endemic areas, where re-infection is frequent. In non-immune patients, antibodies fall more rapidly after treatment for a single infection and are undetectable in 3-6 months. Re-infection/relapse induces a secondary response with a rapidly increasing antibody titer.
Malarial antibodies can be detected by immunofluorescence or enzyme immuno assay. It is useful in epidemiological surveys, for screening potential blood donors and occasionally for providing evidence of recent infection in non-immunes. In future, detection of protective antibodies will be important in assessing the response to malaria vaccines.

D. Flowcytometry[1,8,9,10]: Flowcytometry and automated hematology analyzers have been found to be useful in indicating a diagnosis of malaria during routine blood counts. In cases of malaria, abnormal cell clusters and small particles with DNA fluorescence, probably free malarial parasites, have been seen on automated hematology analyzers and it is suggested that malaria can be suspected based on the scatter plots produced on the analyzer. Automated detection of malaria

pigment in white blood cells may also suggest a possibility of malaria with a sensitivity of 95% and specificity of 88%. On flow cytometric depolarized side scatter, the average relative frequency of pigment carrying monocytes was found to differ among semi-immune, non-immune and malaria negative patients.

E. Mass spectrometry [11,12,13]: A novel method for the in vitro detection of the malarial parasite at a sensitivity of 10 parasites/µL of blood has been recently reported. It comprises a protocol for cleanup of whole blood samples, followed by direct ultraviolet laser desorption time-of-flight mass spectrometry. Intense ion signals are observed from intact ferriprotoporphyrin IX (heme), sequestered by malaria parasites during their growth in human red blood cells. The laser desorption mass spectrum of the heme is structure-specific, and the signal intensities are correlated with the sample parasitemia. Many samples could be prepared in parallel and measurement per sample may not take longer than a second or so. However, the remote rural areas without electricity are not hospitable for existing high-tech mass spectrometers. Future improvements in the equipment and technique can make this method deployable and useful.

Other investigations: Total and differential count, hemoglobin, blood glucose, serum bilirubin, serum creatinine, BUN, SGPT, Prothrombin time, urine analysis etc. may be done as needed.

Widal test may be positive, even up to a dilution of 1:320 for 'O' and H' and at lower titres for 'AH' and 'BH'. Any or all the four may be positive, suggesting a non-specific response. A positive Widal test in a patient with confirmed malaria should not therefore be considered as suggestive of typhoid fever.

Discussion: Spectrum of techniques are available for diagnosis of malaria.Present comparative study show its benefits and drawbacks, each and every test is having its own place. While mass population is from poor socio economical background ,peripheral smear examination by an expert pathologist is gold standard for a tertiary care center but when availability of skilled microscopist, particularly at remote area Rapid diagnostic tests can be used but it has it's own disadvantages. An attempt is to be made to establish QBC system at medical colleges and however patient's with low parasitemia and complicated malaria needs other support like various non- microscopic methods or PCR especially when patient presented with rare symptoms. Flowcytometry should be restricted up to research purpose only because of it's cost and mass spectrometry can be definitely utilize at pandemic area. Other supportive investigation as mentioned should be considered the world is floating in a large number of mosquito borne diseases especially Dengue and Chikungunya.

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