AN EXPOSITORY ON MALARIA DIAGNOSTIC TECHNIQUES IN THE TROPICS

AMALU, Christopher Tochukwu and OKAFOR, Fabian Chukwuemenam
Animal Parasitology and Public Health Research Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Corresponding Author: Amalu**,** C. T., Animal Parasitology and Public Health Research Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria. **Email:** cityamalu@yahoo.com **Phone:** +234 803 7240369,

ABSTRACT

The need for new perspectives for malaria diagnostic techniques in the tropics is on the increase due to rising prevalence rate of malaria mortality and morbidity. The microscopic examination of venous thin and thick blood films is the current "gold standard" for routine laboratory diagnosis of malaria. Other available methods include:- Fluorescence microscopy, which has improved the sensitivity, but not the specificity of diagnosis, Polymerase Chain Reaction (PCR) diagnosis, which is sensitive for all four species of human Plasmodium spp. and can be expected to exceed the sensitivity of microscopy; but poses time and technical problems, Immunochromatographic dipsticks that offer the possibility of more rapid, non-microscopic methods for malaria diagnosis, thereby saving on training, time and Self-diagnosis. Kits for self-diagnosis by travellers in remote areas has been developed.

Keywords: Microscopy, Immunochromatographic dipsticks**,** *Plasmodium* spp.**,** Polymerase chain reaction, Diagnostic techniques, Gold standard, Mortality, Morbidity

INTRODUCTION

Malaria threatens more than 40% of the world's population. Every year, there are 300 – 500 million cases of malaria and it kills 1.5 – 2.7 million people worldwide, with pregnant women and children being more vulnerable (WHO, 2000a). Malaria does not only affect the developing world. Isolated, locally transmitted cases still occur in North America, and a growing number of western travellers are developing malaria. There are 12, 000 malaria cases per year in Western Europe (WHO, 2000a). Deaths linked to malaria in Africa are on the increase due to changes in climate, movement of populations arising from political instability and civil strife, resistance of malaria to common and inexpensive medicines, resistance of mosquitoes to insecticides, and limitations in national services (WHO, 2000b).

Malaria keeps Africa's people poor. It prevents adults from working and children from attending school. Each year a family spends several months' earnings on malaria treatment and prevention. Malaria turns pregnancy, a normal life process into a nightmare, in which the mother may die or her baby born too small to survive (WHO, 2000b).

South and Central America, South and East Asia, the Caribbean, Oceania, Central Asia and Middle East are all affected by malaria, but Africa bears the heaviest malaria burden. Ninety percent (90%) of all malaria deaths occur in the tropical Africa, and in some parts of the continents, sickness and deaths due to malaria have been increasing steadily due to resurgence of resistant *Plasmodium* species and *Anopheles* species vectors. The burden of malaria in Africa is particularly dangerous, causing 900,000 deaths, and every 30 seconds an African child

dies. Everyday, at least 1000 Africans die of malaria and at least 20% of all are children under five. The morbidity is high with about 500,000 African children suffering from cerebral malaria and high incidence of mortality during pregnancy and neonatal deaths (within the first 28 days of birth), about 8 – 14% low birth weight and 3 – 8% of all infant deaths in endemic areas of Africa (Akukwe, 2004).

The disease is transmitted in human by a protozoan parasite of genus *Plasmodium,* which include *Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*; in order of decreasing virulence.

Malaria can be suspected based on a patient's symptoms and physical findings at examination. However, for a definitive diagnosis to be made, laboratory tests must reveal the malaria parasites or their components (CDC, 2005).

Prompt and accurate diagnosis is the key to effective disease management, one of the main interventions of the Global Malaria Control Strategy. Poor diagnosis hinders effective malaria control. This is due to a combination of factors, including non-specific clinical presentation of the disease, high prevalence of asymptomatic infection in some areas, lack of resources and insufficient access to trained health care providers and health facilities, and widespread practice of self-treatment for clinically suspected malaria.

A major contributing factor, however, is that the laboratory diagnosis of malaria has up till now relied nearly exclusively on microscopy, which is a valuable technique when performed correctly, but unreliable and wasteful when poorly executed. A better utilization of microscopy and the development of alternative diagnostic techniques could substantially improve malaria diagnosis. Such objectives prove particularly relevant to the Roll Back Malaria initiative. According to WHO (1991), the most commonly used technique for blood examination is the stained blood films. Giemsa stain (one of the Romanwosky stains) is usually used to stain the films. Field's stain is an alternative where rapid diagnosis is required.

The development during the past ten years of rapid diagnostic tests (RDTs) for malaria using immunochromatographic test strips that offers a valid alternative to or complement microscopy. Various RDT kits have been tested in clinical and field situations, marketed and have found limited use in some malaria control programmes, as well as in special situations such as complex emergencies, epidemics and the diagnosis of malaria in returning travellers. The overall results have been encouraging, and several manufacturers are currently developing improved kits for the global market. The viable market for such kits is illustrated by the fact that one manufacturer alone has introduced 3 – 6 million test kits to date.

The time has come for serious consideration of how RDTs can most effectively be applied to the very diverse situations of malaria occurrences. To that effect, an informal consultation was convened in Geneva on 25 – 27 October 1999, bringing together the developers, manufacturers and potential users of RDTs, representatives of other interested agencies, and other stakeholders to discuss future actions to ensure their optimal deployment to control malaria (WHO, 2000b).

MATERIALS AND METHODS

A comprehensive search was made from the Internet, various journal articles and textbooks of reports on malaria diagnostic techniques in the tropics. Such articles were assembled and studied and this opinion report developed.

RESULTS

Different Types of Malaria Diagnostic Techniques

Clinical diagnosis: This is based on the patient's symptoms and on physical findings at examination. The characteristic feature of malaria is fever caused by the release of toxins (when erythrocytic schizonts ruptures) which stimulate the secretion of cytokines from leucocytes and other cells. In the early stages of infection the fever is irregular or continuous. As schizogony cycles synchronize, fever begins to recur at regular intervals particularly in quartan

malaria (every 72 hour), vivax and ovale malaria (every 48 hour). This method of diagnosis is widely practiced by physicians and some health workers including the indigenous medicine practitioners in remote areas due to absence of microscopy diagnostic facilities and unavailability of rapid diagnostic dipstick tests. The diagnosis of malaria is based upon detecting the asexual forms of the parasites, in the blood smear stained with Giemsa or Field's stain. In cases where the laboratory diagnosis is unavailable or unreliable, a history of exposure within the last year, particularly within the preceding 10 weeks and the suggestive clinical picture should prompt the physician to start anti-malarial treatment. The need to recognize severe malaria in both adults and children is important as this allows the immediate institution of optimal treatment in the hospital - either with increased nursing care in a general ward or preferably by admission to an intensive care unit (ICU) or high dependency unit (HDU), where requisite monitoring, nursing and therapeutic facilities are available (Njuguna and Newton, 2004).

Typical malaria fever attack: The first symptoms of malaria (most often fever, chills, sweats, headaches, muscle pains, nausea and vomiting) are often not specific and are also found in other diseases (such as the "flu" and common viral infections). Likewise, the physical findings are often not specific (elevated temperature, perspiration, tiredness). It starts with a cold stage (rigor) in which the patient shivers and feels cold, even though his or her temperature is rising. A hot stage follows in which the temperature rises to its maximum, headache is severe, and there are back and joint pains, vomiting, and diarrhea. The final stage known as paroxysm is when the patient perspires, the temperature falls, the headache and other pains are relieved, and the patient feels exhausted. Splenomegaly occurs in forms of malaria with repeated attack causing a great enlarged spleen. Anaemia and jaundice are also features of malaria, particularly *P. falciparum* malaria. In severe malaria (caused by *P. falciparum*), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the suspicion index for malaria. Thus, in most cases the early clinical findings in malaria are not typical and need to be confirmed by a laboratory test.

Presumptive diagnosis and treatment: In highly endemic areas (particularly in Africa), the great prevalence of asymptomatic infections and lack of resources (such as microscopes and trained microscopists) have led peripheral health facilities to use "presumptive treatment". Patients who suffer from a fever that does not have any obvious cause are presumed to have malaria and are treated for that disease, based only on clinical suspicion, and without the benefit of laboratory confirmation. This practice is dictated by practical considerations and allows the treatment of a potentially fatal disease. But it also leads frequently to incorrect diagnoses and unnecessary use of antimalarial drugs. This results in additional expenses and increases the risk of selecting for drug-resistant parasites.

Advantages of clinical diagnosis: The advantages of clinical diagnosis include; prompt and/ or presumptive treatment in malaria endemic regions, especially in rural areas lacking health facilities, cost effective or cheap, diagnosis is based on signs and symptoms, except in some asymptomatic individuals, diagnosis and treatment are based on indigenous knowledge and no special training and rigid protocols are adhered to.

Disadvantages of clinical diagnosis: This may result in a delay before the correct diagnosis is made and which may lead to death before appropriate treatment can be initiated. It increases the dangers of self-medication. It may result to drug abuse, over dosage, under dosage, and consequently the insurgence of wide drug resistance to the resistant strain of *P. falciparum.* Small outbreaks of malaria may occur in countries considered free of the disease, such outbreaks are most likely the result of an infected person entering the country asymptomatic and where suitable mosquito vectors are present. No measurable sensitivity and specificity may be achieved and diagnosis is

based on assumptions, because signs and symptoms could be mimic as in the case of black fever.

Routine Laboratory Diagnosis / Microscopic Examination

Advantages of microscopy

Sensitive: When used by skilled and careful technicians, microscopy can detect densities as low as 5–10 parasites per µl of blood. Under general field conditions, the detection capabilities of a typical microscopist can be more realistically placed at 100 parasites per µl of blood.

Informative: Observed parasites are can be characterized in terms of their species (*P. falciparum, P. vivax, P. ovale*, and/or *P. malariae)* and of the circulating stage (e.g. trophozoites, schizonts, gametocytes). Occasionally, expert microscopists can detect morphological alterations induced by recent drug treatment. Also, the parasite densities can be quantified (from ratio of parasites per number of leukocytes or erythrocytes). Such quantifications are needed to demonstrate hyperparasitaemia (which may be associated with severe malaria) or to assess parasitological response to chemotherapy.

Relatively inexpensive: Cost estimates for endemic countries range from about 0.12 USD to 0.40 USD per slide examined. Such figures do not reflect the true cost to the health system or to the patient, which may be substantially higher. The cost per test will increase if utilization is low, or if microscopy in the health facility is used only for malaria diagnosis. It is a general diagnostic technique that can be shared with other disease control programmes, such as those against tuberculosis or sexually transmitted diseases. It can provide a permanent record (the smears) of the diagnostic findings and be subject to quality control.

Disadvantages of microscopy: During preparation of the thin and thick films, the parasites may appear distorted making species identification difficult. It is labour-intensive and time-consuming, normally requiring at least 60 minutes from specimen collection to result.
Thin and thick films preparations depend absolutely on good techniques, reagents, microscopes and, most importantly, well trained and well supervised technicians. Unfortunately these conditions are often not met, particularly at the more peripheral levels of the health care system. Thus, microscopic diagnosis may be becoming riskful an unreliable tool that uses up scarce resources for doubtful results. Long delays in providing the results to the clinician, that decision on treatment are often taken without the benefit of the results. False-positive results may result due to improper collection, preparation, examination, and identification of parasite density and species differentiation. Extremely difficult to find or recognize malaria parasites in blood films within few hours of treatment (expect in drug resistance situation). In patients who live in malarious areas and who are partially immune to the disease, malaria infections may be asymptomatic and of little clinical significance. Repeated blood films may be necessary to detect malaria parasites in non immune patients, in whom symptoms can arise from very low parasitemia. Danger of transmitting other diseases, such as AIDS or hepatitis B, through the use of contaminated lancets, the collection of blood samples raises significant biosafety issues. The transportation and examination of blood slides for epidemiologic surveys can be both cumbersome and logistically difficult, particularly in remote areas. There is a backlog of slides to be examined, and results may not be available for many months. High cost of materials, reagents and microscopes.

Fluorescence Microscopy

Before the introduction of rapid immunodiagnostic test strips for diagnosing of malaria, two fluorescence techniques were developed to improve the sensitivity and speed of detecting parasites using microscopy. These are: Becton Dickinson Quantitative Buffy Coat (QBC) system using the ParaLens objective to

obtain incident light fluorescence and Kawamoto AO Interference Filter system using transmitted light fluorescence.

QBC system: In the QBC system, malaria parasites are concentrated by centrifuging blood in a special capillary tube. The tube is coated with acridine orange and an anticoagulant. It contains a small plastic cylinder (float). Following centrifugation, the white cells, platelets, and upper layer of the red cells which contain the malaria can be found in the capillary. When examined by fluorescence microscopy at X600 (using a ParaLens objective), the acridine orange stained malaria parasites fluoresce green-yellow against a dark red-black background with the nucleus (chromatin dot) of trophoziotes and meroziotes of schizonts fluorescing bright green. Schizonts and gametocytes can be seen in the white cell layers. Although more rapid than examining thick stained blood films, field evaluations of the QBC system have shown it to be less sensitive than examining thick stained blood films in detecting low parasitaemia (<100/ul) and less sensitive than ParaSight F.

Kawamoto AO interference filter: This system, designed by Dr. F Kawamoto, requires an interference filter specially designed for the fluorochrome AO and located in the sub stage filter holder of an ordinary transmitted light microscope, a barrier filter (inserted in the eyepiece) and a 150 W or 200 W halogen lamp. The nuclei of malaria parasites and white cells fluorescent bright green and the cytoplasm of the parasites fluoresces red (particularly the cytoplasm of gametocytes). Compared with the QBC system, the Kawamoto fluorescence system is inexpensive. Most field evaluation of the system have found it to be as sensitive, but more rapid than Giemsa stained thick films in detecting *P. falciparum* parasites. Blood films must be prepared and stained correctly.

Advantages of fluorescence microscopy: Useful in survey work for screening blood donors and reduces wastage. The AO stained films can be washed with methanol and restained with Giemsa.

Disadvantages of fluorescence microscopy: They are of little value in the "acute" malaria situation. The equipment required for the QBC system is very expensive. The special disposable capillary tubes are also more than can be affordable by most hospital laboratories. Although more rapid than examining thick stained blood films, field evaluations of the QBC system have shown it to be less sensitive than examining thick stained blood films in detecting low parasitaemia (< 100/µl) and less sensitive than ParaSight F. Species differentiation is difficult thus identity of the actual cause of malaria is problematic. Considerable skill and experience are required to process and examine the tubes correctly and confidently. Microcoagulation, the adverse effects of heat and humidity, strong background fluorescence, scattered lyzed white blood cells and the incorrect positioning of the float, have been reported as making it difficult to examine some tubes. The tubes must be centrifuged promptly. Over staining or under staining make the parasites difficult to be recognized

Molecular Diagnosis

Polymerase chain reaction (PCR): Another relatively new method is the polymerase chain reaction (PCR) which uses a non-isotopically labelled probe following PCR amplification. PCR may yet prove to be a valuable addition to the examination of blood films for the diagnosis and speciation of malaria (Moody, 2002).

Advantages of PCR: It is possible to detect <10 parasites per 10uL of blood. PCR is a valuable addition to the examination of blood films for the diagnosis and it is useful in speciation of malaria parasites.

Disadvantages of PCR: The special equipment required precludes practical field application for rapid diagnosis of malaria. Some researchers have claimed that PCR (and Elisa) techniques are as sensitive as blood films, however they are infinitely more expensive, requires specialized equipment and takes a longer time to complete.

Rapid Diagnostic Test (RDT)/Immunochromatographic Tests (ICT)

A new generation of easy to perform tests has been developed to diagnose *falciparum* malaria rapidly and reliably without the need of a microscope. The three main groups of antigens detected by commercially available RDTs are: (1) histidine-rich protein 2 (HRP2), specific to *P. falciparum (2) Plasmodium* lactate dehydrogenase (pLDH), currently used in products that include *P. falciparum*-specific, pan-specific, and *P. vivax*-specific pLDH antibodies and (3) aldolase (pan-specific).

The targeted antigens of commercially-available malaria rapid diagnostic tests include; HRP2, pLDH, aldolase, *P. falciparum*-specific, Pan-specific (all species) and *P. vivax*-specific. Most of the commercial products include antibodies such as: HRP2 alone (*P. falciparum*), HRP2 and aldolase (distinguishing *P. falciparum*/mixed infection from non-falciparum alone), Falciparum-specific pLDH and pan-specific pLDH (distinguishing *P. falciparum* / mixed infection from non-falciparum alone), HRP2 and pan-specific pLDH, HRP2, pan-specific pLDH and vivax-specific pLDH or Pan-specific aldolase only. RDT detecting both falciparum-specific and non-falciparum (or pan-specific) target antigens are commonly called combination or 'combo' tests.

The products come in a number of formats such as: plastic cassette, card, dipstick and hybrid cassette-dipsticks. Cassettes tend to be simpler to perform than dipsticks, and this is likely to affect test accuracy (WHO, 2005).

The most recently developed tests can also diagnose vivax malaria. Three blood tests are available for diagnosing falciparum malaria based on the immunochromographic detection of antigen HRP 2 (histidine-rich protein 2) or specific pLDH (parasite lactate dehydrogenase). Both HRP 2 and pLDH are produced by the parasites during their growth and multiplication in red cells. The tests are ParaSight F and ICT Malaria pf (both producing antigen HRP 2) and OptiMAL (producing antigen pLDH).

HRP 2 antigen: This is produced by *P. falciparum* and is released from parasitized cells into the circulation. Following successful treatment, HRP 2 can be found in the blood several days after parasites have cleared. In heavy infections, HRP 2 may persist for up to 14 days or more. According to Cheesbrough and Precott (1998), a small number (2 – 3%) of locally found *P. falciparum* strains in Mali were naturally lacking the gene which produces HRP 2 (HRP 2 gene) and therefore gave false ParaSight F test results.

Performance of ParaSight F: ParaSight F was first rapid immunochromographic malaria test to be developed. It has been evaluated extensively in tropical and developing countries, in stable and unstable areas of malaria transmission and in both laboratory and non-laboratory situations including village health workers. The evaluations have shown ParaSight F to be sensitive and specific, performing as well as, and sometimes better than microscopy in field situations.

ICT combined test to diagnose falciparum and vivax malaria: ICT diagnostics have developed a test that can diagnose both falciparum and vivax malaria, and can be performed as easily as the ICT Malaria pf test. The new combined test is expected to be available in 1998.

ICT Malaria pf for diagnosing falciparum malaria: ICT malaria pf kit contains 25 tests and has a shelf-life of one year. ICT Diagnostics also manufactures 2 and 4 test kits for travellers visiting falciparum malaria endemic areas. These kits are called Malapachol.

ICT Combined Test to Diagnose Falciparum and Vivax Malaria: ICT Diagnostics have developed a test that can diagnose both falciparum and vivax malaria, and can be performed as easily as the ICT Malaria pf test.

pLDH antigen test to diagnose falciparum and vivax malaria: The metabolic malaria parasite enzyme pLDH is actively produced by

all human parasite species during their growth in red cells. It is found in the blood and although present in urine, it is not sufficiently constant to be useful. Differentiation of malaria species in the OptiMAL test is based on antigenic differences between pLDH isoforms. Unlike HRP 2, pLDH does not persist in the blood, but clears about the same time as the parasites following successful treatment.

OptiMAL test to diagnose falciparum and vivax malaria: This test, based on the detection of pLDH is the most recently developed of the immunochromographic rapid malaria strip tests. It is able to monitor responses to drug therapy and detect drug-resistant malaria because pLDH reflect the presence of viable malaria parasites in the blood. pLDH levels follow closely parasitaemia. *OptiMal* test detects pLDH enzyme with a series of monoclonal and polyclonal antibodies against *Plasmoduim* species. Studies so far carried out indicated that the tests were sensitive and able to differentiate between *P. vivax* and *P. falciparum*.

Sensitivity: This has been reported as between 84.20 – 96.60. Most studies have found the lower limit of detection for ParaSight F to be equivalent to 25 – 60 parasites /µl detected in thick film. Although an experienced microscopist working under optimal conditions is able to detect as few as 10 – 20 parasites/µl in a thick blood film, this level of sensitivity is rarely achieved in most district laboratories. Crag and Sharp (1997) found a sensitivity of 84 parasites /µl for Giemsa thick films and a sensitivity of 30 parasites/µl for ParaSight F.

Specificity: Most evaluations have estimated this to be between 81 – 99.5% with variations being found in different areas of malaria transmission. Positive predictive values ranged from 80 – 98.7% although lower values have been found in hypoendemic areas. Negative predictive values ranged from 72 – 100%.

Rapid Malaria Strip Tests: The cost of the new non-microscopical malaria tests in developing countries (in 1997, 1.230 – 2.25 USD or more, depending on country) is more than the cost of microscopy to diagnose falciparum malaria. In the many places where microscopy is not possible and malaria is diagnosed presumptively from clinical symptoms (shown repeatedly to be unreliable)**,** the savings in drug costs more than offset the cost of using HRP 2 or pLDH malaria test, particularly in areas of drug resistance where expensive second line antimalarial drugs may be need for used. In a recent study in Zimbabwe the use of ParaSight F reduced mistreatment for malaria, relative to clinical diagnosis, by up to 81%, especially in a hypoendemic area.

Besides the financial savings from unnecessary treatments, the use of non-microscopical rapid malaria tests is of value in the early investigation and management of malaria epidemics. The rapid tests are also of value in the diagnosing malaria in those who have taken antimalarial drugs (can make microscopical diagnosis difficult). Most important, extending and improving the accuracy of malaria diagnosis with subsequent correct use of drugs will reduce significantly the spread of parasite resistance to antimalarials.

Advantages of RDT: Rapid diagnostic test provides speedy result. The accuracy of diagnosing *P. falciparum,* particularly in non specialized laboratories where inexperienced or junior staff may be involved makes RDT preferable for diagnosis and mass chemotherapy associated with malaria. Very little training is required for these techniques. Dipstick kits are very useful in screening or confirmatory tests, especially when there is difficulty in identifying scanty ring forms in blood films. ParaSight F is available in tropical countries; kits contain 20 tests or 100 tests. It does not require refrigeration, but the strips and reagents must be kept out of direct sunlight and the strips kept dry. The kits have a 2 year shelf-life. The test is easy to perform and takes about 7 minutes.

Disadvantages of RDT: However dipstick methods are unable to indicate parasite load. In some countries, the cost may be prohibitive. A potential problem with these methods is that

the circulating antigen may be detected for many days (up to 2 weeks in our laboratory) after the elimination of viable parasites from the circulation. It must therefore be remembered that a positive test may not always be due to an active infection.

Other Diagnostic Techniques: Daily measurement of haemoglobin level especially at heavy parasitaemia, particularly in children under 5 years, when anaemia resulting from haemolysis, and anoxia, can be fatal. Blood urea or serum creatinine sensitivity test in suspected renal damage. Urine test for haemoglobin if black water fever is suspected. Haemoglobin sensitivity in Glucose 6 Phosphate Dehydrogenase (G6PD) deficiency. Liver Function Test (LFT), if liver cell is damage is suspected. Measurement of serum bilirubin, albumin and aspartate aminotransferase is a clue. Platelet count and measurement of plasma fibrinogen, FDPs (fibrin/fibrinogen degradation products), Activated Partial Thromboplastin time Test (APTT) and P0rothrombin Time (PT) if Disseminated Intravascular Coagulation (DIC) or other abnormal bleeding is suspected (Cheesbrough, 1987). An Indirect Haemagglutination Antibody (IHA) Test has also been used in epidemiological survey, but compared with the IFA test, it lacks sensitivity and specificity.

Serology: Serology detects antibodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). Serology does not detect current infection but rather measures past experience. Also are the Enzyme Linked Immunoabsorbent Assay (ELISA) (Voller, 1979) and Radioimmunoassay (RIA). These tests may become more important than the IFA test when monoclonal antibodies are used to produce specific purified antigens. Such tests enable large number of samples to be tested rapidly (Sulzer and Wilson, 1972).

Drug Resistance Tests: Drug resistance tests are performed in specialized laboratories to assess the susceptibility to antimalarial compounds of parasites collected from a specific patient. Two main laboratory methods are available:

In vitro tests: The parasites are grown in culture in the presence of increasing concentrations of drugs; the drug concentration that inhibits parasite growth is used as endpoint.

Molecular characterization: molecular markers assessed by PCR or gene sequencing allow also the prediction, to some degree, of resistance to some drugs; however, the predictive values of these molecular tests are still being evaluated.

Sensitivity and specificity: These are ratios comparing test results to True disease, which means test results from individuals known to have the particular condition are compared with test result of individual known not to have the condition. Actually, tests are used the other way round. Specimens are taken from individuals whose disease state is not known. It is the testy that is used to predict whether the individual have the disease, hence the term predictive value. Predictive value depends upon sensitivity, specificity and upon the disease in the population being tested.

DISCUSSION

A marked improvement in diagnosis would greatly enhance the applicability of the tests and/or their reliability: an increase in sensitivity, aiming at 100% sensitivity for densities of >100 parasites per µl blood in all four species; reduction or suppression of time-critical steps, or development of methods for self-timing; improvement in stability at high temperatures and against short temperature surges; improvement in the robustness of the test kits; reduction in the number of steps and test components; improvement in the readability of the tests (applies to better signal intensity as well as to the avoidance of mix-ups); development of safer methods of blood handling; and development of non-blood-based immunological tests (e.g. saliva and urine). It is suggested that the body itching and the

characteristic odour that occur during severe malaria may be due to acidosis and hypoglycemia as proposed by researchers and thus are symptoms for clinical diagnosis. Intradermal blood film provides a more complete picture both of the stage of infection and total body parasite burden than examining the peripheral blood alone, but it is unlikely to replace peripheral blood film as the primary tool for malaria diagnosis, as they fail to pick-up the none-sequestering *P. vivax.* Meanwhile, they may turn-out to be useful adjunct to the peripheral blood film for both diagnosis and provision of prognostic information either alone or more probably in combination with examination for more phagocyte pigment. However, venous blood films are the best for diagnosis because of the availability of free parasitized red cells in the circulating blood and with the pressure exerted by the syringe in drawing sequestered red cells from the capillaries during blood collection. In-addition, placental histological microscopy is the best diagnostic method for malaria in pregnancy. *If one is asked, identify the "*gold standard" *against which malaria diagnostic tests should be assessed?* While microscopy is acknowledged to be an imperfect diagnostic tool, it has practically always been used as the standard against which other tests such as RDTs are assessed. This work emphasizes that these dipstick methods are regarded as useful additional tests to the long established method of examining thick and thin blood films which is still regarded as the "gold standard", NOT as replacement methods. The highest density of malaria occurs in countries least able to afford sophisticated and expensive diagnostic tools. Tests such as PCR are more sensitive and specific, but may not reflect accurately the presence of live parasites. The identification of a better "gold standard" would not only provide an improved tool for the development of new diagnostic tests, but might also offer a better understanding of the biology of malaria in the human host. The main research questions are as follows. Which of the currently available methods should be used as the "gold standard"? Is there a combination of diagnostic findings that might yield a better approach to a "gold standard"? Can the same "gold standard" be used for all epidemiological situations?

Conclusion: It is suggested that a more proactive approach by government and stakeholders in equipping the laboratories in the tropics with diagnostic facilities that can give 100% sensitivity and specificity at a reduced cost and to ensure availability. Currently, the use of venipunture in microscopy is highly advocated for a better diagnostic result and as a "gold standard" to malaria diagnosis. There should be regular reporting of the malaria burden to health authorities to increase the opportunities of getting support for prevention and intervention in the control of malaria in the tropics. Malaria is still curable, the key to this, is prompt identification of the parasite and early institution of anti-malarial therapy.

REFERENCES

AKUKWE, C. (2004). Malaria and Tuberculosis: The Forgotten Diseases. *Excerpts of Continuing Medical Education (CME). Lecture delivered by at the International Health and Travel Medicine Seminar*. Organized by the Association of Scientists and Physicians of African Descent (ASPAD), Washington, DC, April 2004.

CDC. (2005). *Malaria Treatment.* Department of Health and Human Services, Centre for Disease Control (CDC), Atlanta, USA. Available at http://www.cdc.gov/malaria/diagnosis_treatment/treatment.htm. Assessed 23 October, 2011.

CHEESBROUGH, M. and PRECOTT, L. M. (1987). *Manual of Basic Techniques for a Health Laboratory*. World Health Organization, 239-250.

CRAIG, M. H. and SHARP, B. L. (1997). Comparative evaluation of four techniques for the diagnosis of *Plasmodium falciparum* infections. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 91(3): 279 – 282.

MOODY, A. (2002). Rapid diagnostic tests for malaria parasite. *Clinical Microbiology Reviews,* 15(1): 66 – 78.

NJUGUNA, P. W. and NEWTON, C. R. (2004). Management of severe falciparum malaria. *Journal of Postgraduate Medicine,* 50: 45 – 50.

SULZER, A. and WILSON, M. (1972). The indirect fluorescent antibody test for the detection of occult malaria in donors. *Bulletin of the World Health Organization,* 45: 131 – 133.

VOLLER, A. (1979). Methods for the Detection of Antibodies to the Asexual Blood Forms and Merozoites with Special Attention to Solid Phase Immunoassays. Immunodiagnostic Techniques in Malaria*, Chapter 7. UNDP/World Bank/World Health Organization*, 93-103.

WHO. (1991). *Basic Laboratory Methods in Medical Parasitology*. World Health Organization, Geneva.

WHO. (2000a). *New Perspectives: Malaria Diagnosis*, Report of a Joint WHO/USAID Informal Consultation 25-27 October, 1999, Geneva. (WHO/CDC/RBM)

WHO. (2000b). *Roll Back Malaria, Promise for Progress*. Roll Back Malaria Cabinet Project, WHO Geneva, 2-6.

WHO. (2005). *World Malaria Report 2005.* Map showing Malaria Regional Distribution (http://rbm.who.int/wmr2005/html/profile). Assessed Oct 2005.