Review
of
Literature
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Malaria is an acute and chronic protozoan illness characterized by paroxysms of fever, chills, sweat, fatigue, anemia and splenomegaly, occasional severe lesions of nervous system, liver, kidney and other organs.

History of Malaria


♦ The term malaria (meaning ‘bad air of marsh and swamp land’) first originated in 17th century (Rao G.P.1998).

♦ This fever was recognized in ancient India, China and Mesopotamia.

♦ Cincona bark was recognized to be effective against fever in Peru in 1600 A.D (Park J.E 1974).

♦ Giovanni Maria Lancisi (1654 – 1720) came close to understanding that a insect transmitted malaria believing that swamp emitted animate creature (later found to be mosquito and also inanimate particle that produce disease (Albert S. L 1987).

♦ Bright 1831 noted the pigmented appearance of spleen and brain at autopsy (Albert S. L 1987).

♦ In 1881 Charles Louis Alphonse Laveron (1845 – 1922) actually demonstrated the causative organism a protozoan but the mechanism of transmission was not proved (Albert S. L 1987).
Marchiafava in 1883 used methylene blue for staining of malarial parasite (Kaul S.M 1997).

Romanasky in 1891 developed a technique of staining for an accurate study of erythrocytic stage of malaria (Rao G.P 1998).

Ronald Ross, who while working in Secundrabad (Andhra Pradesh, India) discovered the transmission of malaria by anopheles mosquito in 1897. Ross found malarial parasite growing as cyst (oocyst) on stomach wall of an anopheles mosquito which had previously fed on malaria patient. This was subsequently proved in 1898 by Grassi (Kaul S.M 1987).

Manson in 1900 A.D confirmed the mosquito transmission of malaria (Albert S.L 1987).

In 1948 Shorff Garnham and Bray confirmed the presence of pre-erythrocytic stage in man (Rao G.P 1998).

Lanar (1989), Persing (1993), and Weiss (1993) used Acridine orange staining for detection of parasite specific antigen or DNA provide enhanced sensitivity and specificity but are generally not appropriate or available for smaller laboratories (Thomas R. F et al, 1998).

Aetiology of Malaria and life cycle of malarial parasite

Malaria is caused by intracellular plasmodium protozoa transmitted to human by female anopheles mosquito. Four species of plasmodium cause malaria in human: plasmodium falciparum, plasmodium vivax, plasmodium malariae and plasmodium ovale.

Plasmodium species exist in a variety of forms and have a complex life cycle that enables them to survive in different cellular
environment in human as intermediate host (Asexual phase) and the mosquito vector as definitive host (Sexual phase).

**Asexual cycle**

A marked amplification of plasmodium organism from approximately $10^2$ to as many as $10^{14}$ occurs during a two step process in human. The first occurs in the cells of liver (exoerythrocytic phase) and the second in the red blood cells (erythrocytic phase).

**Pre-erythrocytic Schizogony** (Intrahepatic schizogony or Merogony)

Human infection begins when a female anopheles mosquito inoculates sporozoits from its salivary gland during a blood meal. Within 30 minutes of bite, the sporozoits disappear from the blood and invade liver and reticuloendothelial tissues. Some of these are destroyed by phagocytes, while other reach hepatocytes and begin a period (for plasmodium falciparum 5.5 days, plasmodium vivax 8 days, plasmodium ovale 9 days, plasmodium malariae 15 days) of asexual reproduction (Loban, Polazak 1985). By this amplification process a single sporozoite of plasmodium falciparum produces 30000 daughter merozoits, plasmodium vivax 10000, plasmodium ovale 15000, plasmodium malariae 15000.

**Erythrocytic Schizogony**

Merozoits rapidly (within 30 seconds) invade erythrocytes and become trophozoits (Ladda et al, 1976). Attachment is mediated via a specific erythrocyte surface receptor. In case of plasmodium vivax, this receptor is related to the Duffy blood group
antigen $A_y^a$ or $F_y^b$ and for plasmodium falciparum it is glycophorines of erythrocytes membrane. During the early stage of intraerythrocytic development the small ring form of the four parasites species, appear similar under light microscopy. As trophozoits enlarge species specific characteristic become evident, pigment becomes visible and the parasite assumes an irregular or amoeboid shape. By the end of the 48 hrs (72 hours for plasmodium malariae) intraerythrocytic life cycle, the parasite has consumed nearly all the haemoglobin and grow to occupy most of the red cells. Multiple nuclear division takes place (Merogony) and the red cell ruptures to release 6 – 30 daughter merozoits, each capable of invading a new red cell and repeating the cycle.

**Gametogony**

After a series of asexual cycle (plasmodium falciparum) or immediately (plasmodium vivax, ovale and malariae) some of the parasites develop into morphologically distinct long lived sexual forms (gametocytes).

**Sexual Cycle (Sporogony)**

After being ingested in the blood meal of a biting female anopheles mosquito, the male and female gametocytes form a zygote in the insects midgut. This zygote matures into an ookinite, which penetrates and encysts in the mosquito’s gut wall. The resulting oocyst expands by asexual division until it bursts to liberate myriad motile sporozoits, which then migrate in the hemolymph to salivary gland of mosquito to await inoculation into another human at the next feeding.
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>P. falciparum</th>
<th>P. vivax</th>
<th>P. ovale</th>
<th>P. malariae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Pre-erythrocytic phase</strong></td>
<td>(<strong>2. Erythrocytic phase</strong>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Duration</td>
<td>5.5 days</td>
<td>8 days</td>
<td>9 days</td>
<td>15 days</td>
</tr>
<tr>
<td>b. Number of merozoits</td>
<td>30,000</td>
<td>10,000</td>
<td>15,000</td>
<td>15,000</td>
</tr>
<tr>
<td><strong>2. Erythrocytic phase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Duration</td>
<td>36 – 48 hours</td>
<td>48 hours</td>
<td>50 hours</td>
<td>72 hours</td>
</tr>
<tr>
<td>b. Red cell preference</td>
<td>All RBCs</td>
<td>Reticulocytes</td>
<td>Reticulocytes</td>
<td>Older RBCs</td>
</tr>
<tr>
<td>c. Maximum duration of erythrocyte infection if untreated</td>
<td>2 years</td>
<td>4 years</td>
<td>4 years</td>
<td>40 years</td>
</tr>
<tr>
<td><strong>3. Multiple infectivity</strong></td>
<td>Common</td>
<td>Occasional</td>
<td>Occasional</td>
<td>Rare</td>
</tr>
<tr>
<td><strong>4. Chloroquine resistance</strong></td>
<td>Yes (Widespread)</td>
<td>Rare</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>5. Sequestration of trophozoits and schizonts</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>6. Relapse</strong></td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>7. Recrudescence</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>8. Microscopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A Erythrocyte</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Shape and size</td>
<td>Normal size, Normal shape</td>
<td>1½ to 2 times larger than normal, oval to round</td>
<td>60% of the cells larger (1½ to 1½) than normal, oval, 20% have irregular frayed edges</td>
<td>Size normal, shape normal</td>
</tr>
<tr>
<td>(ii) Schuffner's dots</td>
<td>Absent (Maurer's dots occasionally seen)</td>
<td>Present with all stages except early ring form</td>
<td>Present with all stages except early ring form, dots may be larger and darker than in vivax</td>
<td>Absent (Zeimanns dots rarely seen)</td>
</tr>
<tr>
<td><strong>B Parasite</strong></td>
<td>(<strong>2. Erythrocytic phase</strong>)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Number of merozoits</td>
<td>6 – 32 (average 24)</td>
<td>12 – 24 (average 16)</td>
<td>6 – 14 (average 8)</td>
<td>6 – 14 (average 8)</td>
</tr>
<tr>
<td>(ii)</td>
<td>Stages in peripheral blood smear</td>
<td>Ring form and / or gametocytes, other stages develop in blood vessel of internal organ</td>
<td>All stages</td>
<td>All stages</td>
</tr>
<tr>
<td>(iii)</td>
<td>Pigments</td>
<td>Black, coarse and conspicuous in gametocytes</td>
<td>Golden brown conspicuous</td>
<td>Dark brown conspicuous</td>
</tr>
<tr>
<td>(iv)</td>
<td>Early trophozoits (ring form)</td>
<td>Delicate small ring, small chromatin dots scanty cytoplasm around small vacuoles or filamentous cylinder form may have multiple ring per cell</td>
<td>Ring is 1/3 diameter of the cell, cytoplasmic circles around vacuoles, heavy chromatin dots</td>
<td>Ring is larger and more amoeboid than in vivax, otherwise similar to vivax</td>
</tr>
<tr>
<td>(v)</td>
<td>Mature trophozoits</td>
<td>Not seen in peripheral blood (except in severe infection)</td>
<td>Irregular amoeboid mass fills almost entire cell, fine brown pigment</td>
<td>Compact vacuoles disappear, pigment dark brown, less than in p.malariae</td>
</tr>
<tr>
<td>(vi)</td>
<td>Schizont (presegmental)</td>
<td>Not seen in peripheral blood</td>
<td>Progressive chromatin division, cytoplasmic band containing clump of brown pigment</td>
<td>Smaller and more compact than in vivax</td>
</tr>
<tr>
<td>(vii)</td>
<td>Mature schizont</td>
<td>Not seen in peripheral blood (rare exception)</td>
<td>Chromatin and cytoplasm present, fills entire red cell which can hardly be seen</td>
<td>Occupy ¼ of red cell, rosette or irregular cluster</td>
</tr>
<tr>
<td>(viii)</td>
<td><strong>Macrogametocyte</strong></td>
<td>Sex differentiation difficult, crescent or sausage shaped characteristic, may appear in shower black pigment</td>
<td>Rounded or oval, light brown pigment throughout parasite, eccentric compact chromatin</td>
<td>Smaller than vivax</td>
</tr>
<tr>
<td>(ix)</td>
<td><strong>Microgametocyte</strong></td>
<td>Sex differentiation difficult, crescent or sausage shaped characteristic, may appear in shower black pigment</td>
<td>Large, pink to purple chromatin mass surrounded by pale and colourless halo, evenly distributed pigment</td>
<td>Smaller than vivax</td>
</tr>
<tr>
<td>9.</td>
<td><strong>Main criteria</strong></td>
<td>Delicate ring form, crescent shaped gametocyte are only forms normally seen in peripheral blood</td>
<td>Large pale red cells, trophozoits irregular, pigment usually present, schuffner's dots not always present, several phase of growths seen in one smear, gametocytes appear early</td>
<td>Red cells enlarged, oval with fimbriated edge, schuffner's dots seen in all stages</td>
</tr>
</tbody>
</table>
Malaria Toxins

- Histotoxic - Direct tissue damage
- Pyrogenic - Causing paroxysms of fever
- Hemolytic - Causes hemolysis
- Endotheliotoxic - Causing damage to capillary and tissue endothelium

Clinical Features

Wide variability in malaria’s clinical presentation poses great difficulties in clinical diagnosis and management. Malaria can be divided into complicated and uncomplicated malaria. For all practical purposes complicated malaria is caused by plasmodium falciparum.

Uncomplicated Malaria

The usual incubation periods are as follows: plasmodium falciparum 9 – 14 days, plasmodium vivax 12 – 17 days, plasmodium ovale 16 – 18 days and plasmodium malariae 18 – 40 days.

Malaria caused by plasmodium vivax, plasmodium ovale, plasmodium malariae usually results in parasitemia of < 2% whereas that of plasmodium falciparum can be ≥ 60% because plasmodium falciparum infects immature and mature erythrocytes. Plasmodium malariae malaria is the mildest and most chronic of all malarial infections.

Plasmodium ovale malaria is the least common type of malaria. It is similar to plasmodium vivax malaria and commonly is found in conjunction with plasmodium falciparum malaria. Clinical manifestation varies a great deal in different pediatric ages. Malaria is invariably mild in neonates and young infants.
Splenomegaly, anaemia with or without fever is usual presentation in this age group. Presence of passively acquired maternal protective antibodies, high number of HbF containing senescent RBCs and milk diet deficient in para-amino benzoic acid are factors and believed to exert malarialstatic effect at this age.

Late infancy and early childhood becomes the most susceptible age for malarial infection. Respiratory, gastrointestinal and convulsive symptoms dominate in this age group. Marked vomiting, greenish mucoid loose stools associated with abdominal colic and tenesmus are present. Convulsion commonly occurs during malarial fever (Febrile convulsion). Absence of organic neurological involvement differentiates these patients from cerebral malaria. By late childhood, the malarial presentation starts evolving into classical paroxysmal pattern. Malaria paroxysm comprises of three successive stages, namely cold stage, hot stage and sweating stage. This paroxysm form of malarial fever invariably is absent in plasmodium falciparum infection, where pyrexia is irregular and devoid of specific pattern.

Anaemia and splenohepatomegaly are constant features with all forms of malarial presentations.

**Complicated Malaria (WHO 2000)**

A patient with severe falciparum malaria may present with confusion or drowsiness with extreme weakness. In addition the following may develop:

- Cerebral malaria – defined as unarousable coma not attributable to any other cause in a patient with falciparum malaria.
- Generalized convulsions.
• Severe normocytic anaemia.
• Hypoglycemia.
• Metabolic acidosis with respiratory distress.
• Fluid and electrolyte disturbances.
• Acute renal failure.
• Acute pulmonary oedema and adult respiratory distress syndrome (ARDS).
• Circulatory collapse, shock, septicemia (Algid malaria).
• Abnormal bleeding.
• Jaundice.
• Haemoglobinuria.
• High fever.
• Hyperparasitemia.

These severe manifestations can occur singly or more commonly in combination in the same patient.

Relapse
Relapse is defined as reappearance of parasitemia in a sporozoite induced infection following adequate blood schizonticidal therapy. Some of the sporozoits do not immediately start to grow and divide but remain in a dormant stage for weeks or months in liver cells. These dormant parasites are known as hypnozoits.

Only plasmodium vivax and plasmodium ovale produces hypnozoits and thus are capable of relapsing.
Recrudescence

Plasmodium falciparum and plasmodium malariae may produce secondary waves of parasitemia by two mechanisms. The erythrocytic stages of these parasites may survive on a long-term basis or continue to undergo schizogony in the blood stream at low levels for a prolonged period of time. These recurrent attacks are known as recrudescence that may continue to occur for months or years together.

Prognostic Indicators

Major indicators of a poor prognosis are:

*Clinical indicators:*
- Age under 3 years.
- Deep coma.
- Witnessed or reported convulsion.
- Absent corneal reflexes.
- Decerebrate / decorticate rigidity or opisthotonus.
- Clinical signs of organ dysfunction (e.g. renal failure, pulmonary oedema).
- Respiratory distress (acidosis).
- Circulatory collapse.
- Papilloedema and / or retinal oedema.

*Laboratory Indicators:*
- Hyperparasitemia (250000 / μl or > 5%).
- Peripheral Schizontaemia.

- Peripheral blood polymorphonuclear leucocytosis (> 12000 / μl).
- Mature pigmented parasites (> 20% of parasites).
- Peripheral blood polymorphonuclear leucocytosis with visible malaria pigment (> 5%).
- Packed cell volume less than < 15%.
- Haemoglobin concentration < 5 gm%.
- Blood glucose less than < 40 mg% (2.2 mol / L).
- Blood urea > 60 mg%.
- Serum creatinine > 3 mg% (265 μmol / L).
- High CSF lactic acid (> 6 mmol / L) and low CSF glucose.
- Raised venous lactic acid (> 5 mmol / L).
- More than 3 folds elevation of serum enzymes (aminotransferase. Plasma 5 nucleotidase).
- Low antithrombin III level.
- Very high plasma concentration of TNF.

**Difference between severe malaria in adults and in Children**

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>Adults</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of Cough</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Convulsion</td>
<td>Common</td>
<td>Very common</td>
</tr>
<tr>
<td>Duration of illness</td>
<td>5 – 7 days</td>
<td>1 – 2 days</td>
</tr>
<tr>
<td>Resolution of coma</td>
<td>2 – 4 days</td>
<td>1 – 2 days</td>
</tr>
<tr>
<td>Neurological sequelae</td>
<td>&lt; 5%</td>
<td>&gt; 10%</td>
</tr>
<tr>
<td>Jaundice</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Pretreatment hypoglycemia</td>
<td>Uncommon</td>
<td>Common</td>
</tr>
<tr>
<td>Pulmonary oedema</td>
<td>Uncommon</td>
<td>Rare</td>
</tr>
<tr>
<td>Renal failure</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>CSF opening pressure</td>
<td>Usually normal</td>
<td>Usually raised</td>
</tr>
<tr>
<td>Respiratory distress (acidosis)</td>
<td>Sometimes</td>
<td>Common</td>
</tr>
<tr>
<td>Bleeding / clotting disturbance</td>
<td>Upto 10%</td>
<td>Rare</td>
</tr>
<tr>
<td>Abnormality of brainstem reflexes</td>
<td>Rare</td>
<td>More common</td>
</tr>
</tbody>
</table>
Diagnosis of Malaria

Most important element in the clinical diagnosis of malaria in both endemic and non-endemic areas, is to have a high index of suspicion.

Peripheral blood smear

In 1981, Laveron discovered malarial parasites in the blood smear of a dying patient. Since then, demonstrating parasites in blood film has remained a gold standard for malarial diagnosis.

Type of smear

Both thick and thin blood smears should be examined. The concentration of erythrocytes on thick smear is approximately 20 – 40 times that on a thin smear, so thick smear is used for parasite detection and thin smear for species identification.

A thick smear can pick up to 4 – 10 parasites per microlitre, while a thin smear can pick up to 100 parasites per microlitre (Bruce Chwatt LJ 1985).

Blood sample

It is better to take sample by prick of heal or finger because these sites pricks often yield parasites when venipuncture specimen fails (Murry Wittner 1996).

Stain

Giemsa stain is superior to Wright or Leishman stain (Murry Wittner 1996).
Time of sampling

Blood can be taken at any time with plasmodium vivax, but in plasmodium falciparum infection however, ring form may be present only immediately after fever peaks. Plasmodium falciparum is most likely to be identified from blood just after a febrile paroxysm. Timing of smear is less important than obtaining them several times a day (4 – 6 hourly) over a period of 3 successive days.

Sensitivity

Expert microscopy can detect as few as 4 asexual parasites / microlitre.

Advantages (WHO 2000)

Low direct cost, sensitive, can be used to differentiate between species and determine parasite density level. It can also be used to diagnose many other conditions.

Disadvantages

In clinical situation many a times for various technical and scientific reasons, blood film could falsely be negative. This problem is particularly marked with plasmodium falciparum (Ashok SK 2002).

The reliability and cost effectiveness of light microscopy is questionable (Payne 1988. WHO 1988). It is rather lengthy, needs trained personnel and maintenance cost.

So, need of cost effective technique is crucial for the implementation of Global strategy for malaria control (WHO 1993).
Indirect evidences

Anaemia is normocytic and may be severe (<4 gm%). Thrombocytopenia (< 100000 / µl) is usually present and peripheral leucocytosis is found in patient with the most severe disease. Elevation of serum creatinine, bilirubin and enzymes e.g. aminotransferase may be found. Severely ill patients are commonly acidotic with low capillary plasma pH and bicarbonate concentration. Fluid and electrolyte disturbances (Sodium, Potassium, Chloride, Calcium and Phosphate) are variable. Concentration of lactic acid in the blood and CSF are often high.

QBC

A modification of light microscopy, the quantitative buffy-coat method (Becton Dickinson USA) was originally developed to screen, large number of specimens for complete blood cells count, adopted for malaria diagnosis. The technique involved the use of a special fluorescent stain (Acridine orange) to highlight malaria parasite and centrifugation to concentrate parasites at a predictable location, on a specially prepared capillary tube (Wardlow Sc et al 1983, Spielman A et al 1988, Tharavanij 1990).

Method

60 microlitres EDTA blood is taken in QBC capillary tube coated with fluorescent dye upto the blue mark. The capillary is centrifuged at the rate of 1200 rpm for 5 – 7 minutes, different columns of blood are formed.

- Plasmodium falciparum rings are circular, smooth and walled.

  Nucleus and cytoplasm ratio 1:1 and nucleus is embedded into
cytoplasm (Nucleus bright green and cytoplasm appear pale green or pale orange).

- Plasmodium vivax rings are irregular in shape, nucleus and cytoplasm ratio 1:5 to 6 and nucleus is outside the cytoplasm.

The QBC technique was found to be a rapid technique, with a sensitivity of 83% and specificity of 94%. Malaria species identification was also possible. However, quantitation of parasitemia could not be made by this technique (Bijay R. et al 1998).

This technique can detect 3 – 4 parasites / ml of blood (Bijay R et al, 1998).

Advantages of QBC are that less staining is required to operate the system, and the test is typically quicker to perform.

Disadvantages to QBC technique are that, it requires special equipment, electricity, and the cost is more and species specific diagnosis is not reliable. Field trial have shown that QBC may marginally be more sensitive than conventional microscopy under ideal conditions (Wardlow SC et al 1983).

**DNA Probes**

The DNA of plasmodium falciparum has abundant repetitive sequences that are not released in blood stream after parasite lysis and that can be detected using hybridization. The two complement strands are separated and bound to a solid surface. They are mixed with a probe DNA which is also denatured and contain a nucleotide sequence complementary to a repetitive sequence in the parasite DNA. A second treatment allows strands to hybridize with the parasite DNA. This reaction may be visualized
by detecting isotope emission or the activity of any enzyme bound to the probe DNA.

DNA probes provide enhanced sensitivity and specificity, but are generally not appropriate or available for smaller laboratories (Thomas R. Fritsche et al 1998).

**Antibodies detection**

A number of methods are available for detection of malaria antibodies. The long lasting nature of antibodies restricts their use as diagnostic test. It has a special value on epidemiological studies.

Indirect hemagglutination test (IHA), Indirect fluorescent antibody (IFA) test, Gel precipitation test, ELISA have all been used for serodiagnostic purposes.

(a). Indirect hemagglutination test (IHA): The serological test have been found good as an indicator of endemicity and transmission of malaria. Of large number of tests, Indirect hemagglutination test (IHA) has been found to be simple, reliable, reproducible, specific, sensitive (plasmodium vivax 61.9% and for plasmodium falciparum 60.5%) (Rastogi SK et al 1988).

(b). Indirect fluorescent antibody (IFA) test: Sensitivity of technique is good and quantitative evaluation of antibody level can be obtained. Disadvantage of this technique is need of ultraviolet microscope and special reagent.

(c). ELISA: It can be used under field condition, can be read visually. It has relatively poor sensitivity and specificity when parasitized RBC extract are used.
ANTIGEN DETECTION

Inhibition ELISA

Parasite antigen may be detected in human blood during current malarial infection in inhibition ELISA. The test was found to be adequately sensitive and specific and provide a good immunodiagnostic tool in selective clinical and epidemiological situation (Mackey et al, 1982). The test was found to be quite sensitive, being able to detect 5 parasites / $10^6$ RBC in case of natural plasmodium falciparum infection (Krishna Ray et al 1992).

Antigen detection of plasmodium falciparum

The antigen detection parasight F Dipstick method for diagnosis of plasmodium falciparum malaria (Becton Dickinson USA) (Shiff et al 1993, 1994) appear to have highly acceptable level of sensitivity and specificity, almost equally those of microscopy, the standard method of malaria diagnosis.

The method based on the detection of soluble plasmodium falciparum antigen histidine rich protein 2 (HRP 2) (Parra et al 1991) in the circulation has been shown by Beadle et al (1994), Premji et al (1994), Dietze et al (1995), Backongason et al (1996), and Kaushik et al (2000) to have several advantages over microscopy, in endemic situation. These include ease and speed of performance, which suggest that it may be suitable for clinical use.

These dipstick tests (Parasight F, ICT malaria pf and PATH falciparum malaria IC Strip) detect only plasmodium falciparum infection. All three assays detect histidine rich protein 2 (HRP 2) an antigen expressed only by plasmodium falciparum trophozoits.
Antigen detection of plasmodium falciparum and plasmodium vivax

The two newer tests (the OptiMal assay and ICT malaria pf/pv assay) have advantage of detecting infection with plasmodium falciparum and plasmodium vivax both.

OptiMal Assay

The OptiMal assay detect parasite lactate dehydrogenase (PLDH) and can distinguish between plasmodium falciparum and plasmodium vivax because of the antigenic difference between their lactate dehydrogenase (PLDH) isoenzymes, because only live parasites produce PLDH. This test can be of clinical relevance because it distinguishes between living (clinical infection) and dead parasite (recently treated).

ICT malaria pf / pv (AMRAD Australia)

This test is a rapid manual test for plasmodium falciparum and plasmodium vivax. The test is simple to perform and provide a definite answer in about 10 minutes. It requires minimal training, no special equipment and requires only a single drop of blood.

It is an antigen capture test detecting histidine rich protein 2 (HRP 2) found only in plasmodium falciparum and panmalaria antigen found in plasmodium falciparum and plasmodium vivax both, allowing the two species to be differentiated. The test uses two antibodies which have been immobilized as two separate lines across a test strip. One antibody (test area 1) is specific for histidine rich protein 2 antigen of plasmodium falciparum. The other antibody (test area 2) is specific for a malarial antigen which is common to both (Panmalaria Ag). When a positive sample is
applied, malarial antigens bind to the gold coupled antibodies in the pad and immune complex formed migrate along the test strip where they are captured by the immobilized antibodies. When capture occurs a pink line forms.


The time taken for a patient to revert to negativity by this test is significantly longer upto 14 days, in contrast to microscopy which reverts negative almost immediately after cure.

Several studies have been done in respect to sensitivity and specificity of ICT malaria pf / pv

Gatti et al (2001), carried out a prospective, multicentric study, was carried out in Italy, to assess the sensitivity and specificity of a rapid dipstick test (ICT malaria pf/pv) in the diagnosis of imported malaria, caused by plasmodium falciparum and other plasmodium species. The 241 subjects were International travelers or migrants from area, where malaria is endemic. When compared with microscopic examination of blood smear (used as gold standard), the dipstick was found to be 94.4% sensitive and 94.5% specific for pure infection with plasmodium falciparum.

The performance of the test when used on patients infected with species other than plasmodium falciparum or more than one plasmodium species, showed a high degree of variability. Although, the dipstick represents a very simple, rapid and valuable
diagnostic aid, they should not be considered a complete substitute for direct microscopic diagnosis, using stained blood smear.

Tarimo DS et al (2001), carried study with children from a holoendemic region of Tanzania, were checked for malarial parasites by microscopy, and by using two rapid immunochromatographic tests (RIT) for diagnosis of malaria (ICT malaria pf/pv and OptiMal). The rapid immunochromatographic test, both had very high corresponding sensitivities (of 100% for ICT and 94% for OptiMal), but the specificity of ICT (74%), was significantly lower than that for OptiMal(100%).

Emiliana Tjitra et al (1998), evaluated the new combined plasmodium falciparum, plasmodium vivax immunochromatographic test (ICT malaria pf / pv) in eastern Indonesia with 560 symptomatic adults and children with presumptive clinical diagnosis of malaria. Blinded microscopy was used as the gold standard with all discordant and 20% of concordant results cross checked blindly. Only 50% of those with presumptive clinical diagnosis were parasitemic. The ICT malaria pf / pv was sensitive (95.5%) and specific (89.9%) for diagnosis of falciparum malaria HRP 2 and panmalaria antigen line intensities were associated with parasitemia density for both species. Although the specificity and negative predictive value for the vivax malaria were 94.8% and 98.2% respectively, the overall sensitivity (75%) and PPV (50%) for the diagnosis of vivax malaria were less than the desirable levels. The sensitivity for diagnosis of plasmodium vivax malaria was 96% with parasitemias of > 500/µl, but only 29% with parasitemias of < 500/µl.
Mason DP et al (2002), tested two rapid malaria immunochromatographic kits, the OptiMal assay and ICT malaria pf / pv. Both OptiMal and ICT gave lower sensitivities than previously reported. ICT sensitivity to plasmodium falciparum and non-falciparum parasites were 86.2% and 29% respectively. Specificity was 76.9% and 100% respectively. OptiMal sensitivity for plasmodium falciparum and non-falciparum parasites were 42.6% and 47.1% respectively, specificity was 97% and 96.9% respectively.