About IKP Centre for Technologies in Public Health

IKP Centre for Technologies in Public Health (ICTPH) is a research centre that aims to improve the health of poor populations by focusing on designing, developing and delivering innovative solutions in healthcare concerning India and the developing world through an inclusive process that scientifically integrates knowledge of factors influencing health and diseases in India, regular evaluation and impact assessment of existing health systems and integration of appropriate technology for optimal health care delivery. ICTPH aims to learn, discover and apply relevant innovative solutions for health care leading to improved health for the people of India and other developing countries and to integrate technological advances with delivery of affordable, accountable and accessible health care. ICTPH has prioritized the diseases of its interest as malaria, tuberculosis, reproductive health, diarrhoeal diseases and diabetes.

IKP Centre for Technologies in Public Health is structured as an autonomous centre within ICICI Knowledge Park (IKP), a not-for-profit Research Park in Hyderabad, India focusing on Life Sciences.

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Foreword

The IKP Centre for Technologies in Public Health (ICTPH) aims to identify the most important evidence gaps in the current knowledge that India and developing countries have about health technologies. ICTPH has prioritized the diseases of its interest as malaria, tuberculosis, reproductive health, diarrhoeal diseases and diabetes. Pertinent to Malaria, ICTPH is interested in the current status of preventive, curative and therapeutic interventions with a focus on determining gaps in knowledge as well as potential areas for improvement. This report will be utilized by ICTPH to explore potential product development and/or development of product diffusion and scale up strategies. We welcome comments and suggestions from our readers.

Reports on strategies for tuberculosis, reproductive health, diarrhoeal diseases and diabetes are also available as a part of our Working Paper Series.

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Executive Summary

Early intervention is the key to effective management of Malaria. Early intervention is dependent on an early and accurate diagnosis of the disease. A satisfactory diagnosis in terms of accuracy and speed is difficult with the two diagnostic approaches currently used. Clinical diagnosis, the most widely used approach, is unreliable because the symptoms of malaria are very non-specific. Microscopic diagnosis, the established method for laboratory confirmation of malaria, presents technical and personnel requirements that often cannot be met, particularly in facilities at the periphery of the health care system. The delays in the provision of the microscopy results to the clinician often result in treatment provided without the benefit of the results.

Rapid diagnostic tests (RDT) in combination with current methods of diagnosis may improve early detection of malaria. The tests can be performed in less than 30 minutes by individuals with minimal training, using test kits that require no electricity and no special equipment. The RDTs have detection capabilities that are in general comparable to those generally achieved by microscopy in the health services. The existing RDTs have several limitations compared to microscopy. Lack of sensitivity at low levels of parasitaemia; inability to quantify parasite density; inability to differentiate between *P. vivax (Plasmodium vivax)*, *P. ovale* and *P. malariae*, as well as between the sexual and asexual stages of the parasite; persistently positive tests (for some antigens) in spite of parasite clearance following chemotherapy; and relatively high cost per test.

The emergence of multidrug resistance is a major incentive for the development and deployment of RDTs to obtain reliable laboratory confirmation of the disease. RDTs can be performed by local health workers or community volunteers to diagnose malaria leading to early treatment. Where multidrug resistance occurs, the cost of the recommended Antimalarial drugs is higher, thus justifying the use of RDTs when microscopy is not available.

WHO (World Health Organization) has provided certain recommendations on the scope of RDTs for Malaria, which can form the basis for developing new RDTs or improving upon existing RDTs. A major area of focus has to be on patient and organizational impacts of the RDT, as well as cost effectiveness of the RDTs besides the robustness in the field. Delivery and deployment strategies have to be developed as well as follow up studies on the diffusion of the technology.

There is evidence for the benefits of ITNs (Insecticide Treated Nets) in prevention of malaria. ITNs are highly effective in reducing childhood mortality and morbidity from malaria. However, strategies to increase use and for development of newer products like long lasting ITNs are urgently needed. Widespread access to ITNs is currently being advocated by Roll Back Malaria, but universal deployment will require major financial, technical, and operational inputs besides commitment from national governments.

There is evidence for benefits of artemisinin derivatives in the treatment of Malaria. In uncomplicated malaria, artemisinin drugs clear malaria parasites from the blood more effectively than standard treatment drugs. In areas where malaria parasites are more resistant to existing drugs, such as South East Asia, artemisinin drugs are not better at sustained parasite clearance than standard treatment with quinine or meoquine.

Combination treatment using an artemisinin drug together with the longer acting antimalarial drug meoquine improves sustained clearance of parasites, but meoquine is associated with adverse effects. In severe malaria, compared with quinine, artemisinin drugs showed faster parasite clearance from the blood and similar adverse effects. The evidence suggests that artemisinin drugs are no worse than quinine in preventing death in severe or complicated malaria.

There are several potential areas of interest in malaria. The priorities are the ability to diagnose malaria appropriately and early, and the improved strategies to prevent malaria. These include development and deployment of RDTs and development of strategies to deploy ITNs besides development of better ITNs. On the public health research front, there is an urgent need to explore the patient, organizational and societal impact besides cost effectiveness and cost benefits of these newer developments. Several new initiatives focus on the development of appropriate vaccines for malaria and newer medications for malaria.

A. Background – Malaria as a Public Health Priority

A.1 Causes of Malaria

Malaria infections in humans are caused by four species of plasmodium: P falciparum, P. vivax, P. ovale and P. malariae. Human infection occurs when the female anopheline mosquito (the malaria vector) inoculates from its salivary glands sporozoites of plasmodium into humans during the process of sucking blood from the human body. The sporozoites mature in the liver of the human and are subsequently released into the blood stream as merozoites. The merozoites invade the red blood cells. Some forms of the parasites (gametocytes) are ingested by the anopheline mosquito restarting the whole cycle.

A.2 Epidemiology

Plasmodium falciparum is common in sub Saharan Africa, Haiti and Papua New Guinea. Plasmodium vivax is more common in Central America. The prevalence of these two species is almost equal in the Indian subcontinent, eastern Asia, Oceania and South America. Plasmodium malariae is less common than the other species and is found in most endemic areas especially sub Saharan Africa. Plasmodium ovale is very uncommon outside Africa.

More than one human bite per infected mosquito can occur in high transmission areas. There is a considerable morbidity and mortality in childhood in such areas. Survivors, in such areas, develop some immunity against the disease and by adulthood most malarial infections in such persons maybe asymptomatic. Such a situation with frequent, intense, year round transmission is called as *stable malaria*. In contrast, full protective immunity is not acquired in areas where transmission is low, erratic or focal and leads to the presence of symptomatic disease at all ages- *unstable malaria*.

More than 3 billion people live in areas at risk for malaria and the disease causes between 1 and 3 million deaths annually.[1],[2]

1. Breman JG, Alilio MS, Mills Conquering the intolerable burden of malaria: What's new, what's needed: A Summary. *American Journal of Tropical Medicine and Hygiene* **2004**; *71* (Suppl 2):1-15

2. Snow RW, Craig MH, Newton CRJC, Steketee RW. The public health burden of plasmodium falciparum malaria in Africa: deriving the numbers. *Working Paper 11, Disease Control Priorities project, Bethesda, MD* **2003**

Five hundred and fifteen (inter quartile range 298 to 659) million cases of falciparum malaria occur annually. This figure is 92% higher than the 278 million cases of malaria estimated by World Health Organization (WHO) in 1998 (which also included estimates for plasmodium vivax), and 200% higher than previous estimates for areas outside of Africa.

The global incidence of malaria is 236 episodes per 1000 persons per year in all endemic areas. The incidence ranges from 400 to 2000 episodes (median 830 episodes) per 1000 persons per year in areas with intense and stable transmission-these areas represent 38% of all falciparum endemic areas.

The WHO listed malaria as the eighth highest contributor to global disease burden as reflected in disability adjusted life years (DALYs).[3] Worldwide, deaths from malaria represented 2.00% of all deaths, and 2.90% of all DALYs. In Africa, deaths from malaria represented 9.00% of all deaths, and 10.10% of all DALYs. The DALYs attributable to malaria are estimated largely from the effects of falciparum infection as a direct cause of death and the much smaller contributions of short duration, self limiting or treatable febrile illnesses, as well as consequences of anemia and neurological disability following cerebral malaria.

A.3 Malaria in India

1314 million persons are at risk for malaria and 119 million cases (inter quartile range 66 to 224 million) occur each year in Southeast Asia. The falciparum attack rate in Southeast Asia is 91 per 1000 persons at risk per year. [4] In South East Asia, deaths from malaria represented 0.70% of all deaths, and 0.90% of all DALYs.[5]

Malaria is endemic in India with varying levels of transmission. The bulk of malaria cases are found in the flood plains of northern India and coastal plains of the east and west coasts. The north-eastern region (population 28.5 million), and the forests and forest fringes on the hill ranges of peninsular India occupied by minority ethnic groups (population 71 million) are highly endemic to malaria.

3. WHO 2002a. Monitoring Antimalarial Drug Resistance. WHO/CDS/CSR/EPH/2002.1WHO/CDS /RBM /2002.39. Geneva: WHO

4. Snow RW, Guerra CA, Noor AM, Myint HW, Hay SI. The global distribution of clinical episodes of plasmodium falciparum malaria. *Nature* **2005**; *434*:214-17

5. WHO 2002b. World Health Report **2002**:Reducing Risks, Promoting Healthy life. Geneva: WHO

Other Indian states that contribute most to the morbidity from Malaria are Bihar, Jharkhand, Orissa, Gujarat, Maharashtra, Goa and Madhya Pradesh. An estimated 1044.7 million people live in areas that are at risk for malaria in India. About 1.8 million people are reported with malaria in India and approximately 1000 deaths from malaria are reported per annum. The reported figures appear to be gross underestimates compared to the projected morbidity (15 million to 80 million cases) and projected mortality of 83,099 per annum for India. Mortality from Malaria is low due to the large proportion of P.vivax infections that have lower mortality and the possible gross underreporting of deaths from Malaria.

In addition to rural malaria, urban malaria has emerged as an important challenge accounting for 14 to 15% of the total malaria cases. Regions, where large-scale irrigation has been developed, have now become regions of endemic malaria. DDT (Dichloro-Diphenyl-Trichloroethane) is still used as a cheap insecticide to control malaria with very limited use of insecticide treated bed nets. The percentage of bed net use does not exceed 50% in most states at risk for malaria. India has pockets where larvivorous fish is utilized as a means of vector control.

About 100 million blood smears were examined in India for malaria in 2004. There were 13,818 malaria clinics, 71,053 multipurpose workers trained for malaria services, and an additional 66,354 auxiliary nurse midwives (ANM) trained for malaria services in India in 2004. 1,193 district level and 63,644 Block/PHC (Primary Health Care) level hatcheries have been established in India to promote the use of larvivorous fish for vector control.

A.4 Consequences of Malaria

Mortality from malaria is a major problem with deaths from malaria representing 2.00% of all deaths worldwide. Cerebral malaria and moderate-to-severe anemia are significant contributors to mortality from malaria. Lactic acidosis and hypoglycemia is also an important predictor of death from malaria. Case fatality rates from cerebral malaria are high with reports of mean case fatality rates ranging from 17.5% to 19.2%.[6,7] These rates do not include fatality for cerebral malaria patients that do not access the health care system and succumb at home.

6. Murphy, S.C., and J.G. Breman. Gaps in the childhood malaria burden in Africa: Cerebral Malaria, Neurological Sequelae, Anemia, Respiratory Distress, Hypoglycemia, and Complications of Pregnancy. *American Journal of Tropical Medicine and Hygiene* **2001**; *64* (suppl.1): 57-56

Neurological sequelae after cerebral malaria have been reported in 3 to 28% of survivors.[8] Such sequelae include coma, seizures, hemi-and-quadriparesis, hearing and visual impairments, speech and language difficulties, behavioral problems, epilepsy and other problems.

The median transfusion rate for children presenting to hospital with hemoglobin levels ≤ 5 grams per deciliter is approximately 80.1%.

The high rates of blood transfusion have several implications by itself. Considering that Malaria is highly prevalent in populations that are more at risk and have a high prevalence of HIV, the potential for HIV (Human Immunodeficiency Virus) transmission through blood cannot be underestimated.[9]

The number of blood units that may need to be screened for HIV is staggering given the magnitude of transfusion required.

Malaria has several indirect consequences that often do not contribute to the DALY estimation of malaria. Low birth weight and its consequences including infant mortality may be linked indirectly to malaria during pregnancy. Low birth weight can also lead to a whole range of persistent problems like behavioral problems, mental retardation, cerebral palsy, blindness and deafness. Malaria associated low birth weight has been reported to account for 62,000 to 363,000 infant deaths.[10]

Undernourishment and growth retardation of children can be linked to malaria infection and may enhance the risk for other concomitant co-morbid infectious diseases through suppression of the immune system.

7. Snow RW, Craig MH, Newton CRJC, Steketee RW. **2003**. The public health burden of plasmodium falciparum malaria in Africa: deriving the numbers. *Working Paper 11, Disease Control Priorities project, Bethesda, MD*

8. Mung'Ala-Odera, V., R.W.Snow, and C.R.J.C. Newton. The burden of the neurocognitive impairment associated with falciparum malaria in sub Saharan Africa. *American Journal of Tropical Medicine and Hygiene* **2004**; *71* (suppl.2): 64-70

9. Greenberg, A.E., P.Nguyen-Dinh, J.M. Mann., N. Kabote., R.L. Coleblunders., H.Francis and others. The association between malaria, blood transfusions, and HIV seropositivity in a pediatric population in Kinshasa, Zaire. *Journal of the American Medical Association* **1988**; *259*: 545-59

10. Murphy, S.C., and J.G. Breman. Gaps in the childhood malaria burden in Africa: Cerebral Malaria, Neurological Sequelae, Anemia, Respiratory Distress, Hypoglycemia, and Complications of Pregnancy. *American Journal of Tropical Medicine and Hygiene* **2001**; *64* (suppl.1): 57-56

Iron, zinc and protein-calorie deficits are associated with morbidity and mortality from malaria.

Approximately 57.3% of deaths of underweight children under five in Africa is attributed to nutritional deficiencies.[11] Malaria also accounts for 13% to 15% absenteeism from school for medical reasons besides the impact on performance and retention related to undernourishment.[12]

11. Caulfield.L., S.A. Richard., and R. Black. Undernutrition as an underlying cause of malaria morbidity and mortality. *American Journal of Tropical Medicine and Hygiene* **2004**; *71* (suppl 2): 55-63

12. Holding, P.A., and P.K. Kitsao-Wekulo. Describing the burden of malaria on child development: what should we be measuring and how should we be measuring it? *American Journal of Tropical Medicine and Hygiene* **2004**; *71* (suppl 2): 71-79

B. Prevention of Malaria

Several preventive measures for malaria exist, primarily environmental in nature. These include fogging, the use of insecticides, environmental sanitation focused on stagnant water, and larvicidal measures including larvivorous fish. The most promising preventive measures mentioned are insecticide – treated bed nets and curtains, collectively known as Insecticide-Treated Nets (ITNs).[13] The wide scale use of ITNs is one of the four main strategies of the Roll Back Malaria program to reduce morbidity and mortality from malaria. Studies of ITNs showed that pyrethroids were safe and that ITNs had an impact on various measures of mosquito biting (such as the proportion of mosquitoes successfully feeding on humans and the number of times a mosquito bit humans in one night). These studies showed that pyrethroids worked by both repelling and killing mosquitoes. In addition, researchers determined optimal doses of various insecticides with different materials.[14, 15, 16, 17, 18] These include 200 mg/m^2 permethrin or etofenprox; 30 mg/m^2 cyuthrin; 20 mg/m^2 alphacypermethrin; 10 mg/m^2 deltamethrin/ lambdacyhalothrin.

B.1 Question of Interest

What is the impact of insecticide-treated bed nets or curtains on malaria related mortality and morbidity, parasitemia, anemia, and spleen rates?

13. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database of Systematic Reviews* **2004**, *Issue 2*. Art. No.: CD000363. DOI: 10.1002/14651858.CD000363.pub2.

14. Curtis CF, Lines JD, Carnevale P, Robert V, Boudin C, Halna J-M,et al. Impregnated bed nets and curtains against malaria mosquitoes. In: CurtisCF editor(s). *Control of disease vectors in the community*.London: Wolfe, **1991**.

15. Curtis CF, Myamba J, Wilkes TJ. Various pyrethroids on bednets and curtains.*Memorias do Instituto Oswaldo Cruz* **1992**; *87* Suppl 3: 363-70.

16. Curtis CF, Myamba J, Wilkes TJ. Comparison of different insecticides and fabrics for anti-mosquito bednets and curtains. *Medical and Veterinary Entomology* **1996**; *10(1):* 1-11.

17. Lines JD. The technical issues. In: LengelerC, CattaniJ, deSavignyDH editor(s). *Net gain: a new method to prevent malaria deaths*. Ottowa: International Development Research Centre, **1996**: 17-53

18. Rozendaal J. Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Diseases Bulletin* **1989**; *86*: R1-R41.

Methodology

A systematic review of the scientific literature focused on the prevention of malaria using Insecticide Treated Nets (ITNs) was performed. The search covered The Cochrane Library, PUBMED, MEDLINE and EMBASE as the central databases and also covered the WHO database of publications.

The search was performed using one or a combination of the following key words: insecticide treated nets, insecticide treated curtains, prevention of malaria, malaria mortality, malaria morbidity, impact, and evaluation

Studies on adults or children with any form of malaria were considered to be eligible for inclusion. Any study that compared an ITN with a reference was also included. Studies that did not compare ITN with either a control net or no nets were not considered. No restriction was placed on study settings and included studies from any country. The major proportion of studies were from developing countries especially Africa mimicking the geographic distribution of malaria. Randomized controlled trials were included for the review as randomized controlled trials are considered the highest level of evidence.

The outcome measures of interest included child mortality, parasite prevalence, parasitemia, anemia, and splenomegaly.

Only articles in the English language were considered for review. The initial review of articles was followed by a review of the cross referenced articles. The related article link in PUBMED was used sequentially to identify other similar articles of interest.

B.2 Results – Prevention with ITN

Five cluster randomized controlled trials examined child mortality from all causes. They were all conducted in areas with stable malaria in sub-Saharan Africa: (Burkina; Gambia; Ghana; and Kenya). Four of the trials did not use any nets as the control group, and one trial used untreated nets. When the five trials were pooled regardless of the type of control group, the summary relative rate was 0.82 (95% CI: 0.76 to 0.89), giving a summary protective efficacy of 18%. A regression analysis of the protective efficacy (ln) on the transmission intensity as measured by the entomological inoculation rate was statistically significant at the 5% level ($r2 = 0.88$, $F = 22.1$ on 1,3 degrees of freedom, $P = 0.05$).

The protective efficacy appeared to be lower in areas with a higher entomological inoculation rate, consistent with the hypothesis that relative impact is lower in areas with higher entomological inoculation rates. The summary rate difference, which expresses how many lives can be saved for every 1000 children protected, was 5.53 deaths averted per 1000 children protected per year (95% CI 3.39 to 7.67). In contrast to protective efficacies, the risk differences seemed to have a tendency towards a higher effect with a higher entomological inoculation rate. This apparent paradox is because the baseline mortality rates are higher in areas with high entomological inoculation rates.

A trial using passive and hospital-based case ascertainment observed a 45% (cluster-adjusted 95% CI 20 to 63) reduction in the frequency of severe malaria episodes following the introduction of ITNs. The effect of ITNs on uncomplicated clinical episodes of malaria is shown by large effect estimates in all trials. Overall, the reduction in clinical episodes was around 50% for all subgroups (stable and unstable malaria; no nets and untreated nets) and for both *P. falciparum* and *P. vivax*. The protective efficacy is higher (at least 11% for *P. falciparum*) when the control group had no nets. In areas with stable malaria (entomological inoculation rate > 1) the differences in protective efficacies against uncomplicated malaria was 11% (50% no nets versus 39% untreated nets).

In areas with unstable malaria (entomological inoculation rate < 1), the differences were bigger: 23% (62% no nets versus 39% untreated nets) for *P. falciparum*, and 41% (52% no nets versus 11% untreated nets) for *P. vivax*. In areas of unstable malaria (entomological inoculation rate<1), the impact against *P. falciparum* episodes seemed to be higher than the impact against *P. vivax* episodes.

In areas of stable malaria, impact on prevalence of infection (measured through cross-sectional surveys) was small: 13% reduction when the control group did not have any nets and 10% reduction when the control group had untreated nets. In areas with unstable malaria, the results are of limited value because there was only a single trial in each subgroup (treated versus no nets; and treated versus untreated nets).

Overall, the packed cell volume of children in the ITN group was higher by 1.7 absolute packed cell volume per cent compared to children not using nets. When the control group used untreated nets, the difference was 0.4 absolute packed cell volume per cent. Splenomegaly was significantly reduced for both types of controls: there is a 30% protective efficacy when controls were not using nets; and a 23% protective efficacy when the control group used untreated nets.

In The Gambia, mean z-scores of weight-for-age and weight-for-height were higher in children from treated villages (-1.36 and -0.98, respectively) than in those from untreated villages (-1.46 and -1.13, respectively). The differences were statistically significant after adjustment for area, age, differential bed net use, and gender (P = 0.008 and P = 0.001, respectively). There was no statistically significant difference in mean z-scores for height-for-age.[19]

In the trial carried out in Kenya,[20] infants sleeping under ITNs in the intervention areas had statistically significantly higher z-scores for weight-for-age than control infants not under treated nets (analysis of variance allowing for season, gender, and age: F = 21.63, P = 0.03). Mean mid-upper arm circumference z-scores were also statistically significantly higher among infants in the intervention communities (analysis of variance allowing for survey, gender, and age: F = 19.0, P = 0.005).[21] In Kenya[22], protected children under two years of age had a statistically significantly better weight-for-age z-score than unprotected children (P< 0.04).

No other statistically significant differences were measured for other parameters or other age groups, although all z-score differences between intervention and control groups were in favour of the protected group.

19. D'Alessandro U, Olaleye B, McGuire W, Langerock P, Bennett S, Aikins MK, et al.Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet* **1995**; *345(8948):* 479-83.

20. Nevill CG, Some ES,Mung'alaVO,MutemiW,New L,Marsh K, et al. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine & International Health* **1996**; *1(2)*: 139-46.

21. Snow RW,Molyneux CS, Njeru EK, Omumbo J,Nevill CG,Munui E, et al. The effects of malaria control on nutritional status in infancy. *Acta Tropica* **1997**; *65(1)*: 1-10.

22. Phillips-Howard PA, Nahlen BL, Kolczak MS, Hightower AW, ter Kuile FO, Alaii JA, et al. Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in western Kenya. *American Journal of Tropical Medicine and Hygiene* **2003**; *68 Suppl(4):* 23-9.

A cost-effectiveness assessment has shown that ITN programs compare well in terms of cost-effectiveness with other child survival interventions such as the Expanded Program on Immunization (EPI).[23]

C. Early Diagnosis

Early diagnosis and effective intervention can cure infection, prevent progression to severe disease, further morbidity and possible mortality, and arrest transmission. Early intervention needs timely diagnosis, use of effective medicines, and preventive and service delivery strategies with a system for appropriate referral. If left untreated, up to 1 in 250 cases of falciparum malaria infections may progress to severe disease within a few hours to a few days.[24] The globally agreed goal is that diagnosis and effective treatment has to be given *within 24 hours* of the onset of signs and symptoms. An accurate diagnosis of malaria is based on the detection of the parasite, and clinical symptoms and signs if laboratory diagnosis is not possible.

Clinical Diagnosis

Clinical diagnosis is the most widely used approach and often the only approach especially in rural areas and at the periphery of the health care system where laboratory support to clinical diagnosis does not exist. The most prominent symptom is fever, often accompanied by chills, perspiration, anorexia, headaches, vomiting and malaise. Residents of endemic areas are often familiar with this combination of symptoms, and frequently self-diagnose malaria based on symptoms alone. In addition to these symptoms of uncomplicated malaria, other manifestations may develop that signal severe malaria, which is almost always due to *Plasmodium falciparum*. These include confusion or drowsiness with prostration together with severe manifestations such as cerebral malaria, severe anemia and others. Although clinical diagnosis is inexpensive to perform and requires no special equipment or supplies, the symptoms of malaria are very non-specific and overlap with those of other febrile illnesses. A diagnosis of malaria based on clinical grounds alone is therefore unreliable, and when possible should be confirmed by laboratory tests.

Laboratory Diagnosis

23. Goodman CA, Coleman PG,Mills AJ. Cost-effectiveness of malaria control in sub-Saharan Africa. *Lancet* **1999;** *354(9176):* 378-85.

24. Greenwood BM, Bojang K, Whitty CJM, Targett TAG, Malaria. *Lancet* **2005**; *365 (9469);* 1487-98

Laboratory diagnosis is usually detection using a microscope of the asexual forms (gametocytes) of the parasite in stained peripheral blood smears. Conventional light microscopy is the established method for the laboratory confirmation of malaria. The careful examination by an expert of a well prepared and well stained blood film remains currently the "gold standard" for detecting and identifying malaria parasites. In most settings, the procedure consists of: collecting a finger-prick blood sample; preparing a thick/thin blood smear; staining the smear (most frequently with Giemsa); and examining the smear through a microscope (preferably with a 100X oil immersion objective) for the presence of malaria parasites.[25] Microscopy offers many advantages. It is sensitive when used by skilled and careful technicians; microscopy can detect densities as low as 5–10 parasites per µl of blood.[26]

Under general field conditions, however, the detection capabilities of a typical expert might be more realistically placed at 100 parasites per µl of blood.[27] When parasites are found, they can be characterized in terms of their species and of the circulating stage. Occasionally, experts can detect morphological alterations induced by recent drug treatment. In addition, 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. Microscopy is also relatively inexpensive to perform and is a general diagnostic technique that can be shared with other disease control programmes such as those against tuberculosis or sexually transmitted diseases. Microscopy also provides a permanent record (the smears) of the diagnostic findings and can be subject to quality control.

Several concerns remain in the early diagnosis of malaria using microscopy including the reliability of the diagnosis, the training of the personnel, the availability of lab facilities, the turn- around time, and the preservation of the blood samples.

There are often long delays in providing the microscopy results to the clinician, so that decisions on treatment are often taken without the benefit of the results.

25. Payne D. Use and limitations of light microscopy for diagnosing malaria at the primary health care level. *Bulletin of the World Health Organization*, **1988**, *66*: 621–626

26. World Health Organization. Severe and complicated malaria. Second edition. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **1990**, *84 (Suppl. 2*): 23–25.

27. Malaria diagnosis: Memorandum from a WHO meeting. *Bulletin of the World Health Organization*, **1988**, *66*: 575–594

These concerns have led to the development of several rapid diagnostic tests (RDT) for malaria. These RDT have their own limitations including species sensitivity, parasite quantification, field feasibility and costs.

C.1 Questions of Interest

We aimed to review the literature available on the effectiveness and limitations of rapid diagnostic tests (RDT) relative to conventional microscopic diagnosis of malaria.

The following questions were included in the scope of review:

a) How accurate are the different immunochromatographic tests for the rapid diagnosis of malaria?

b) How accurate are the PCR based techniques including sequence based nucleic acid amplification and real time PCR for the rapid diagnosis of malaria?

c) How accurate are tests based on the loop-mediated isothermal amplification technology for the rapid diagnosis of malaria?

d) How accurate are the different fluorescent microscopy techniques for the rapid diagnosis of malaria?

e) How do these tests perform at diverse levels of parasitemia and in diverse clinical settings including community/field settings?

Methodology

A systematic review of the scientific literature focused on diagnosis of malaria was performed. The search covered The Cochrane Library, PUBMED, MEDLINE and EMBASE as the central databases pertinent to technology. The search also covered the WHO database of publications.

The search was performed using one or a combination of the following key words: rapid diagnostic tests, diagnosis, malaria, cost effectiveness, effectiveness, accuracy, sensitivity, specificity, positive predictive value, and negative predictive value.

Studies on adults or children with any form of malaria were considered to be eligible for inclusion. Any study that compared a rapid diagnostic test for malaria with a reference study was included. A rapid diagnostic test was defined as a test that could be completed with provision of results within one hour. Studies that focused only on the utility of the test for post diagnostic purposes (like monitoring of post treatment effects) were not considered. The use of microscopy and/or PCR as reference standards was considered.

Microscopy has several limitations including the dependence on the level of training and skills of the person reading the smears. Microscopy cannot distinguish between dead and live parasites and hence may overestimate positivity. Additionally, microscopy may not be useful to identify sequestered parasites that do not reach the peripheral blood and hence may miss plasmodium falciparum. However, microscopy is still considered the current gold standard. The sensitivity of PCR with its ability to pick up ≤5 parasites/µl of blood is an advantage. However, differences in techniques for collection and storage of the specimens, methods for DNA extraction and selection of primers, amplification conditions, and analysis of amplified product may lead to variation in results from PCR based techniques. Studies that did not compare new tests with any of these two lab methods or if they used only presumptive clinical diagnosis for the confirmation of malaria were not considered.

No restriction on study settings were placed and included studies from any country. The major proportion of studies was from developing countries mimicking the geographic distribution of malaria. Randomized controlled trials, cohort study designs or case series type studies that compared a diagnostic test with an established reference standard in patients suspected to have malaria were included. Randomized controlled trials were considered the highest level of evidence. Case–control studies where the performance of a test is compared in two or more groups of patients potentially ranging from those with confirmed malaria to those with diseases other than malaria or even no known disease (healthy controls) were considered the least level of evidence because of the potential bias and the potential unrepresentative mix of subjects.

The establishment of accuracy of the test was the focus of the review. Studies that focused on the establishment of the technical efficacy alone were not considered. Studies that reported on sensitivity, specificity, predictive values, and accuracy were considered for the review. Studies that reported only summary statistics without raw data were considered lower in the hierarchy of evidence. Studies that did not report confidence intervals around the point estimates were also considered lower in the hierarchy of evidence.

Only articles in the English language were included for review. The initial review of articles was followed by a review of the cross referenced articles. The related article link in PUBMED was used sequentially to identify other similar articles of interest.

D. A Description of Technologies for the Diagnosis of Malaria

D.1 Diagnosis by Oil Immersion Microscopy

The routine method of laboratory diagnosis is described since it is the gold standard test for the diagnosis of malaria. However, there is no accepted single standard method in current use by all investigators for the quantification of parasites. Nevertheless, the blood film is still the only widely available result against which the newer methods for diagnosis of malaria can be compared.

The diagnosis of malaria is made based on microscopy examination of a blood sample stained with Giemsa, Wright or Field's stain.[28] A finger prick or earlobe prick blood sample is the ideal because the density of mature trophozoites or schizonts is greater in blood from these capillary rich areas.[29] Blood obtained by venipuncture collected in heparin or Sequestrine (EDTA) anticoagulant-coated tubes is also acceptable. However, such samples have to be used shortly after collection to prevent alteration in the morphology of white blood cells (WBC) and malaria parasites. The collected blood should be used to prepare both thick and thin blood smear films.

Thick blood films: The thick blood film essentially concentrates the layers of red blood cells (RBC) by a factor of 20 to 30 on a small surface. The film is stained as an unfixed preparation using Field's stain or diluted Wright's or Giemsa stain. The thick blood film provides enhanced sensitivity and is better than the thin film for detection of low levels of parasitemia and reappearance of circulating parasites during infection recrudescence or relapse.

The number of parasites present in 1µl of blood is estimated using a standard value for the white blood cell (WBC) count (8,000 WBC/µl). The number of parasites present is counted until 200 WBCs are seen. The parasite count is then multiplied by 40 to give the parasite count per microliter of blood. If an accurate WBC count is known, this can be used to give a more accurate figure with appropriate adjustment of the multiplication factor.

28. Warhurst, D. C., and J. E. Williams. Laboratory diagnosis of malaria. *J. Clin. Pathol.* ***1996;*** 49: 533–538

29. Gilles, H. 1993. Diagnostic methods in malaria, p. 78. *In* H. M. Gilles and D. A. Warrell (ed.), Essential malariology, 3rd ed. P Edward Arnold, London, United Kingdom

The most accurate counts are obtained by counting the total parasites seen in a measured volume of blood. The blood volume should not be less than 2 µl and is spread over an area of 1 cm^2. However, this method is time-consuming, and a count of parasites against 200 WBC in a thick film in the presence of an accurate total WBC count gives satisfactory accuracy for most clinical purposes.

The expected sensitivity achieved by an experienced technician for the examination of the thick blood film procedure is about 50 parasites/µl of blood (assuming a total RBC count of 5 X 10^6/µl of blood), which is equivalent to 0.001% of RBC infected. Most routine diagnostic laboratories generally achieved a lower sensitivity of detection (average, 0.01% RBC infected, 500 parasites/µl).

The major disadvantage of the thick blood film is the lysis of the RBCs during the staining process, making the slide more difficult to read with the absence of RBC features and irregularities in the thickness of the film. Using the thick film to estimate the intensity of the parasite infection, the examiner should only count parasites in thick-film fields containing no more than 20 WBC/100X oil immersion field. However, fields containing 20 WBC are often too thick to count whereas the fields that are easiest to read are those that contain only 5 or 6 WBC. Transfer of parasites from positive slides to a negative one is a distinct possibility, and care should be taken when bulk staining thick blood film slides. The use of 50X or 60X oil immersion objectives may be insufficient to identify some smaller ring forms seen on the thick film, and 10X eyepieces for the microscope are essential in these circumstances.

Thin blood films: The thin blood film is often preferred for routine estimation of the parasitemia because the organisms are easier to see and count. The morphological identification of the parasite to the species level is much easier because of the fixed monolayer of RBC available in this procedure. This is especially important for sequential blood film counts that are used to monitor response to therapy. The thin blood film is methanol fixed and stained with diluted Giemsa or Wright's stain using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC.

The parasitemia is usually estimated by noting the number of parasitized RBC (not individual parasites) seen in 10,000 RBC (equal to approximately 40 monolayer cell fields of a standard microscope using the 100X oil immersion objective.

Technicians are advised to calculate the average number of cells per microscope field of view for their own microscopes and express the number of parasitized cells seen as a percentage. The approximate numbers of parasites present in 1µl of blood can be calculated by assuming that 1µl of blood contains $5X10^6$ RBC; therefore, a 1% parasitemia will contain 1 parasite/100 RBC or 50,000 parasites/µl of blood. This may be corrected to exact counts if the total RBC count per microliter is known. The expression of the parasitemia as a percentage infection of RBC is the method of choice for routine practice in non endemic areas.

Examination of thin blood films is only 1/10 as sensitive as examination of thick blood films for the quantification of malarial parasites, although morphological identification of the *Plasmodium* species present is much easier using thin films. The thin blood film, however, provides greater specificity than the thick-film examination.

Disadvantages of Oil Immersion Microscopy

The blood film technique has undergone very little improvement since its development in the early 1900s. The staining process may take up to 60 min of preparation time to produce a stained thin or thick film and is labor-intensive. Interpretation requires considerable expertise, particularly at low levels of parasitemia. A *P. falciparum* infection may easily be missed because there are insufficient numbers of parasites for detection in the peripheral blood films (as the parasite may be sequestered in the deep capillaries). Oil immersion microscopy cannot distinguish between dead and live parasites thus prolonging the assumed positivity of the patient and extending treatment.

D.2 Diagnosis by Alternate Microscopic Methods: Fluorescence Microscopy

Certain fluorescent dyes have an affinity for the nucleic acid in the parasite nucleus and will attach to the nuclei. When excited by UV light at an appropriate wavelength, the nucleus will fluoresce strongly. Two fluorochromes have frequently been used for this purpose, acridine orange (AO) and benzothiocarboxypurine (BCP), which are both excited at 490 nm and exhibit apple green or yellow fluorescence. Rhodamine-123 is also useful for assessing the viable state of parasites, since its uptake relies on an intact, working parasitic membrane.

The inability to easily differentiate among *Plasmodium* sp. is a major limitation of methods involving both AO and BCP. The requirements for special training and expensive equipment and supplies are a limitation for most resource poor countries. However, fluorescence microscopy remains a viable and rapid alternative to oil immersion microscopy.

Acridine Orange

AO is used either as a direct-staining technique or combined with a concentration method such as a thick blood film.[30,31] The centrifugal quantitative buffy coat or QBC II (QBC) method uses a combination of an AO-coated capillary tube and an internal float to separate layers of WBC and platelets using centrifugation. The parasites concentrate below this layer of cells, appearing in the upper layer of RBC but also sometimes appearing within the layers of platelets and WBC. Parasites can be viewed through the capillary tube using a special long-focal-length objective (paralens) with a fluorescence microscope.[32,33,34,35] A simpler technique, the Kawamoto technique, uses an excitation filter mounted in the pathway of the transmitted light beam and allows the excitation wavelength of AO (470 to 490 nm) to pass to the stained film. The fluorescing parasites stained with AO are viewed with a second filter (510 nm) placed in the ocular for viewing.

30. Delacollett, D., and P. Van der Stuyft. Direct acridine orange staining is not a 'miracle' solution to the problems of malaria diagnosis in the field. *Trans. R. Soc. Trop. Med. Hyg.* **1994**; *88*:187–188.

31. Gay, F., B. Traore, J. Zanoni, M. Danis, and M. Gentilini. Direct acridine orange fluorescence examination of blood slides compared to current techniques for malaria diagnosis. *Trans. R. Soc. Trop. Med. Hyg.* **1996**; *90*: 516–518.

32. Baird, J. K., Purnomo, and T. R. Jones. Diagnosis of malaria in the field by fluorescence microscopy of QBC capillary tube´s. *Trans. R. Soc. Trop. Med. Hyg.* **1992**; *86*: 3–5.

33. Clendeman, T. E., G. W. Long, and J. K. Baird. QBC® and Giemsa stained thick blood films: diagnostic performance of laboratory technologists. *Trans. R. Soc. Trop. Med. Hyg.* **1995**; *86*: 378.

34. Craig, M., and B. L. Sharp. Comparative evaluation of four techniques for the diagnosis of *Plasmodium* infections. *Trans. R. Soc. Trop. Med. Hyg.* **1997**; *91*: 279–282.

35. Gaye, O., M. Diouf, and S. Diallo. A comparison of thick films, QBC malaria, PCR and PATH falciparum malaria test strip in *Plasmodium falciparum* diagnosis. *Parasite* **1999**; *6*: 273–275.

This method can make use of strong sunlight (if a shield to surround the observer's eyes is used) or a quartz halogen source as the exciting wavelength source.[36] [,37,38,39]The QBC fluorescence method is more technically demanding and requires specialized equipment to separate the cell layers by centrifugation and a good fluorescence microscope with a high-intensity mercury vapor or quartz halogen lamp to provide the excitation wavelength.

Although AO is a very intense fluorescent stain, it is nonspecific and stains nucleic acids from all cell types. The technician using AO has to be trained to distinguish fluorescence-stained parasites from other cells and cellular debris containing nucleic acids especially in patients with hemolytic anemia.[40]

Accuracy of AO

The sensitivity of AO staining for detection of malaria parasites in infections with parasite levels of <100 parasites/µl (0.002% parasitemia) has been reported to range from 41 to 93%.[41] The specificity for infections with *P. falciparum* is excellent (>93%), with most observers recognizing the small ring forms.[42] Ring forms or early trophozoites of other species may result in misdiagnosis, particularly in the early phase of the asexual cycle, when only ring forms may be present.

36. Hakim, S. L., T. Furuta, A. N. Rain, Y. Normaznah, M. R. Zamri, S. Kojima, and J. W. Mak. Diagnosis of malaria using a light microscope fitted with an interference filter for the diagnosis of malaria. Trans. *R. Soc. Trop. Med. Hyg.* **1993**; *56*: 44–48

37. Hind, M., M. Agabani, I. A. El Hag, I. A. El Toum, M. Satti, and A. M. El Hassan. 1994. Fluorescence microscopy using a light microscope fitted with an interference filter for the diagnosis of malaria. Trans. *R. Soc. Trop. Med. Hyg.* **1994**; *88*: 61

38. Kawamoto, F. Rapid diagnosis of malaria by fluorescence microscopy. *Lancet* **1991**; 624–625.

39. Kawamoto, F., H. Miyake, O. Kaneko, M. Kimura, T. D. Nguyen, Q. Lui, M. Zhou, D. D. Le, S. Kawai, S. Isomura, and Y. Wataya. Sequence variation in the 18S rRNA gene, a target for PCR-based malaria diagnosis, in *Plasmodium ovale* from southern Vietnam. *J. Clin. Microbiol.* **1996;** *34*: 2287–2289.

40. Delacollett, D., and P. Van der Stuyft. Direct acridine orange staining is not a 'miracle' solution to the problems of malaria diagnosis in the field. *Trans. R. Soc. Trop. Med. Hyg.* **1994**; *88*: 187–188

41. Wongsrichanalai, C., J. Pornsilapatip, V. Namsiriponpun, H. K. Webster, A. Luccini, P. Pansamdang, H. Wilde, and M. Prasittisuk. Acridine orange fluorescent microscopy and the detection of malaria in populations with low-density parasitemia. *Am. J. Trop. Med. Hyg.* **1991***; 44*: 17–20.

42. Gaye, O., M. Diouf, and S. Diallo. 1999. A comparison of thick films, QBC malaria, PCR and PATH falciparum malaria test strip in *Plasmodium falciparum* diagnosis. *Parasite* **1999**; *6*: 273–275.

For plasmodium vivax and other non-plasmodium falciparum infections, particularly during later stages of development, the specificity when using AO staining has been found to be lower (52%), particularly for the QBC centrifugal method, where the denser late-stage parasites may be hidden in the separated mononuclear cell layer.

Benzothiocarboxypurine

BCP can be applied directly to a lysed blood suspension or to an unfixed but dry thick blood film.[43 , 44,45] BCP intensely stains the nucleic acid of viable *P. falciparum* parasites and stains poorly RBC inclusions and the nuclei of leukocytes, preventing fading or precipitation of the dye.

Accuracy of BCP

The method has been reported to be a sensitive and rapid diagnostic method, comparable to Giemsa staining, with a reported sensitivity and specificity of >95% for *P. falciparum*. The non *P. falciparum* parasites are more easily distinguished in the thick-film preparation than in the buffy coat of the QBC.

D.3 Detection of Specific Nucleic Acid Sequences

The value of PCR lies in its sensitivity with its ability to detect less than 5 or less parasites/µl of blood.[46] Nested and multiplex PCR methods can give valuable information helpful to identify parasites to the species level.

43. Cooke, A. H., A. H. Moody, K. Lemon, P. L. Chiodini, and J. Horton. Use of the fluorochrome benzothiocarboxypurine in malaria diagnosis. *Trans. R. Soc. Trop. Med. Hyg*. **1992**; *87*: 549

44. Cooke, A. H., S. Morris-Jones, J. Horton, B. M. Greenwood, A. H. Moody, and P. L. Chiodini. Evaluation of benzothiocarboxypurine for malaria diagnosis in an endemic area. *Trans. R. Soc. Trop. Med. Hyg*. **1993**; *87*: 549.

45. Makler, M. T., L. K. Ries, J. Ries, R. J. Horton, and D. J. Hinrichs. Detection of *Plasmodium falciparum* infection with the fluorescent dye Benzothiocarboxypurine. *Am. J. Trop. Med. Hyg*. **1991**; *44*: 11–16.

46. Snounou, G., S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown. Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol. Biochem. Parasitol*. **1993**; *58*: 283–292.

There are several PCR assays that have been developed for the detection of malaria DNA from whole blood as either single or multiplex methods.[47, 48, 49, 50, 51] These assays have been used for the initial diagnosis, and as sensitive standards against which other non-molecular methods have been evaluated. The small-subunit 18S rRNA and circumsporozoite (CS) genes have been used as targets for the differentiation of *Plasmodium* species. Methods using nested PCR and reverse transcription-PCR enable all four species to be identified.[52, 53, 54, 55, 56, 57]

47. Seesod, N., J. Lundeberg, A. Hedrum, L. Asland, A. Holder, S. Thaithong, and M. Uhlen. Immunomagnetic purification to facilitate DNA diagnosis of *Plasmodium falciparum*. *J. Clin. Microbiol*. **1993**; *31*: 2715–2719.

48. Sethabutr, O., A. E. Brown, S. Panyim, K. C. Kain, K. Webster, and P. Echeverria. Detection of *Plasmodium falciparum* by polymerase chain reaction in a field study. *J. Infect. Dis.* **1992**; *166*: 145–148.

49. Snounou, G., S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown. Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol. Biochem. Parasitol*. **1993**; *58*: 283–292.

50. Wataya, Y., M. Arai, F. Kubochi, C. Mizuloshi, T. Kakutani, N. Ohta, and A. Ishii. DNA diagnosis of *falciparum* malaria using a double PCR technique: a field trial in the Solomon Island. *Mol. Biochem. Parasitol*. **1993;** *58*: 65–168.

51. Watya, Y., F. Kubochi, C. Mizukoshi, Y. Ohya, K. Watanabe, M. Arai, A. Ishii, and A. Nakagam. DNA diagnosis of falciparum malaria. *Nucleic Acids Symp. Ser.* **1991**; *25*: 155–156

52. Barker, R. H., N. T. Banchongaksorm, M. M. Courval, W. Suwonkerd, K. Rimwungtragoon, and D. R. Wirth. A simple method to detect *Plasmodium falciparum* infection in human patients: a comparison of the DNA probe method to microscopic diagnosis. *Am. J. Trop. Med. Hyg*. **1992**; *41*:266–272.

53. Kawamoto, F., H. Miyake, O. Kaneko, M. Kimura, T. D. Nguyen, Q. Lui, M. Zhou, D. D. Le, S. Kawai, S. Isomura, and Y. Wataya. Sequence variation in the 18S rRNA gene, a target for PCR-based malaria diagnosis, in *Plasmodium ovale* from southern Vietnam. *J. Clin. Microbiol*. **1996;** *34*: 2287–2289.

54. Pieroni, P., C. D. Mills, C. Ohrt, M. A. Harrington, and K. C. Kain. Comparison of the ParaSight® F and the ICT® malaria *Pf* test with the polymerase chain reaction for the diagnosis of *Plasmodium falciparum* malaria in travellers. *Trans. R. Soc. Trop. Med. Hyg*. **1998**; *92*: 166–169.

55. Singh, N., M. Valencha, and V. P. Sharma. Malaria diagnosis by field workers using a immunochromatographic test. *Trans. R. Soc. Trop. Med. Hyg*. **1997**; *91*: 396–397.

56. Snounou, G., S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown. Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Mol. Biochem. Parasitol*. **1993**; *58*: 283–292.

57. Warhurst, D. C., and J. E. Williams. Laboratory diagnosis of malaria.*J. Clin. Pathol*. **1996**; *49*: 533–538.

The large-subunit RNA gene is extensively conserved among *Plasmodium* species and is also suitable as a genus-specific DNA target region, with the amplified target sequence being detected by internal probes or analyzed by gel electrophoresis.[58] Other DNA targets, such as the CS gene, have been used for species-specific regions and have been coupled with specific fluorescein or radio labeled probes for detection of *P. vivax*.

The major advantages of using a PCR-based technique are

a) The ability to detect malaria parasites in patients with low levels of parasitemia

b) Ability to identify up to the species level.

c) Infection with five parasites or less per µl can be detected with 100% sensitivity and 100% specificity.

d) The additional sensitivity obtained using PCR may provide positive results from sub clinical infections. Parasites may be released from sequestered capillary sites into circulation but in insufficient numbers to be detected by peripheral-blood microscopy alone.

PCR remained positive for a median of 144 hours compared to 66 hours for microscopy.[59] If PCR yielded positive results for 5 to 8 days after treatment, therapeutic failure, possibly due to drug resistance, may be predicted.

However, PCR may detect DNA from circulating nonviable parasites after successful therapy and may lead to clinical confusion.[60] Differences in techniques for collection and storage of the specimens, methods for DNA extraction and selection of primers, amplification conditions, and analysis of amplified product may lead to variation in results.

58. Kain, K. C., A. E. Brown, L. Mirabelli, and H. K. Webster. Detection of *Plasmodium vivax* by polymerase chain reaction in a field study. *J. Infect. Dis.* **1993**; *168*: 1323–1326

59. Kain, K. C., D. E. Kyle, C. Wongrichanalai, and A. E. Brown. Qualitative and semi-quantitative polymerase chain reaction to predict *Plasmodium falciparum* treatment failure. *J. Infect. Dis.* **1994**; *170*: 1626–1630.

60. Srinavasan, S., A. H. Moody, and P. L. Chiodini. Comparison of blood-film microscopy, the OptiMAL® dipstick, Rhodamine 123 and PCR for monitoring anti-malarial treatment. *Ann. Trop. Med. Parasitol.* **2000**; *94*: 227–232.

The ability to detect all species of *Plasmodium* in nested or multiplex assays and the enhanced sensitivity make them ideal procedures for the diagnosis of malaria. However, the turnaround time to provide results is a limitation. Progress in rapid DNA extraction methods and in methods to amplify DNA may allow the test to be performed in a time frame that is clinically relevant for acute diagnosis in both field and laboratory settings.

Loop-Mediated Isothermal Amplification (LAMP)

The loop-mediated isothermal amplification (LAMP) technology is also used to identify 18S rRNA gene of p. falciparum without cross reaction with other species of plasmodium.

A recent study from Hong Kong[61] reported a proof-of-study concept comparing LAMP with PCR. The authors chose PCR as the gold standard comparator given that PCR may be more specific and sensitive than routine microscopy under optimized conditions. The authors stated that the LAMP test for detection of *P. falciparum* is simple; does not require thermal cyclers, expensive reagents (or time-consuming steps) for DNA purification, or downstream processing for amplicon detection. The DNA amplification step in the assay is highly robust. The technician "hands-on" time for the assay is estimated to be one third that of a manual PCR test. In resource-limited situations, rather than using a real-time turbidity meter, one can perform this test with only a water bath.

More importantly, the interpretation of results from this closed-tube test does not require highly experienced staff. The authors estimate the running cost for a LAMP test would be less than €0.25. These features make the LAMP assay an option for the molecular diagnosis of *P. falciparum* even in basic healthcare settings. However, further work in evaluating the performance of this test in bedside or clinical situations is needed.

Accuracy of LAMP

The sensitivity and specificity of the LAMP assay were 95% and 99%, respectively, compared to PCR in this study. Inhibitors like heme in blood can severely affect the amplification of DNA by PCR. These PCR inhibitors appear to have little impact on the LAMP reactions.

61. Leo L.M. Poon, Bonnie W.Y. Wong, Edmund H.T. Ma, Kwok H. Chan, Larry M.C.Chow, Wimal Abeyewickreme, Noppadon Tangpukdee, Kwok Y. Yuen, Yi Guan, Sornchai Looareesuwan, and J.S. Malik Peiris. Sensitive and Inexpensive Molecular Test for Falciparum Malaria: Detecting *Plasmodium falciparum* DNA Directly from Heat-Treated Blood by Loop-Mediated Isothermal Amplification. *Clinical Chemistry* **2006**; *52*, No. 2, 303-306

DNA polymerases have different susceptibilities to PCR inhibitors and it may be that the *Bacillus stearothermophilus* (*Bst*) DNA polymerase used in the LAMP reactions is more resistant.

A major advantage of LAMP is its ability to generate a large amount of white precipitate of magnesium pyrophosphate in a positive reaction allowing for easy identification of a positive reaction via visual inspection. The authors of this study reported that it was feasible to visually interpret the results of LAMP and that these visual interpretations (positive, negative or ambiguous) are consistent with those deduced from a real time turbidity meter.

The authors also evaluated if the LAMP assay is more sensitive than a microscopic test and reported that positive signals could be observed in reactions with 1000-fold diluted samples for samples that had an initial high parasite count. However, only those samples with less dilution were positive for samples with low parasite load. The number of parasites estimated in the LAMP assay correlated positively with the parasite count deduced from the microscopic study.

Real Time Sequence Based Nucleic Acid Amplification and Real Time PCR

Real time sequence based nucleic acid amplification and real time PCR are new developments for rapid detection of malaria parasites. A QT-NASBA based on the detection of *Plasmodium falciparum* 18S rRNA with a sensitivity of 10 to 50 parasites/ml has been developed.[62] This method can be applied to 50-µl finger prick blood samples and has complete specificity for *P. falciparum*. This QT-NASBA assay is a combination of RNA extraction, amplification of the RNA target plus an internal control, and end point detection of amplification products by electrochemiluminescence (ECL). Competitive co-amplification of an internal control enables accurate quantification.[63]

A study compared real time quantitative sequence based nucleic acid amplification (QT-NASBA) with real time quantitative PCR (QT-PCR) for the diagnosis of plasmodium falciparum.[64]

62. Schoone, G. J., L. Oskam, N. C. M. Kroon, H. D. F. H. Schallig, and S. A. Omar. Detection and quantification of *Plasmodium falciparum* in blood samples using quantitative nucleic acid sequence-based amplification. *J. Clin. Microbiol.* **2000**; *38*: 4072–4075.

63. Schallig, H. D., G. J. Schoone, E. J. Lommerse, C. C. Kroon, P. J. de Vries, and T. van Gool. Usefulness of quantitative nucleic acid sequence based amplification for diagnosis of malaria in an academic hospital setting. *Eur. J. Clin. Microbiol. Infect. Dis.* **2003**; 22: 555–557.

64. Petra Schneider, Liselotte Wolters, Gerard Schoone, Henk Schallig, Peter Sillekens, Rob Hermsen, and Robert Sauerwein. Real-Time Nucleic Acid Sequence-Based Amplification Is More Convenient than Real-Time PCR for Quantification of *Plasmodium falciparum*. *Journal of Clinical Microbiology* **2005**; 402–405

This study adapted the QT-NASBA to real-time molecular beacon technology to avoid inaccurate quantification at high parasite densities[65], which may occur with end point detection due to depletion of reagents during the amplification reaction. Moreover, the closed-tube format of the assay greatly reduces the risk of contamination and thus of false-positive results. The real-time QT-NASBA allows accurate quantification of as many as 48 samples in 4 h, including RNA extraction. The QT-PCR is based on detection of DNA encoding 18S rRNA. This real-time QT-PCR has a sensitivity of 20 parasites/ml of blood when 500-µl blood samples are used and provides accurate quantification of 48 samples within 16 h.

The advantages of real-time QT-NASBA are that quantification results are obtained more rapidly and quantification of high parasite densities is not adversely affected by possible depletion of reagents in the amplification process, due to real-time detection of amplicons. Furthermore, results are obtained with less manual handling of samples than the ECL-based QTNASBA requires, and the risk of carryover contamination and of amplicons spreading in the laboratory is greatly reduced due to the closed-tube format. Another advantage of QT-NASBA is the ability to use small sample volumes for analysis, enabling the use of finger prick blood samples as opposed to venous blood collection and mandatory filtering of blood samples for real-time QT-PCR. The specificity of QT-NASBA for RNA, without the need for complicated RNA extraction to remove all genomic DNA from the samples, enables further development of the assay for detection of various life stages of *P. falciparum* .

The modification of ECL-based QT-NASBA to real-time QT-NASBA also allows multiplexing, i.e., the detection of multiple targets within one reaction.

65. Tyagi, S., and F. R. Kramer. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol*. **1996**; *14*: 303–308.

Accuracy of QT-NASBA and QT-PCR

There was significant correlation for the quantification of ring stage parasites and gametocytes by NASBA and of ring stage parasites by PCR. For real-time QT-NASBA, the inter-assay variation was low and relatively stable over the range of parasite concentrations tested. The inter-assay variation appeared to increase at the lowest tested parasite concentrations of 10 asexual parasites per ml of blood. The inter-assay variation of real-time QT-PCR is similar to that of real-time QT-NASBA but could not be calculated accurately for the lower concentrations. The detection limit for both real-time QT-NASBA and real-time QT-PCR is 20 parasites/ml of blood. For real-time QT-NASBA, parasite densities of 10/ml can also be detected, but the detection limit is set to 20 parasites per ml of blood, i.e., 1 parasite per blood sample when 50-µl blood samples are used. For real-time QT-PCR, detection of 10 parasites/ml of blood is feasible but increases the risk of false negative results. When the volumes of blood used for nucleic acid extraction and the amount of extract used in both real-time assays are taken into account, the sensitivity of real-time QT-NASBA is higher than that of real-time QT-PCR, with absolute detection limits of 0.05 and 1 parasite per reaction in QT-NASBA and QT-PCR, respectively.

The higher sensitivity of the real-time QT-NASBA is attributed to the abundance of rRNA compared to that of 18S rRNA, the target of real-time QT-PCR.

D.4 Non Microscopic Rapid Diagnostic Tests

Immunochromatographic Tests (ICT)

ICTs are based on the initial capture of parasite antigen from peripheral blood using monoclonal antibodies prepared against a malaria antigen target. The captured antigens are then conjugated to either a liposome containing selenium dye or gold particles in a mobile phase. A second or third capture monoclonal antibody applied to a strip of nitrocellulose acts as the immobile phase. The migration of the antigen-antibody complex in the mobile phase along the strip enables the labeled antigen to be captured by the monoclonal antibody of the immobile phase. The capture of the antigen by the monoclonal antibody produces a visible colored line. Incorporation of a labeled goat antimouse antibody capture ensures that the system is controlled for migration.

Migration depends on several physical characteristics of the component reagents, primarily the porosity of the membrane controlling the flow rate and the components of the buffer solution used to transport the labeled antigen-antibody complex in the lysed blood sample. Malaria antigens currently targeted by RDT are Histidine Rich Protein (HRP-2)[66], pLDH[67], and *Plasmodium* aldolase.[68]

P. falciparum-infected RBC (IRBC) synthesize three histidine-rich proteins, HRP-1 (the knob-associated HRP), HRP-2, and HRP-3. HRP-1 (*M*r 80,000 to 115,000) was identified in all knob-positive *P. falciparum* parasites but small amounts only were present in Gambian isolates and in several culture-adapted strains. HRP-2 (*M*r 60,000 to 105,000) was identified in all *P. falciparum* parasites regardless of the knob phenotype and was recovered from culture supernatants as a secreted water-soluble protein. HRP-2 was shown to be a surface-exposed protein complex of several close bands. HRP-3 (*M*r 40,000 to 55,000) was present at the lowest abundance compared to HRP-1 and HRP-2. Neither HRP-1 nor HRP-2 was found in a range of other knob-positive and -negative strains of non-*P. falciparum* malaria. HRP-2 is a water-soluble protein produced by asexual stages and young gametocytes of *P. falciparum*. It is expressed on the RBC membrane surface, and because of its abundance in *P. falciparum*, it was the first antigen to be used to develop an RDT for its detection.

HRP-2 Assays

There are several commercially available assays for HRP-2. ParaSight F [Becton Dickinson, Franklin Lakes, N.J.], ICT *Pf* or *Pf/Pv* [Amrad ICT, Sydney, Australia], and PATH Falciparum Malaria IC test [PATH, Seattle, Wash] are the commonly used tests.

Although HRP-2-based ICTs permit rapid diagnosis of *P. falciparum* malaria, these tests will give negative results with samples containing only *P. vivax, P. ovale*, or *P. malariae* since HPR-2 is expressed only by *P. falciparum*. Many cases of non-*falciparum* malaria may therefore be misdiagnosed as malaria negative.

66. Rock, E. P., K. Marsh, S. J. Saul, T. E. Wellems, D. W. Taylor, W. L. Maloy, and R. J. Howard. Comparative analysis of the *Plasmodium falciparum* histidine-rich proteins HRP1, HRP2 and HRP3 in malaria diagnosis of diverse origin. *Parasitology* **1987**; *95*: 209–227.

67. Makler, M. T., R. C. Piper, and W. Milhous. Lactate dehydrogenase and diagnosis of malaria. *Parasitol. Today* **1998**; *14*: 376–377.

68. Meier, B., H. Dobeli, and U. Certa. Stage-specific expression of aldolase isoenzymes in the rodent malaria parasite *Plasmodium bergei*. *Mol. Biochem. Parasitol*. **1992**; *52*: 15–27.

HRP-2 persists in the blood and is detectable after the clinical symptoms of malaria have disappeared and the parasites have apparently been cleared from the host.[69, 70] Circulating HRP-2 antigen was detected in 68% of treated patients on day 7, and in 27% it was still present on day 28. The reason for the persistence of the HRP-2 antigen is not well understood and may reflect the presence of latent, viable parasites (possibly the result of treatment failure or circulating antigen-antibody complexes). The action of antimalarial therapy on the parasite may also potentially influence the persistence of HRP-2. Low-level parasitemias seen in areas of endemic infection because of constant exposure to the malarial parasites may result in positive results with doubtful clinical significance. There is evidence that certain individuals may actually have a gene deletion for the production of HRP-2 and so will never give a positive result with these tests. Other limitations of tests for this antigen relate specifically to technical aspects of the HRP-2 test system- the monoclonal IgG antibody used in the ParaSight F cross-reacts with serum rheumatoid factor causing false-positive results. The Amrad ICT *Pf* and PATH Falciparum Malaria tests use a monoclonal IgM antibody, and reports of false-positive reactions occurring with rheumatoid factor are less frequent.

Accuracy of HRP-2 Tests

ParaSight F immunochromatographic test for HRP-2 for the detection of *P. falciparum* in blood samples have shown an overall average sensitivity of 77 to 98% when >100 parasites/µl are present (0.002% parasitemia), with a specificity of 83 to 98% for *P.falciparum* compared with thick blood film microscopy.[71, 72, 73, 74, 75, 76, 77]

69. Laferi, H., K. Kandel, and H. Pichler. False positive dipstick test for malaria. *N. Engl. J. Med*. **1997**; *337*: 1635–1636.

70. Miller, R. L., S. Ikram, G. L. Armelagos, R. Walker, W. B. Harer, C. J. Chief, D. Baggett, M. Carrigan, and S. M. Manet. Diagnosis of *Plasmodium falciparum* infections in mummies using the rapid manual ParaSight® F test. *Trans. R. Soc. Trop. Med. Hyg.* **1994**; *88*:31–32.

71. Beadle, C., G. W. Long, W. R. Weiss, P. D. Meelroy, S. M. Maret, A. J. Oloo, and S. L. Hoffman. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick-capture assay. *Lancet* **1994**; 564–568

72. Brenier-Pinchart, M. P., C. Pinel, A. Croisonnier, J. P. Brion, O. Faure, D. Ponard, and P. Ambroise-Thomas. Diagnosis of malaria in non-endemic countries by the Parasight-F test. *Am. J. Trop. Med. Hyg.* **2000**; *63*: 150–152.

73. Dietz, R., M. Perkins, M. Boulos, F. Luz, B. Reller, and C. R. Corey. The diagnosis of *Plasmodium* using a new antigen detection system. *Am. J. Trop. Med. Hyg.* **1995**; *52*: 45–59

74. Kilian, A. H. D., E. B. Mughusu, G. Kabagambe, and F. Von Sonnenburg. Comparison of two rapid HRP-2 based diagnostic tests for *Plasmodium falciparum*. *Trans. R. Soc. Trop. Med. Hyg.* **1997**; *91*: 666–667.

The lower sensitivity range obtained from different studies may indicate the inability of the observer to detect parasites at densities as low as 200 parasites/µl by microscopy (many laboratories examine only 10 fields of a thick film as a routine) or the failure to read faint positive lines from the test strip. The low level or absence of HRP-2 secretion by sexual forms may explain the negative results in some cases.

The sensitivity and specificity of the ParaSight F antigen capture test for *P. falciparum* compared with PCR has demonstrated a sensitivity and specificity for the ParaSight F test of 88 and 97%, respectively.[78, 79] The reduced sensitivity was indicative of the greater ability of PCR to detect low levels of parasitemia.

The ParaHIT *f* dipstick test (Code No. 25977, Span diagnostic Ltd, Surat, India) was evaluated as a diagnostic test in 4 districts of Central India with different transmission potential namely Jabalpur (Medical College hospital, a tertiary facility), Dindori (high transmission area), Mandla (very low/ no transmission due to intensive malaria control measures) and Korea district in Chhattisgarh (meso-endemic).[80] The performance of the ParaHIT *f* for detecting *P. falciparum* infections was evaluated against microscope examination of thick blood smears in both field and hospital settings. In the high transmission district, the sensitivity, specificity, PPV and NPV were respectively 84% (95% CI, 60.4–96.6), 69% (95% CI, 57.6–79.5), 41% (95% CI, 25.6–57.9) and 94.5% (95% CI, 84.9–98.9).

75. Kodisinghe, H. M., K. L. Perera, S. D. T. Premawansa, S. Naotunne, A. R. Wickramainghe, and K. M. Mendis. The ParaSight® F test as a routine diagnostic tool for malaria in Sri Lanka. *Trans. R. Soc. Trop. Med. Hyg.* **1997**; *91*: 398–402.

76. Premji, Z., J. N. Minjas, and C. J. Schiff. Laboratory diagnosis of malaria by village health workers using the rapid ParaSight® F test. *Trans. R. Soc. Trop. Med. Hyg.* **1994**; *88*: 418.

77. Snounou, G., S. Viriyakosol, W. Jarra, S. Thaithong, and K. N. Brown. 1993.Identification of the four human malarial species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol. Biochem. *Parasitol.* **1993**; *58*: 283–292

78. Humar, A., C. Ohrt, M. A. Harrington, D. Pillai, and K. C. Kain. ParaSight® F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travellers. *Am. J. Trop. Med. Hyg.* **1997**; *56*: 44–48.

79. Pieroni, P., C. D. Mills, C. Ohrt, M. A. Harrington, and K. C. Kain. Comparison of the ParaSight® F and the ICT® malaria *Pf* test with the polymerase chain reaction for the diagnosis of *Plasmodium falciparum* malaria in travellers. *Trans. R. Soc. Trop. Med. Hyg.* **1998**; *92*: 166–169.

80. Neeru Singh, AK Mishra, MM Shukla, SK Chand and Praveen Kumar Bharti. Diagnostic and prognostic utility of an inexpensive rapid on site malaria diagnostic test (ParaHIT *f*) among ethnic tribal population in areas of high, low and no transmission in central India. 2005. *BMC Infectious Diseases* **2005**, *5*: 50-55

The accuracy of test was 72.3% and J index 0.53 as compared to microscopy. In the high to low transmission district, the sensitivity and specificity of the test were 97% (95%CI, 88.5–99.6) and 95% (95% CI, 92–97) respectively with PPV 75% (95% CI, 64–85) and an NPV of 99.4% (95%CI, 98–100). When compared with microscopy, the accuracy of the test was 95.2% and J-index 0.92. In the low transmission area, the sensitivity of the test was 70% (95% CI, 35–93) with 93% specificity (95% CI, 88.4–96). The PPV and NPV were 33% (95%CI, 15–57) and 98.4% (95%CI, 95.4–99.7) respectively. The accuracy of the test was 92% and J-index 0.63. In 102 samples from a no transmission district, the RDT picked only one case as positive; this case was negative on smear examination. The cost of the test worked out to Indian Rupees 28 per test (approximately US$ 0.50). A systematic review of the Parasight™-F test demonstrated 0.90 (95%, confidence intervals 0.88–0.93) sensitivity and 0.94 (0.92–0.96) specificity. Both sensitivity and specificity were significantly higher in the non-resident than in the resident population.

The post-test probability indicates that in settings of low malaria prevalence, a negative test almost absolutely excludes infection, while in settings of high prevalence; the same result still gives a substantial chance of infection being present.[81]

AMRAD ICT *Pf* and PATH Falciparum Malaria test strip has produced results similar to that found with the ParaSight F test. A comparison of the PATH Falciparum Malaria IC Strip impregnated with Immunoglobulin M (IgM) monoclonal anti HRP-2, with results obtained using microscopy and PCR reported a sensitivity and specificity of 96 and 99% with HRP-2 detection for *P. falciparum*, with discrepant results having >100 parasites/µl (0.002% parasitemia).[82] An assessment of the ICT *Pf* test for detection of asexual *P. falciparum* parasitemia in several field situations in Thailand (symptomatic patients self referring for diagnosis, villagers in a screening survey, and patients recently treated for *P. falciparum* malaria) used expert light microscopy was the reference standard.[83]

81. Cruciani M, Nordi S, Malena M, et al. Systematic review of the accuracy of the ParaSight™-F test in the diagnosis of Plasmodium falciparum malaria. *Med Sci Monit*, **2004**; *10(7)*: MT81-88

82. Mills, C. D., D. C. H. Burgess, H. J. Taylor, and K. C. Kain. Evaluation of a rapid and inexpensive dipstick assay for the diagnosis of *Plasmodium falciparum* malaria. Bull. *W. H. Org.* **1999**; *77*: 553–559.

83. Wongsrichanalai, C., J. Pornsilapatip, V. Namsiriponpun, H. K. Webster, A. Luccini, P. Pansamdang, H. Wilde, and M. Prasittisuk. Acridine orange fluorescent microscopy and the detection of malaria in populations with low-density parasitemia. *Am. J. Trop. Med. Hyg.* **1991**; *44*: 17–20.

This study reported that the ICT test performance was similar for diagnostic and screening modes. Four findings emerged: (i) test sensitivity correlated directly with parasite density, (ii) test band intensity correlated directly with parasite density, (iii) persistent test positivity after parasite clearance precluded its use for monitoring early therapeutic responses, and (iv) a false-negative test at high parasite load (18,000 parasites/µl) was unexplained. The study concluded that a strong positive ICT test was highly predictive of *P. falciparum* asexual parasitemia for the diagnosis of new cases of *P. falciparum* malaria in Thailand but that a negative test result was inadequate to exclude parasitemia of <300 parasites/µl (0.006% parasitemia) and, in some instances, an even higher parasitemia.

pLDH Assays

pLDH, an enzyme found in the glycolytic pathway of the malaria parasite, is produced by sexual and asexual stages of the parasite. Different isomers of pLDH for each of the four *Plasmodium* spp. infecting humans exist. Several other enzymes of the malaria parasite glycolytic pathway, notably aldolase have been suggested as target antigens for RDT for species other than *P. falciparum*.

Specific measurement of pLDH from *Plasmodium* species in the presence of human host LDH can be measured by using the substrate 3-acetylpyridine adenine dinucleotide (APAD), an analogue of NAD, in an immunocapture assay (IC assay).

A panel of monoclonal antibodies that can bind to active pLDH was developed from *P. falciparum*-infected erythrocytes.

Three of these monoclonal antibodies are used in an RDT immunochromatographic dipstick test (OptiMAL; Flow Inc., Portland, Oreg.). Two of the monoclonal antibodies are pan specific, recognizing all four species of malaria; a third monoclonal antibody is specific only for *P. falciparum* LDH. One panspecific antibody (6C9), conjugated to gold particles as the indicator, is used to capture the entire malaria pLDH antigen present from a blood sample. The other two monoclonal antibodies act as separate immobilized capture sites on an immunochromatographic dipstick. One of the monoclonal antibodies (17E4) is specific for the capture of *P. falciparum* pLDH, and the other (19G7) is a panspecific pLDH antibody.

The malaria antigen/labeled-antibody complex will be captured by either or both of the immobilized capture lines (*P.falciparum* species) or by the pan specific line only (non-*P. falciparum* species). The gold-conjugated antigen-antibody complex builds as a purple line at the capture stripe. The presence of a goat anti-mouse monoclonal antibody capture control line indicates a successful test. In the presence of mixed infections of *P. falciparum* and a nonfalciparum species, the results would indicate *P. falciparum*.

The monoclonal antibodies used in the OptiMAL test have been exhaustively tested for cross-reactivity with LDH from other blood protozoa such as *Leishmania, Babesia*, and pathogenic bacteria or fungi; no evidence of such cross-reactivity has been found.

Accuracy of pLDH Tests

The ability of OptiMAL to detect *P. vivax* and *P. falciparum* during an outbreak of malaria has been reported previously from Honduras.[84] Results obtained with the OptiMAL tests were compared to those obtained with Giemsa-stained thick blood films from the same sample (n=202).

The overall sensitivity obtained with the OptiMAL test for *P. falciparum* and *P. vivax* in this series was 94 and 88%, respectively, with a specificity of 100 and 99%, respectively. Samples found positive by microscopy but negative by OptiMAL (3%) had <100 parasites/µl of blood (0.002% parasitemia).

In another field study of symptomatic hospital patients and asymptomatic volunteers that was conducted on hospitalized patients, *P. falciparum* and *P. vivax* parasites were detected by thick-film microscopy and PCR with a mean parasite density of 590/mm^3.[85] When OptiMAL dipstick results for the same samples were compared to the results obtained by microscopy and PCR, OptiMAL had a sensitivity of 100% and a specificity of 95% for samples containing *P.falciparum*.

84. Palmer, C. J., J. F. Lindo, W. I. Klaskala, J. A. Quesada, R. Kaminsky, M. K. Baum, and A. L. Ager. 1998. Evaluation of the OptiMAL test for rapid diagnosis *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J. Clin. Microbiol*. **1998**; *36*: 203–206

85. Quitana, M., R. Piper, H. Boling, M. Makler, G. E. Sherman, E. Fernandez, and S. Martin. Malaria diagnosis by dipstick assay in a Honduran population with co-endemic *Plasmodium falciparum* and *vivax*. *Am. J. Trop. Med. Hyg.* **1998**; *59*: 868–871.

Although the sensitivity and specificity of the dipstick in this study were similar to those of thick-film microscopy for *P. vivax*, when compared with PCR the dipstick test was unable to correctly identify mixed infections owing to the common capture obtained with the panspecific antibody band.

Further studies in India[86] and Gambia[87] with *P. falciparum* and *P. vivax*-positive samples from a cohort of malaria-exposed semi immune individuals confirmed the sensitivity and specificity found by other workers for OptiMAL. Iqbal et al. looked at pLDH detection compared to microscopy and PCR in 550 immigrants from malaria-endemic areas who were entering Kuwait, where malaria is not endemic.[88] They concluded that for parasite levels of >100 parasites/µl (0.002% parasitemia), the sensitivity obtained for OptiMAL was 97%.

However, because half of the samples with <50 parasites/µl (0.001% parasitemia) detected by microscopy were not detected, the authors recommended that the test should be used with great caution and should not replace conventional microscopy in the diagnosis of malaria. The performance of the pLDH assay was comparable to that of microscopy to detect *P. falciparum* infection at a parasitemia of >100/µl.

The pLDH assay offers an advantage over the ICT *Pf* assay for HRP-2 since samples infected with *P. vivax* are easily distinguished from those infected with *P. falciparum*; mixed infections with both *P. falciparum* and *P. vivax* can also be detected. [89]

A study of the OptiMAL assay for detection and identification of malaria infections in asymptomatic residents in Indonesia reported 88 to 92% sensitivity for detecting infections of 500 to 1,000 parasites/µl.

However, the system was markedly less sensitive than was expert microscopy for discriminating between malaria species.[90]

86. John, S. M., A. Sundarsanam, U. Sitaram, and A. H. Moody. Evaluation of OptiMAL®, a dipstick test for the diagnosis of malaria. *Ann. Trop.Med. Parasitol.* **1998**; *92*: 621–622.

87. Hunt-Cooke, A. H., P. L. Chiodini, T. Docherty, A. H. Moody, J. Ries, and M. Pinder. Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL®) with microscopy for the detection of malaria parasites in human blood samples. *Am. J. Trop. Med. Hyg.* **1999**; *60*: 20–23.

88. Iqbal, J., A. Sher, P. R. Hira, and R. Al-Owaish. Comparison of the OptiMAL® test with PCR for diagnosis of malaria in immigrants. *J. Clin.Microbiol.* **1999**; 39: 3644–3646

89. Iqbal, J., P. R. Hira, A. Sher, and A. A. Al-Enezi. Diagnosis of imported malaria by *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (PfHRP-2)-based immunocapture assays. *Am. J. Trop. Med. Hyg.* **2001**; *64*: 20–23.

Monoclonal antibodies produced against *Plasmodium* aldolase are panspecific in their reaction and have been used in a combination test with HRP-2 to detect *P. vivax* as well as *P. falciparum* in blood. The specificity and negative predictive values for the diagnosis of *P. vivax* were 94.8 and 98.2% respectively using the combined *Pf*/*Pv* immunochromatographic test (ICT *Pf*/*Pv*) formatted with capture stripes for both HRP-2 and aldolase.

The overall sensitivity of 75% and positive predictive value of 50% for *P. vivax* malaria were less than desirable. The sensitivity obtained for >500 parasites/µl (0.01% parasitemia) was 96%, but for values below this level it was only 29%.[91]

90. Fryauff, D. J., Purnomo, M. A. Sutamihardja, I. R. S. Elyazar, I. Susanti, Krisin, B. Subianto, and H. Marwoto. Performance of the Optimal assay for detection and identification of malaria infections in asymptomatic residents of Irian Jaya, Indonesia. *Am. J. Trop. Med. Hyg.* **2000**; *63:* 139–145.

91. Tjitra, E., S. Suprianto, M. Dyer, B. J. Currie, and N. M. Anstey. Field evaluation of the ICT malaria *Pf*/*Pv* immunochromatographic test for detection of *Plasmodium falciparum* and *Plasmodium vivax* in patients with presumptive clinical diagnosis of malaria in eastern Indonesia. *J. Clin. Microbiol.* **1999**; *37*: 2412–2417.

Table 1. Summary of Different RDT Tests

Parameter	***Microscopy***	***PCR***	***Fluorescence***	***Dipstick HRP-2***	***Dipstick pLDH ICT pf/pv***	***LAMP***	***Sequence based Nucleic Acid Amplification***
Sensitivity	50	5	50	>100	>100	5-50	10-50
Specificity	All	All	*P. falciparum* good, others difficult	*P. falciparum* only	*P. falciparum*, *P. vivax* good, *P. ovale* *P. malariae* only with pLDH	*P. falciparum* *P. vivax* good	*P. falciparum*
Parasite density or parasitemia	Yes	No	No	Crude estimate	Crude estimate	Yes	Yes
Time for result	30–60 min	24 h	30–60 min	20 min	20 min	20 -30 min	4-16 hrs
Skill level	High	Moderate	High	Low	Low	Low	Moderate
Equipment	Microscope	PCR	QBC apparatus or direct fluorescence microscope	Kit only	Kit only	Kit, Water Bath, real time turbidity meter	PCR
Cost	Low	High	Moderate/Low	Moderate	Moderate	Low	High

D.5 Current Limitations

The recommended method and current gold standard used for the routine laboratory diagnosis of malaria, the microscopic examination of stained thin and thick blood films, in the most capable hands, can be expected to detect 50 parasites/µl (0.001% parasitemia) of blood and to identify to the species level 98% of all parasites seen. However, the procedure is difficult and time-consuming and requires considerable training to obtain the necessary skills. Methods using fluorescence microscopy have helped improve the sensitivity but not the specificity. PCR has proven to be a sensitive method for diagnosis of all four species of human malaria parasites and can be expected to exceed the sensitivity of microscopic examination. PCR has several advantages including the ability to detect <5 parasites/µl of blood and ability to identify to the species level that make it an excellent gold standard to compare the sensitivity and specificity of other non-microscopic methods. However, PCR is an impractical standard because of the time involved and the technical experience required.

Sensitivity for RDT remains a problem, particularly for nonimmune populations. It should be possible to detect parasite densities > 100 /µl (0.002% parasitemia) of blood. While this sensitivity is a reasonable target to expect from dipsticks for *P. falciparum* diagnosis, it is at the lower end of the capability of most devices involving capture methods for HRP-2 or pLDH. Test devices available for non-*P. falciparum* malaria (ICT *Pf/Pv* and OptiMAL), have a sensitivity for the diagnosis of *P. vivax* of 90 to 96% (OptiMAL) and 75 to 95% (ICT *Pf/Pv*). Levels of parasitemia encountered for this parasite rarely exceed 1%, and a much lower figure is usually encountered. Reports on the detection of *P. ovale* and *P. malariae* antigen indicate that the panspecific monoclonal antibodies developed from *P. falciparum* in OptiMAL have a lower affinity for these antigens, with the added problem that fewer parasites are encountered than for *P. vivax*. [92]

92. Moody A. Rapid Diagnostic Tests for Malaria Parasites. *Clinical Microbiology Reviews*. **2002**; *15*: 66–78

The clinical and epidemiological significance of recognizing gametocytes of *Plasmodium* is important. In areas of non-transmission of malaria, the fact that a test will not detect all gametocytes is of less importance than in areas of high transmission. HRP-2 from sexual stages of *P. falciparum* is more readily detected than pLDH, which appears to be active in young forms but not so readily in later ones. A negative RDT will need to be confirmed by microscopic examination. The possibility of gene deletion isolates that do not express HRP-2 has been postulated, although the same evidence for pLDH has not yet been discovered.

RDTs currently on the market are easy to use; most are in cassette format with single-application areas for the blood sample and clearing buffer. Most evaluation trials have included temperature and time stability for at least 1 year at 40°C. The new generation of RDT offers a realistic practical chance to move the diagnosis of malaria away from the laboratory and nearer to the patient.

A sensitivity of >100 parasites/µl (0.002% parasitemia) obtainable for *P. falciparum* diagnosis for both HRP-2 and enzyme-based assays is as good as most clinical laboratory staff in non specialized laboratories could expect to attain microscopically with limited exposure to malaria cases.

That life-threatening parasitemia with *P. falciparum* will not be missed is an added advantage particularly for inexperienced laboratory staff in remote areas. The ability to detect the majority of the non-*falciparum* malaria cases also makes these tests ideally suited as major backup procedures for malaria diagnosis.

There are many considerations to be taken into account when reviewing the methods for laboratory diagnosis of malaria not the least of which are the important factors of availability and cost. RDT have still not reached a stage where they replace review of correctly stained thick and thin blood films as the standard operating procedure when malaria is suspected.

Current studies on RDT have focused primarily on the accuracy of the test. Further studies are needed to understand organizational and patient perspectives, and the cost effectiveness of such tests.

D.6 WHO Recommended Guidelines for RDT[93]

93. World Health Organization. **2000.** WHO/MAL/2000.1091. New perspectives in malaria diagnosis. World Health Organization, Geneva, Switzerland

The WHO has placed certain recommendations or guidelines on the optimal performance for a RDT. These include that results from these test devices should be at least as accurate as results derived from microscopy performed by an average technician under routine field conditions. Other criteria include the sensitivity, which should be above 95% compared to microscopy, and the detection of parasitemia, such that levels of 100 parasites/µl (0.002% parasitemia) should be detected reliably with a sensitivity of 100%. A specificity of 90% is desirable. Quantitative or semi-quantitative information on parasite densities in circulating blood is essential. Other essential criteria include the ability to distinguish viable parasites from parasite products such as antigens or nucleic acids not associated with viable organisms and also to indicate the prediction of treatment outcomes or resistance to common anti-malarial drugs.

It will be useful if the test is able to overlap with other diseases that may be similar in clinical and epidemiologic characteristics like dengue. The test kits should have clear illustrated instructions for use, components that are easy to manipulate, a minimum number of reagents and a minimum number of steps with results that are easy to interpret. The kits should not require refrigeration and should be able to tolerate temperatures of at least 40^0C and preferably peaks of up to 50^0C, which may occur during storage under tropical conditions. The addition of a color strip on the box to monitor heat exposure might increase test reliability.

The shelf-life should be at least one year and preferably two following arrival in the country. The production standards (good manufacturing practices) should be provided and followed. Standard reagents, such as positive controls, should be made available for quality control. The tests should be affordable to the larger population at risk. The comparator gold standard needs to be chosen carefully considering the fact that microscopy may be unreliable.

E. Treatment – Artemisinin Derivatives

National antimalarial drug policies normally recommend at least two standard treatment regimens for uncomplicated falciparum malaria. There is a first-line treatment recommended for routine use, and second-line alternatives for treating infections that are not cured by the first-line treatment, or for which the first-line treatment is contraindicated. Standard treatment drugs vary according to local policy and local patterns of malaria parasite resistance to the drugs available. The main options recommended by the WHO for first- or second-line oral treatment of uncomplicated malaria are regimens using the drugs chloroquine, sulfadoxine-pyrimethamine, meoquine and quinine. However, the decision of which drugs to use is becoming more of a problem in areas where malaria parasites are becoming more resistant to these and other established drugs. In several areas of South East Asia a pattern of decreasing effectiveness of anti malarial drugs (multidrug resistance) is emerging in other malaria endemic areas.

The artemisinin derivatives have great potential as Antimalarial drugs. They have a novel structure and mode of action amongst antimalarial compounds. Resistance to the drugs has not yet been demonstrated in malaria parasites, nor has cross resistance with other antimalarial drugs currently used in standard treatment regimens.

Artemisinin (qinghaosu) is the active principle isolated by Chinese scientists in 1972 from the plant Artemisia annua. The plant has been used since ancient times in China as a traditional medicine for fever and malaria. The solubility and activity of artemisinin were improved by developing new formulations and by modifying the parent compound to create several semi-synthetic derivatives. Of the many compounds produced by chemical modification of artemisinin only four, dihyroartemisinin (DHA), artesunate (AS), artemether (AM) and arteether (AE), have reached pharmaceutical development for use in humans. Various formulations are now available, or are being developed, in China, Europe, Thailand and Viet Nam. These formulations include: ARTEMISININ: oral (tablet, capsule), suppository; DIHYDROARTEMISININ: oral (tablet); ARTESUNATE: oral (tablet, capsule), suppository, intramuscular, intravenous; ARTEMETHER: oral (tablet, capsule), intramuscular; CO-ARTEMETHER: oral (artemether plus lumefantrine together in tablet form); ARTEETHER: intramuscular.

The problem with artemisinin drugs is that when they are used alone over short periods (less than 5 days), clearance of malaria parasites from the blood is only temporary in up to 50% of patients. This recrudescence has been attributed to the short time it takes for artemisinin drugs to be eliminated from the body. Longer courses of treatment for uncomplicated malaria raise the problem of poor compliance. An alternative is to use artemisinin drugs in combination with other antimalarial drugs which take longer to be eliminated from the body, such as meoquine or sulfadoxine-pyrimethamine. Combination of a high dose of meoquine at the end of a full course of artemisinin drug has produced a high cure rate, although this still requires at least 5 days of treatment. There is a need for combinations that are effective in shorter courses and suitable for all types of patient.

One drug company (Novartis) has responded by developing a combination tablet of artemether with benumetol (now called lumefantrine), a longer-acting antimalarial drug also originating in China.

Artemisinin drugs are a logical option to clear parasites from the blood quickly, and perhaps combination with a longer-acting drug could allow for a shorter overall course of treatment. An important clinical question, therefore, is whether artemisinin drugs should be combined de novo with a longer-acting antimalarial drug.

In 1996 a WHO report of an informal consultation on management of uncomplicated malaria recommended treatment regimens with artemisinin derivatives as follows (WHO 1996):

Monotherapy

ARTEMISININ: 10mg/kg once a day for 5 days, double divided dose on first day

ARTESUNATE: 2mg/kg once a day for 5 days, double divided dose on first day

ARTEMETHER: 2mg/kg once a day for 5 days, double divided dose on first day

DIHYDROARTEMISININ: 2mg/kg once a day for 5 days, double divided dose on first day

Combination Therapy

ARTEMISININ: 10mg/kg once a day for 3 days, plus meoquine (15-25mg/kg) as a single dose on second or third day

ARTESUNATE: 4mg/kg once a day for 3 days, plus meoquine (15-25mg/kg) as a single dose on second or third day

ACT costs range from US $ 2.00 (artemisin-amodiaquine combination, 3 doses in 48 hours) to US $ 9.12 (artemisin-lumefantrine combination, 6 doses in 48 hours) per adult treatment.[94] WHO provides the artemisin-lumefantrine combination to low income countries at a cost of US$ 2.40 per adult treatment.

E.1 Artemisinin for uncomplicated malaria[95]

Questions of Interest for Uncomplicated Malaria

- Are treatment regimens with artemisinin drugs better than other standard treatment regimens for uncomplicated malaria?
- Do artemisinin drugs have a comparative advantage over other antimalarials in geographical areas where malaria parasites are still sensitive to existing antimalarial drugs, as well as in areas where drug resistance is high?
- Should artemisinin derivatives be combined de novo with all first-line treatment regimens using longer-acting antimalarials?

Results – Artemisinin for Uncomplicated Malaria-Monotherapy

Parasite clearance at day 7 was markedly better with artesunate in Nigeria (OR 61.04, 95%CI 7.82, 476.76) and artemether in Tanzania (OR 7.52, 95%CI 2.59, 21.85) relative to chloroquine which was the comparator drug. The comparative advantage of the artemisinin drugs remained at the end of follow up, 14 days in one study and 28 days in the other.

Parasite clearance at day 7 was better with artemisinin (OR 13.70, 95% CI 1.79, 105.06) relative to quinine. The size of the effect was markedly significant in adults, but not in children.

94. Baird JK. Effectiveness of Antimalarial drugs. *New England Journal of Medicine* **2005**; *352*: 1565-77

95. McIntosh HM, Olliaro P. Artemisinin derivatives for treating uncomplicated malaria. *Cochrane Database of Systematic Reviews* **1999**, *Issue 2*. Art. No.: CD000256. DOI: 10.1002/14651858.CD000256.

By day 28, there was still no difference in children, however, loss to follow-up exceeded 70% in both treatment groups. In adults the benefit shifted in favour of quinine (OR 0.57, 95% CI 0.18, 1.79) due to a higher rate of recrudescence in the artemisinin group; this effect was stable to sensitivity analysis counting the 20% loss to follow up as successes or failures.

Parasite clearance at day 7 was reported in the two comparisons of artemether with meoquine in Thailand. No difference was shown overall (OR 1.41, 95% CI 0.05, 42.18). At day 28, artemether was significantly more effective in patients in one study (OR 10.889, 95% CI 0.99, 119.25), but not when losses to follow-up were counted as failures (OR 2.90, 95% CI 0.63, 13.39). The second study showed no difference in parasite clearance at day 28.

Artesunate was not shown to be better than meoquine at day 7, nor at day 28 in patients in three studies (OR 0.98, 95% CI 0.49, 1.95) nor on intention-to-treat analysis (less than 10% loss to follow-up). Artesunate was significantly better at clearing parasites by day 7 (OR 21.08, 95% CI 2.67, 166.68) relative to sulfadoxine-pyrimethamine and the comparative advantage remained to the end of the 14 day follow-up.

All adverse events reported from artemisinin derivatives were mild and transient; none resulted in discontinuation of treatment. Dizziness tended to be more frequent with meoquine than with artesunate or artemether.

Nausea, dizziness and ringing in the ears (tinnitus) was more common in quinine-treated patients, although a few artemether patients also experienced nausea and dizziness. No difference in vomiting was observed between artesunate and meoquine in three studies. No artemether patient vomited compared to one third of meoquine- treated patients in one study, whereas the other study comparing these drugs showed no difference. Slowing of the heart rate (bradycardia) was more common with artemether (5/12) than meoquine (10/34) in one study reporting this outcome.

All these studies were in adults. Itching was more commonly associated with chloroquine than artemisinin drugs. Abdominal pain and diarrhoea affected a similar number of artemether and meoquine patients. No adverse effect on blood cell count or chemistry was reported with artemether compared with chloroquine or meoquine. To summarize, Artesunate and artemether clear malaria parasites from the blood more effectively than the standard first-line drugs chloroquine and sulfadoxine-pyrimethamine in Tanzania and Nigeria.

In South East Asia, artemisinin drugs tend to be more effective by day 7, although different comparator drugs were used in studies with artemisinin (versus quinine), artemether and artesunate (versus meoquine).

On day 28, however, there appears to be no difference between the artemisinin drugs and the comparator drugs. The same was noted in Brazil at day 35 follow up. It is worth noting that the day 28 cure rates with artemether and artesunate in the South East Asian studies between 1989 and 1994, calculated on an intention-to-treat basis, are less than 85%, and varied between 67 and 91% with meoquine. In contrast, both artesunate and meoquine were highly effective in Brazil in 1994-5.

Single-agent treatment with artemisinin drugs appears to be well tolerated. Dizziness was notably less common than with meoquine or quinine.

Results-Artemisinin for Uncomplicated Malaria-Combination Therapy

Parasite clearance at day 7 favoured combination treatment over meoquine alone (pooled OR 2.58, 95% CI 1.67, 3.13). Artemether with meoquine was more effective in patients (OR 19.36, 95% CI 6.81, 55.01) in the largest of the two studies (n=311), even when the 27% of patients lost to follow- up were counted as failures (OR 3.04, 95% CI 1.89, 4.89). The smaller study (n=76) showed a non-significant tendency in favor of meoquine. Pooling the data using a random effect model showed no overall difference between treatments (OR 3.08, 95% CI 0.05, 191.18). The wide confidence interval rejects the marked difference in effect between these studies. Both were conducted in Thailand and used artemether from the same source (Kunming) in equivalent doses. The former study gave artemether over 3 days, the latter over 2 days.

Parasite clearance at day 28 was reported in 9 artesunate comparisons, and at day 35 in the tenth. Loss to follow-up was around 10-20% per group, with none in two studies. Analysis of patients on day 28 showed a tendency to favor combination therapy in seven out of 9 comparisons, significant in five. The overall odds ratio favors artesunate-meoquine treatment (OR 4.03, 95%CI 1.58, 10.23, random effect model). The difference is still significant if patients lost to follow-up are included as failures.

Over all, parasite clearance in patients followed up for 28 days was significantly better in those treated with a combination of artemisinin drug and meoquine compared with those given only meoquine (OR 4.05, 95% CI 1.88, 8.73, random effect model), based on 12 comparisons (n=2148 patients).

A metanalysis of weighted mean difference in parasite clearance time shows that parasites are cleared faster when artemisinin or artesunate is combined with meoquine compared with meoquine given alone, regardless of variation in the treatment regimen used in different studies (WMD -20.70 hours, 95% CI -36.21, -6.48, random effect model).

A metanalysis of weighted mean difference in fever clearance time shows that fever resolves faster when an artemisinin drug is combined with meoquine compared with meoquine given alone, regardless of variation in the treatment regimen used in different studies (WMD-10.40 hours, 95% CI -18.08, 2.73, random effect model).

Major adverse effects included neuropsychiatric effects like acute psychoses, depressive syndrome, delusions, hallucinations, anxiety, palpitations and sleep disturbance. Severe vomiting was the only adverse event that resulted in discontinuation of treatment. Early vomiting was significantly less common with sequential meoquine treatment in the larger of the two studies reporting this outcome with either artesunate or artemether.

Late vomiting was similar in both treatment groups. Nausea was less for combination treatment (OR 0.67, 95% CI 0.42 to 1.07, random effect model).

The data are consistent with a conclusion that parasite clearance is achieved earlier and faster, and is better at day 28 or 35 follow-up, with combination regimens compared with meoquine alone. Keeping in mind deficiencies in reporting of fever clearance data in some studies and a mixed pattern of effect size across different studies, the available data show that fever clearance is improved by combining meoquine with an artemisinin derivative. Neuropsychiatric adverse reactions were suffered by six patients given meoquine, either alone or in combination with an artemisinin drug. Severe vomiting is less common with combination treatment regimens, although numbers are small. Data on dizziness are inconclusive.

Studies from areas of high and seasonal malaria transmission in Africa reported fewer participants using sulfadoxine-pyrimethamine (SP) plus amodiaquine (AQ) failed treatment by day 28 (RR 0.59, 95% CI 0.42 to 0.83; 652 participants, 3 trials) relative to SP and artesunate (AS). Even excluding new infections, SP plus AQ performed better (RR 0.62, 95% CI 0.40 to 0.96; 649 participants, 3 trials). There was no statistically significant difference between the two treatments for treatment failure at day 14 (RR 1.14, 95% CI 0.47 to 2.78; 775 participants, 4 trials). SP plus AS was more effective at reducing gametocyte carriage at day seven (RR 2.31, 95% CI 1.36 to 3.92; 220 participants, 1 trial). The data suggests that SP plus AQ performed better at controlling treatment failure at day 28, but was not as good as SP plus AS at reducing gametocyte carriage at day seven. Careful consideration of local resistance patterns is required because resistance to sulfadoxine-pyrimethamine and amodiaquine are high in many areas.

In order to delay development of resistance to artesunate, the combination with sulfadoxinepyrimethamine should only be considered where both drugs are known to be effective. Data on adverse events are still lacking.[96]

Studies have reported fewer total failures with artemether-lumefantrine on day 28 (RR 0.29 95% CI 0.26 to 0.34; 724 participants) and day 14 (RR 0.03, 95% CI 0.01 to 0.05; 750 participants) relative to amodiaquine. Gametocyte carriage on day 14 was lower for artemether-lumefantrine (RR 0.32, 95% CI 0.18 to 0.56; 461 participants).

Fewer total failures occurred in the artemether-lumefantrine group, but the results were not statistically significant by day 42 (RR 0.95, 95% CI 0.48 to 1.87; 216 participants), day 28 (RR 0.90, 95% CI 0.46 to 1.77; 427 participants), day 14 (RR 0.44, 95% CI 0.11 to 1.74; 435 participants), or day 7 (RR 0.22, 95% CI 0.01 to 3.48; 410 participants). The parasite clearance time was significantly (P< 0.001) faster with artemether-lumefantrine (2.08 days, 95% CI 2.0 to 2.1; 107 participants) than chloroquine plus sulfadoxine-pyrimethamine (2.9 days, 95% CI 2.8 to 3.0; 102 participants).

The mean fever clearance time was also statistically significantly (P < 0.001) faster with artemether-lumefantrine (23.1 h, 95% CI 20.9 to 25.3; 107 participant) compared with chloroquine plus sulfadoxine-pyrimethamine (40.2 h, 95% CI 35.9 to 44.4; 102 participants). Gametocyte carriage was lower with artemether-lumefantrine by day 28, day 14, and day 7.

96. Bukirwa H, Critchley J. Sulfadoxine-pyrimethamine plus artesunate versus sulfadoxine-pyrimethamine plus amodiaquine for treating uncomplicated malaria. *Cochrane Database of Systematic Reviews* **2006**, *Issue 1*. Art. No.: CD004966. DOI:10.1002/14651858.CD004966.pub2.

The data suggests that total failure at day 28 for artemether-lumefantrine was lower when compared with amodiaquine (270 participants, 1 trial), amodiaquine plus sulfadoxine-pyrimethamine (507 participants, 1 trial), but not with chloroquine plus sulfadoxine-pyrimethamine (201 participants, 2 trials). In comparisons with artemisinin derivative combinations, artemether-lumefantrine performed better than amodiaquine plus artesunate (668 participants, 2 trials), worse than meoquine plus artesunate (270 participants, 4 trials), and no differently to dihydroartemisinin-napthoquine-trimethoprim (89 participants, 1 trial). The six-dose regimen of artemether-lumefantrine appears more effective than antimalarial regimens not containing artemisinin derivatives.[97]

E.2 Artemisinin for severe malaria[98]

Questions of Interest for Severe Malaria

- Are treatment regimens with artemisinin drugs better than standard treatment regimens for severe and complicated malaria?
- Do artemisinin drugs have a comparative advantage over other antimalarials in geographical areas where malaria parasites are still sensitive to existing antimalarial drugs, as well as in areas where drug resistance is high?
- Should artemisinin derivatives be combined de novo with longer-acting antimalarials in the treatment of severe and complicated malaria?

Results-Artemisinin for Severe Malaria

97. Omari AAA, Gamble C, Garner P. Artemether-lumefantrine (six-dose regimen) for treating uncomplicated falciparummalaria. *Cochrane Database of Systematic Reviews* **2005**, *Issue 4.* Art. No.: CD005564. DOI: 10.1002/14651858.CD005564

98. McIntosh HM, Olliaro P. Artemisinin derivatives for treating severe malaria. *Cochrane Database of Systematic Reviews* **2000**, *Issue 2.* Art. No.: CD000527. DOI: 10.1002/14651858.CD000527.

Pooled analysis of all artemisinin drug comparisons with quinine pertaining to mortality from malaria gives a result in favor of artemisinin drugs, with some heterogeneity between studies (OR 0.61, 95% CI 0.46, 0.82, random effect model). The pooled Relative Risk Reduction is 48% (95% CI 54% to 18%) using random effect analysis. The pooled data also gives a number needed to treat (NNT) of 25 (95% CI 16, 65). This is an estimate of the number of patients we would need to treat with an artemisinin drug in order to prevent one more death compared with quinine. These pooled estimates must, however, be interpreted with caution, as there is heterogeneity in the outcome between studies. Furthermore, the statistical difference is barely significant without the six studies that did not report adequate concealment of allocation: RRR 24% (95% CI 40% to 3%); OR 0.72, 95% CI 0.54, 0.96, random effect model.

Pooling all the available data for only patients with cerebral malaria gives an odds ratio in favor of artemisinin drugs (OR 0.63, 95% CI 0.44, 0.88, random effect model) and a Relative Risk Reduction of 31% (48% to 10%) but the difference is not significant when only studies reporting adequate concealment of allocation are analyzed: RRR 19% (40% to -7%); OR 0.78, 95% CI 0.55, 1.10, random effect model. Overall, there is no convincing pattern in favor of either artemisinin derivatives or quinine on coma recovery and neurological sequelae: RRR 16% (40 to -18%); OR 0.80, 95% CI 0.52, 1.25, random effect model.

The pooled odds ratio for parasite clearance at Day 7 was 2.20, 95% CI 0.99, 4.91, random effect model). No significant difference between artemether and quinine was shown in four trials (OR 1.74, 95% CI 0.75, 4.05, random effect model) for parasite clearance at day 28.

Artemisinin drugs are no worse than quinine in preventing death from severe malaria. Aggregate data suggests that at best one more life could be saved in every 25 patients (95% CI 16 to 65) treated with an artemisinin drug compared with quinine, however, this has to be interpreted with caution because of the heterogeneity between studies. Additional supportive care might be more essential to survival than the antimalarial drug in patients with complications of severe malaria, including cerebral malaria.

There is no evidence yet to suggest that early treatment in rural areas with suppositories is inappropriate, being more convenient than parenteral quinolines, whilst patients are being transferred to a higher grade health facility. Combination with a longer-acting antimalarial drug such as meoquine or sulfadoxine-pyrimethamine does reduce the rate of recrudescence according to the available evidence, and could possibly slow development of resistance to artemisinin derivatives. However, the risk of enhanced neurological reactions with meoquine following severe malaria needs to be considered.

Two trials compared arteether with quinine in children with cerebral malaria and reported on similar outcomes. There was no statistically significant difference in the number of deaths (relative risk 0.75, 95% confidence interval 0.43 to 1.30; n = 194, 2 trials), neurological complications (relative risk 1.18, 95% confidence interval 0.31 to 4.46; n = 58, 1 trial), or other outcomes including time to regain consciousness, parasite clearance time, and fever clearance time. The meta-analyses lack statistical power to detect important differences. More trials with a larger number of participants are needed before a firm conclusion about the efficacy and safety of arteether can be reached.[99]

99. Afolabi BB, Okoromah CN. Intramuscular arteether for treating severe malaria. *Cochrane Database of Systematic Reviews* **2004**, *Issue 4*. Art. No.: CD004391. DOI: 10.1002/14651858.CD004391.pub2

F. Potential Directions for ICTPH

F.1 Diagnostic Strategies

A focus on RDTs is a potential area of interest for ICTPH considering that accurate diagnosis of malaria is essential for the therapeutic interventions to become more optimal. ICTPH could explore the following options:

- Improve current test performance characteristics
- Obtain, in several areas, qualitative and quantitative information that could be used to develop a model for the appropriate introduction or expansion of the use of diagnostic tests (especially RDTs) at the peripheral level, aiming at their optimal deployment.
- Assess the feasibility and acceptability of introducing RDTs in selected situations, such as use in isolated communities, use by private health providers, and diagnosis of malaria in travelers
- Develop a bank of reagents and a network of testing sites in support of quality control and test development
- Conduct health economic studies on RDTs
- Develop methods that permit quantification of parasite density with RDTs
- Develop improved tests that reflect viable asexual parasitemia
- Assess the potential role of RDTs in the detection of treatment failures

Potential Tests of Interest

a) Loop-mediated isothermal amplification (LAMP) technology

b) Real time sequence based nucleic acid amplification

The focus should be on testing the accuracy of the test across several levels of parasitemia, and in diverse field and hospital settings. It is also necessary to conduct a comparative cost effectiveness analysis from a societal perspective. The test needs to be evaluated for its ability to detect different strains including from different geographical locations, and the amount of support infrastructure and training needed. The test needs to be evaluated from a patient and organizational perspective including the impact of the kit on several aspects of management of malaria.

F.2 Preventive and Curative Strategies

On the preventive front, ICTPH can explore deployment strategies including scale up of ITNs. On the curative front, ICTPH can partner with pharmaceuticals active in R&D pertaining to drugs for malaria.

ICTPH can also play a major role in designing studies for HTA assessment including health economics pertaining to Malaria.

G. Abbreviations

ACT	Artemisinin Combination Treatment
AE	Arteether
AM	Artemether
ANM	Auxiliary Nurse Midwives
AO	Acridine Orange
APAD	3-acetylpyridine adenine dinucleotide
AQ	Amodiaquine
AS	Artesunate
BCP	Benzothiocarboxypurine
CI	Confidence Interval
CS	Circumsporozoite
DALY	Disability Adjusted Life Years
DDT	Dichloro-Diphenyl-Trichloroethane
DHA	dihydroartemisinin
DNA	Deoxyribo Nucleic Acid
ECL	Electrochemiluminescene
EDTA	Ethylenediamine Tetraacetic Acid
EPI	Expanded Program on Immunization
HIV	Human Immunodeficiency Virus
HRP	Histidine Rich Protein
HTA	Health Technology Assessment
In	Protective efficacy
ICT	Immunochromatographic Tests
ICTPH	ICICI Centre for Technologies in Public Health
IRBC	Infected Red Blood Cell

IgG	monoclonal antibody
IgM	Immunoglobulin M
ITN	Insecticide Treated Nets
LAMP	Loop Mediated Isothermal Amplification
NAD	Nicotinamide Adenine Dinucleotide
nm	nano meter
NPV	Negative Predictive Value
NNT	Number Needed to Treat
PCR	Polymerase Chain Reaction
P. vivax	Plasmodium vivax
PHC	Peripheral Health Centre
pLDH	Plasmodium lactate dehydrogenase
PPV	Positive Predictive Value
QBC	Quantitative Buffer Coat
QT – NASBA	Quantitative Nucleic Acid Sequence Based Amplification
RBC	Red Blood Cell
RDT	Rapid Diagnostic Tests
RR	Relative Risk
RRR	Relative Risk Reduction
RNA	Ribo Nucleic Acid
SP	Sulfadoxine-pyrimethamine
WBC	White Blood Cell
WHO	World Health Organization
WMD	Weighted Mean Difference