The role of laboratory diagnosis to support malaria disease management

Focus on the use of rapid diagnostic tests in areas of high transmission

REPORT OF A WHO TECHNICAL CONSULTATION, 25–26 OCTOBER 2004

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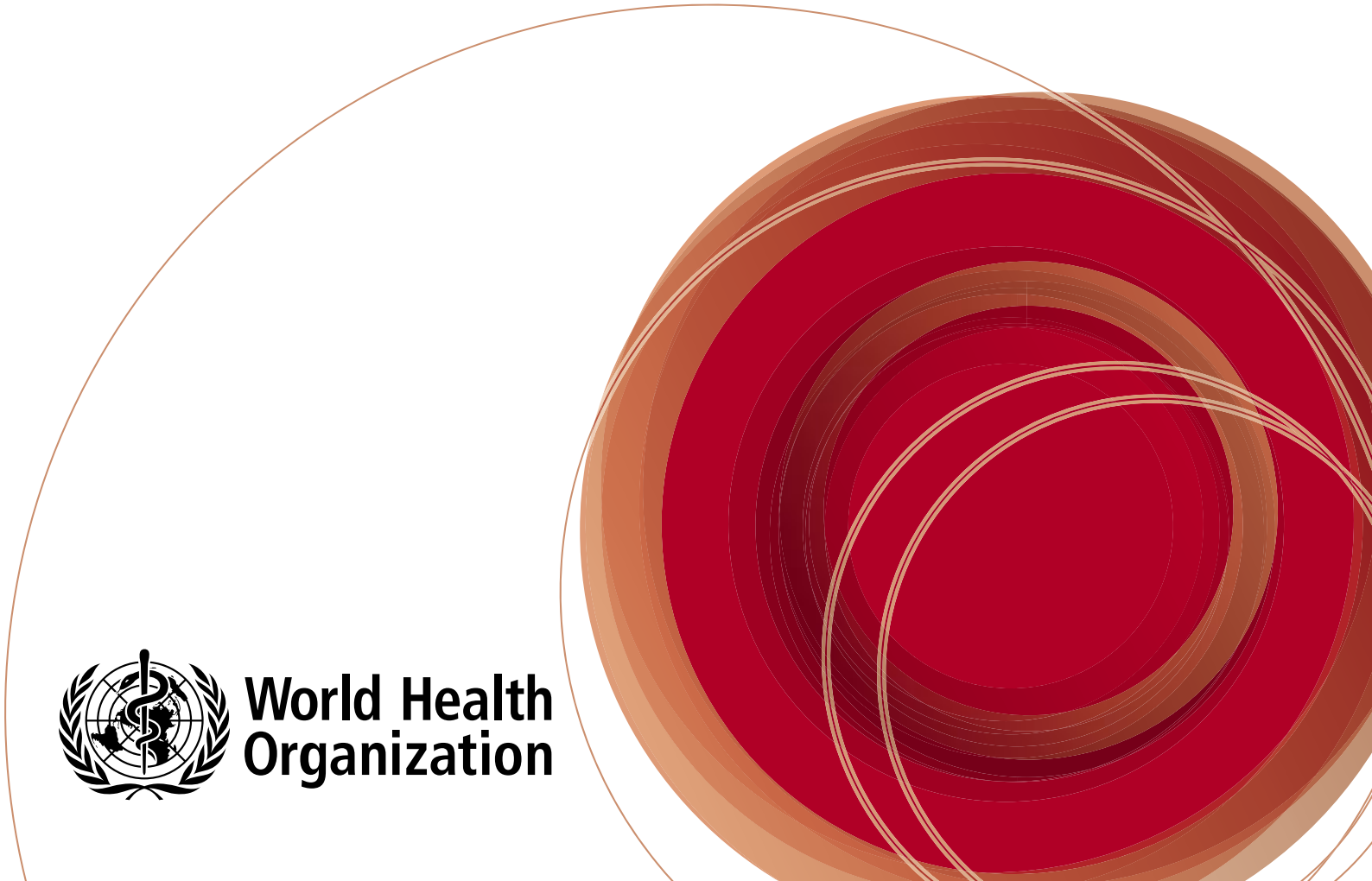

World Health Organization

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Abbreviations

ACT	artemisinin-based combination therapy
GFATM	Global Fund to fight AIDS, Tuberculosis and Malaria
HRP2	histidine-rich protein-2
IMCI	Integrated Management of Childhood Illness
MSF	Médecins Sans Frontières
pLDH	plasmodium lactase dehydrogenase
QA	quality assurance
RDT	rapid diagnostic test
VMW	village malaria workers

Introduction

In areas of intense malaria transmission,[1] such as large parts of tropical Africa, where the burden of malaria is greatest and where severe disease and mortality are largely confined to children under 5 years of age, malaria treatment is often dispensed on the basis of "fever" rather than on the basis of a parasitologically confirmed diagnosis. In these settings, malaria is by far the commonest cause of childhood fevers and most young children have malaria parasitaemia. However, with high malaria transmission and high levels of immunity, a significant proportion of the infections are asymptomatic and detecting parasites in the blood does not always help to distinguish malaria from other causes of fever. Moreover, in most of these areas microscopy and rapid diagnostic tests (RDTs) are not generally available at the periphery of the health services or at community level, where most cases of malaria are managed.

The deployment of more effective and expensive antimalarial medicines, particularly artemisinin-based combination therapies (ACTs), has revived interest in parasitological confirmation of diagnosis at various levels of the health care system. With the increased financial support for malaria control being made available through mechanisms such as the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), many countries are planning to promote parasitological confirmation of diagnosis, often in the context of ACT deployment.

Until now, WHO has recommended parasitological diagnosis of malaria with microscopy or, where microscopy is not available, with RDTs in the following circumstances:

- to guide malaria case management in areas with low to moderate transmission,[2]
- for the assessment of patients with suspected severe malaria,
- for investigating antimalarial treatment failures,
- for confirmation of malaria epidemics, and
- for monitoring changes in malaria prevalence over time.

The high cost of ACTs compared to that of previously recommended medicines has led countries in Asia and Latin America, where malaria transmission is low, to strengthen parasite-based diagnostic facilities so that cost savings may be made by treating with ACTs only those individuals with a positive parasitological

[1] In areas with intense transmission, infants and young children suffer the highest rates of acute malarial illness and mortality. Immunity is acquired with increasing age: older children and adults thus suffer fewer clinical attacks and severe malaria and mortality become rare.

[2] In areas of low transmission, incidence of malaria is lower than in areas of intense transmission, most infections are symptomatic and all age groups are affected more or less equally.

diagnosis. This has led to RDTs being deployed at the periphery of the health services, including at community level, and in the private sector. Although RDTs are costly (US$ 0.6–2.50 per test) and subject to stability problems in the field (sensitivity is affected by high temperatures and high humidity), economic analyses have shown that their use can lead to overall cost savings under certain conditions.

In the light of increasing experience with RDTs over the past five years, WHO convened a Technical Consultation on 25–26 October 2004 with the following objectives:

- To assess the role of a parasite-based diagnosis of malaria in improving the targeting of ACT treatment, particularly in areas of intense transmission.
- To identify priorities for operational research to improve current guidelines for malaria diagnosis in areas of intense transmission where ACTs are being deployed.

Recommendations

The recommendations agreed by consensus during the Consultation are intended to provide guidance on the appropriate use of parasitological diagnosis in areas of intense malaria transmission, with specific focus on RDT use in countries implementing ACT. Each of the numbered sections that follow highlights the recommendations and provides the underlying rationale.

1. Parasitological diagnosis at different levels of the health care system

- Prompt and accurate diagnosis of malaria is the key to effective disease management, guiding the management of febrile patients and reducing the unnecessary use of antimalarial drugs. High sensitivity of malaria diagnosis is important in all settings, and essential for the most vulnerable population groups in which malaria infection produces an acute illness that can rapidly progress to death.
- Parasitological confirmation of clinical diagnosis should be part of good clinical practice to improve the quality of care.
- Existing laboratory services that provide malaria microscopy should be strengthened. Where microscopy is not possible, RDTs should be introduced and appropriate quality assurance systems established.
- Quality assurance of both microscopy and RDTs should be promoted at all health service levels.

Misdiagnosis of malaria results in significant morbidity and mortality. Rapid, accurate and accessible detection of malaria parasites has an important role in reducing malaria burden and in promoting the more rational use of increasingly expensive medicines. Use of RDTs makes it possible to provide accurate diagnosis for remote populations, reaching those who lack access to good-quality microscopy services.

As a priority, existing laboratory services that provide malaria microscopy should be strengthened. Microscopes are essential equipment for laboratory services, offering diagnostic support for the clinical management of many diseases. For example, faecal specimens are examined for the presence of various protozoa cysts and helminth larvae and eggs. Urine specimens are usually examined to detect eggs of *Schistosoma haematobium*, and vaginal and urethral materials are examined for *Trichomonas vaginalis*. Blood is examined by microscopy to detect different species of *Plasmodium*, microfilariae such as *Wuchereria bancrofti* or *Brugia malayi*, and *Trypanosoma* infections. Aspirated material, tissue imprints or smears from cutaneous lesions or bone marrow may be examined for *Leishmania*. Microscopy can be used for detection of *Onchocerca volvulus* in skin specimens and also has several applications in clinical bacteriology and haematology.

Because microscopy has so many and varied diagnostic applications and such an important role in supporting patient management, RDTs should be considered as a means of extending parasite-based diagnosis only to areas where good microscopy cannot be maintained or is non-existent.

To ensure the quality of care (case management), parasitological confirmation of the clinical diagnosis of malaria should be part of good clinical practice wherever possible. Although clinical diagnosis is sensitive and detects almost all malaria cases it is not specific: reliance on clinical diagnosis alone results in a high proportion of misdiagnoses. The result is prolonged and worsening illness and missed opportunities to treat diseases other than malaria, which may be the main cause of illness. Other consequences are the unnecessary use of inappropriate medicines, exposure to potential drug toxicity and wastage of meagre economic resources *(1)*.

In addition, there are public health reasons for confirming suspected malaria. Over-diagnosis inflates perceived levels of malaria morbidity. It also increases the perceived levels of drug resistance (fever is unresponsive to antimalarials). In areas of high transmission, treatment of patients without parasitaemia leads to unnecessary drug pressure on the parasite by exposing new infections to sub-therapeutic drug levels during their slow elimination phase *(2)*. Moreover, in areas of high resistance, over-diagnosis of malaria may lead to misperception of the true efficacy of antimalarial medicines when they appear to "cure" self-limiting febrile conditions mistaken for malaria, against which they are actually ineffective.

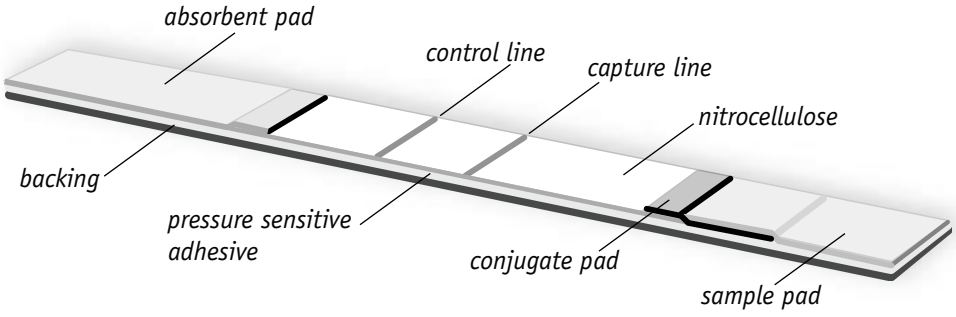
In health facilities, parasitological confirmation of the diagnosis of malaria, as well as its exclusion that will guide the health provider to other diseases, allows better targeting of treatment.

2. Performance requirements of laboratory diagnosis to support clinical management of malaria

- Malaria RDTs should have high sensitivity in all situations. Previous WHO consultations have recommended 95% sensitivity at parasite densities of 100/µlitre. To reduce the risk of missing true-positive cases, RDTs should be able to reliably detect parasite densities close to this level.
- Malaria RDTs should be highly stable, i.e. have long shelf-life. Diagnostic standards must be maintained at all levels by the introduction of a quality assurance system. Performance of RDTs in the field must be regularly compared with the results of microscopy.
- RDTs that detect antigens with short persistence in the circulation have advantages for monitoring disease progression and responses to antimalarial treatment.
- Where *Plasmodium falciparum* is the predominant species (> 90%), RDTs that can detect only *P. falciparum* are appropriate. In areas where *P. vivax* accounts for a significant proportion of the cases of malaria disease, a test that detects falciparum plus pan-malaria antigens or falciparum plus non-falciparum species is preferable.

All RDTs are based on essentially the same principle, using an immunochromatographic capture procedure with monoclonal antibodies that detect parasite-specific antigens in lysed blood. First, the dye-labelled antibody binds to a parasite antigen; the resultant complex is then captured on a nitrocellulose strip by a band of bound antibody, forming a visible line ("capture" or "test" line). A control line gives information on the integrity of the antibody–dye conjugate (see figure 1).

Figure 1. Diagrammatic representation of RDT strip



RDTs have been used experimentally since the early 1990s. The early tests used only histidine-rich protein-2 (HRP2) and could diagnose only *P. falciparum* infections. Tests targeting plasmodium lactase dehydrogenase (pLDH) and the pan-specific aldolase (thus detecting all four *Plasmodium* species that infect humans) have since become commercially available, with monoclonal antibodies specific to the malaria antigens listed below.

- Histidine-rich protein-2, which is a water-soluble protein produced by trophozoites and young (but not mature) gametocytes of *P. falciparum*. The RDTs that use HRP2 detect *P. falciparum* only.
- Plasmodium lactate dehydrogenase, which is produced by both asexual and sexual stages (gametocytes) of malaria parasites. Three different pLDH tests are available:
  - pan-specific tests,
  - tests that are specific to *P. falciparum*, and
  - more recent tests that are specific to *P. vivax*.
- Pan-specific aldolase, which is an antigen common to all four species of human malaria. It is used in conjunction with HRP2 to distinguish falciparum/mixed infections from non-falciparum infections, or in single antigen tests to detect malaria infections of unspecified origin.

Most products detect *P. falciparum*-specific protein, either HRP2 or pLDH. Some detect both falciparum-specific and pan-specific antigens (aldolase or pan-malaria pLDH), and can therefore distinguish a non-falciparum infection from *P. falciparum* or falciparum/mixed infections. Using currently available tests, it is possible to detect all four human malarias, but it is not possible to differentiate infection by *P. falciparum* alone from a falciparum/mixed infection.

Sensitivity and specificity

Ideally, detection of parasite antigens by RDTs should be at least as sensitive as detection of parasites by microscopy; 95% sensitivity at a parasite density of 100/µl of blood is recommended. While this degree of sensitivity may be possible for microscopy in high-quality reference laboratories, it is rarely achievable in hospital laboratories in malaria-endemic countries. The sensitivity and specificity of RDTs are affected by several factors discussed below (see also section 8, "Operational requirements for the use of RDTs").

Procurement of a good-quality test, adherence to the instructions provided by the RDT manufacturer, and ensuring that the product is transported and stored under the correct conditions will help to minimize the number of false-positive and false-negative test results.

A false-positive RDT result (a positive result in the absence of parasites) may result both in the patient receiving antimalarials that are not required and in

the real cause of illness being overlooked and therefore not treated. In addition, false-positive results obtained after previous treatment may suggest that the treatment has not worked: this could be recorded as a treatment failure and result in unnecessary re-treatment with an antimalarial.

Despite adherence to all recommendations, a number of false-positive results will still occur. Various studies have shown that HRP2 RDTs remain positive from 6 to 31 days following radical treatment *(3–6)*. Persistent antigenaemia, 14 days after parasite clearance, was detected by HRP2 RDT in 10% of patients in the United Republic of Tanzania *(7)*. In a Kenyan study, of 134 children who were positive to the same HRP2-detecting test, 11.9% were still positive on the sixth day following radical treatment with quinine and doxycycline *(3)*.

It has been suggested that the presence of *P. falciparum* gametocytes is responsible for some false-positive test results in falciparum infections *(6, 8–11)*, although one person had persistent HRP2 antigenaemia for 31 days following artemisinin combination therapy which had cleared all gametocytes *(6)*. A further study found no significant association between persistent antigenaemia and gametocytaemia *(12)*. In a trial designed to determine the persistence of *P. falciparum* antigens after successful treatment *(12)*, it was found that the pLDH-detecting test was more specific than the HRP2-detecting test for monitoring responses to antimalarial therapy, since pLDH activity offers a good correlation with the presence of viable parasites and its half-life is shorter than that of HRP2 when parasitaemia is cleared.

In the past, the cross-reaction between rheumatoid factor and HRP2 antigen contributed to false-positive results *(13–15)*. This problem is thought to be less common with currently available tests *(16)*. Now, the most common cause of false-positives is probably back-flow, resulting from the test results being read after the time recommended by the manufacturer. False-positive results may also be caused by the presence of heterophile antibodies, e.g. anti-mouse antibodies, but more research is needed in this area (Moody, personal communication).

A false-negative result may have far more serious consequences, such as the patient's not receiving any antimalarial treatment or being given inappropriate medicines.

In the United Republic of Tanzania, it was noted that false-negatives occurred in cases with low-level parasitaemia; most such cases had < 40 parasites/µl blood *(17)*. In Uganda it was shown that sensitivities were significantly lower when parasitaemia was 100 parasites/µl than when it exceeded 100 parasites/µl *(11)*.

The effect of genetic diversity on RDT sensitivity

Evidence indicates extensive antigenic variation in HRP2 from *P. falciparum* isolates within and between countries. This variation is likely to influence the sensitivity of HRP2-detecting RDTs at parasite densities below 500/µl blood.

The extent of antigenic variation in pLDH and parasite aldolase is less well understood. Evidence suggests that pLDH varies less than HRP2, but various isomers that exist in some non-falciparum parasites may perhaps influence the sensitivity of pLDH-detecting RDTs.

Quantitative vs. qualitative aspects

RDTs cannot measure the intensity of parasite infection, the parasite density or biomass. Essentially, they record the presence or absence of antigens. In areas of intense malaria transmission many malaria infections will be asymptomatic. Since RDTs cannot differentiate between symptomatic and asymptomatic malaria-infected persons, the only practical approach is to give antimalarials to any sick person with clinical suspicion of malaria and a positive RDT result. Nevertheless, it must be understood that the positive RDT result does not guarantee that the signs and symptoms are due to malaria.

Selection of tests according to operational requirements

Based on their higher sensitivity, greater stability and lower cost, HRP2-based tests are preferred for areas of stable malaria, where *P. falciparum* represents at least 90% of all malaria infections. In areas where *P. falciparum* and non-*P. falciparum* malaria co-exist, and the latter are clinically important, tests that can detect either *P. falciparum*-specific and pan-specific antigens (pan-specific pLDH or aldolase) or *P. falciparum*-specific and *P. vivax*-specific antigens should be used. This is particularly important where different treatments are recommended for different species.

To maximize the detection of parasites before any treatment is given, HRP2-based RDTs are recommended, despite their remaining positive, due to persistent antigenaemia, for more than 14 days following parasite elimination. The pLDH-based RDTs are more useful for detecting treatment failures, because they become negative within two days of parasite clearance. HRP2-based RDTs should *not* be used to investigate malaria treatment failures, because of the frequent persistence of HRP2 antigens after clearance of parasitaemia. For assessment of therapeutic efficacy, however, microscopy is the preferred tool as it allows quantification of the parasite density and accurate species determination.

3. The clinical benefits of parasitological diagnosis

- The use of parasite-based diagnosis supports the exclusion of malaria in the differential diagnosis of febrile illness, which may contribute to improved clinical management of non-malarial illnesses. The benefits may vary according to prevalence, health impact and practices for the treatment of other causes of febrile illness.
- By reducing malaria over-diagnosis and unnecessary antimalarial treatments, parasitological confirmation of diagnosis can improve adherence to treatment (as fewer medicines are taken) and reduce the risk of adverse drug reactions.

Confirmation of malaria by parasitological tests guides the prescription of more specific treatment with potentially less adverse drug reactions. There is likely to be better adherence to treatment (compliance) as fewer medicines are prescribed and taken. Because parasitological diagnosis allows malaria to be targeted specifically, malaria mortality may be reduced. The following two studies were presented and discussed at the Consultation.

Experience from Mali

In Mali, two RDTs – one based on HRP2 and the other on pLDH – were compared with thick and thin blood smears and with the quantitative buffy coat (QBC) test to determine the most appropriate diagnostic tool for improving malaria diagnosis in the field (for more information, contact: mal-rdt@wpro.who.int).

The results showed that the sensitivity and specificity of the RDTs were comparable to those of the blood films under field conditions. However, the HRP2-detecting RDT remained positive up to 14 days after parasite clearance following treatment, and the pLDH-detecting RDT was negative after 7 days under similar conditions. The HRP2-detecting RDT failed to detect 2–3% of clinical malaria cases, with high parasitaemias confirmed by microscopy, representing false-negative results. The study concluded that the pLDH-detecting RDT could be used to investigate treatment failures in rural areas.

In a paediatric hospital, use of RDTs led to an improvement in case management, allowing rapid (within 20 minutes) diagnosis of malaria and better targeting of malaria treatment.

Experience from Papua New Guinea

In a study carried out in Madang, Papua New Guinea, the role of RDTs in discriminating malaria from other causes of fever was evaluated and compared with microscopy, the Integrated Management of Childhood Illnesses (IMCI)

algorithm, and routine clinical diagnosis. The algorithm promoted by IMCI for the clinical management of febrile children does not differentiate between malarial and non-malarial fevers.[3] Although the IMCI strategy has been shown to be cost-effective in child health care *(18)*, probable over-diagnosis of malaria is among the problems constraining its effectiveness *(19)*.

A total of 1058 children were enrolled in the study, and three RDTs were evaluated – two different HRP2-detecting RDTs, and one RDT detecting both HRP2 and pan-malaria aldolase (for more information, contact: mal-rdt@wpro.who.int).

The main conclusions of the study were:

- HRP2 tests should not be used to exclude malaria, and negative results should be considered also as "non-falciparum" malaria and treated accordingly.
- RDTs could be more effective in younger children (< 2 years of age) because of the lower prevalence of malaria in this age group.
- RDTs may assist in the differential diagnosis of malaria and pneumonia, as defined by the IMCI guidelines.[4]
- The use of RDTs can be economically justified under specific conditions. The cost savings of malaria management with RDTs depend on a combination of factors such as the epidemiological pattern of malaria, the performance of the tests, the cost of the tests, and recommended treatment regimens. In areas of high malaria risk, RDTs seem to be more cost-effective in children under 3 years of age with lower malaria prevalence than they are in children aged 3–5 years.
- RDTs can be used by first-line health workers at primary health care facilities. First-line health workers completed the RDTs in less time than recommended by the manufacturers but still retained high sensitivity and specificity.

[3] According to IMCI guidelines, a child is classified as having "malaria" if he or she has a recent history of fever or feels hot or has an axillary temperature of 37.5 °C or above.

[4] According to IMCI guidelines, a child is classified as having "pneumonia" if he or she has cough or difficult breathing with fast breathing (50 breaths per minute in children aged 2–11 months and 40 breaths per minute in children aged 1–5 years).

4. Use of parasitological diagnosis in different population groups in areas of high transmission

- Children under 5 years of age with a clinical presentation of malaria should receive antimalarial treatment: there is insufficient evidence to recommend withholding treatment on the basis of negative parasitological results (microscopic examination or RDT).
- In children over 5 years of age and in adults, malaria diagnosis should be based on a parasitological confirmation of diagnosis.
- In pregnant women, parasitological diagnosis should be promoted as part of good clinical practice, to improve the differential diagnosis of fever. This may also reduce the unnecessary use of antimalarial drugs, many of which are of questionable safety during pregnancy. The potential clinical benefit of RDTs for detecting antigens from parasites sequestered in the placenta and not detectable by microscopic examination of peripheral blood requires further investigation.

Parasitological diagnosis of malaria in children aged under 5 years

Children aged under 5 years in areas of high transmission are the most vulnerable age group, with the highest malaria morbidity and mortality. They normally have the highest prevalence of malaria infection of all population groups. The risk of mortality in this age group, due to missed malaria diagnosis (false-negative result), may outweigh the risks and costs associated with over-treatment based on clinical diagnosis. In high-transmission settings, all under-5 children with a clinical suspicion of malaria should therefore be treated.

Parasitological diagnosis of malaria in individuals aged over 5 years

In areas of intense malaria, the prevalence of parasitaemia is lower in individuals aged over 5 years. In areas of high transmission, this group also experiences the lowest death rate due to malaria. Because of the high proportion of expected negative results and low risk of mortality in this group, treatment based on clinical signs and symptoms alone leads to considerable over-diagnosis and unnecessary consumption of antimalarials *(20)*. Whenever malaria is suspected, a differential diagnosis should be considered, and treatment should be provided only after parasitological confirmation of the infection.

Parasitological diagnosis of malaria in pregnant women

In areas of high or moderate (stable) malaria transmission, the deleterious impact of malaria is particularly apparent in the first and second pregnancies.

Although parasite prevalence and density are higher among pregnant women compared with non-pregnant women, infections with *P. falciparum* are usually asymptomatic. Clinical malaria is thus not a prominent feature of infection during pregnancy, and the major detrimental effects of infection are low birth weight and maternal anaemia.

During pregnancy, *P. falciparum* parasites are sequestered in the placenta, often without being detectable in the peripheral blood. The evidence is still unclear on the benefits of using RDTs to detect placental malaria. In one study in Cameroon, 20.1% of pregnant women who had placental malaria at the time of giving birth were peripheral blood smear-negative *(21)*, and in most of these (88%) HRP2 was detected. In the same study, a combination of microscopy and RDTs yielded accurate diagnoses in 94% of women with malaria. In a study in Malawi *(22)*, the pLDH-based RDT had a low sensitivity for the detection of placental *P. falciparum* infection and was no more sensitive than conventional microscopy for the detection of parasites in peripheral blood. In a more recent study in Nigeria, a pLDH-based test detected less than half as many peripheral parasitaemias in pregnant women as microscopy, probably because of low parasitaemia *(23)*. The potential clinical benefit of RDTs for detecting antigens from parasites sequestered in the placenta requires further investigation.

5. The economic benefits of parasitological diagnosis

- Modelling estimates based on current prices and on the anticipated use of both diagnostic tests and antimalarial medicines show that cost savings can be achieved through reduced drug consumption. The higher the price of ACTs and the greater the proportion of malaria over-diagnosis detected by parasitological diagnosis, the higher could be the cost savings, assuming that patients with negative results will not be treated.
- The wider adoption of parasitological diagnosis is expected to contribute to more efficient budgeting of antimalarial drug requirements through strengthened health information systems.
- More evidence is required from large-scale operational settings to define the economic and health benefits of parasitological diagnosis.

One of the drawbacks of implementing RDTs as part of a malaria control programme appears to be the cost of individual tests. To date, there have been few in-depth economic studies on the diagnosis of malaria: RDTs are often compared with the assumed "zero" cost of clinical diagnosis and rarely with the true cost of microscopy. It is therefore important to determine the cost-effectiveness and cost-benefit ratios of RDTs in different epidemiological situations, especially in areas of intense transmission.

Two economic models were developed to investigate the cost-benefit of implementing RDTs in areas of intense transmission. The first was based on data from febrile patients treated for malaria in two districts in Mozambique. Analyses compared the costs of disease management based on clinical diagnosis with that based on definitive diagnosis (clinical + parasitological) of all febrile patients and definitive diagnosis limited to febrile children of under 6 years of age. Two antimalarials were evaluated (artesunate + sulfadoxine-pyrimethamine, and artemether-lumefantrine). The analyses looked at:

- total cost 1 = cost of RDTs + cost of antimalarials;
- total cost 2 = cost of RDTs + cost of antimalarials + other treatment costs (including personnel, facility maintenance, administration, utilities, and other supplies).

Sensitivity analyses focused on the following factors:

- different prices of antimalarials,
- the different positivity rates expected in different age groups, and
- the different prices of RDTs.

The model assumed that the RDTs had high sensitivity and specificity and that the price of RDTs included the costs of transport, storage, etc. The costs

and benefits of management of malaria-negative cases were not included in the analysis.

The analysis indicated that RDTs were more cost-effective when a relatively expensive ACT was deployed; they were also more cost-effective when used for all fever cases and not selectively in children alone. The benefits of using RDTs increased when "other treatment costs" were also taken into consideration. Thus, the major determinant of cost saving is the price of antimalarial drugs (ACTs).

The second model, based on analysis using a "decision tree", compared the current practice of malaria treatment based on fever (prioritizing sensitivity and low cost), with chloroquine or sulfadoxine-pyrimethamine as standard treatment, and the "time to reconsider" which incorporates the cost of low specificity and sustainability when ACTs are the first-line treatment.

The most important assumptions in this model are that the relative numbers of false-negatives and false-positives are dependent on prevalence, and that the magnitude of the costs and related health effects is dependent on the characteristics of other febrile illness prevalent in the same area. The model raised the following questions:

- What is the febrile illness when it is not malaria?
- Are RDTs used when they are available?
- Will the therapeutic decisions of the health worker be guided by the result of the RDT?
- How do different diagnostic approaches affect patient adherence to treatment?
- What changes in treatment-seeking behaviour follow the introduction of RDTs and ACT?

In conclusion, this model showed that:

- RDTs are cost-effective in areas of low prevalence.
- The potential for cost savings depends on the price of RDTs, and this is related to RDT specificity.
- More robust conclusions across different settings will require more data to answer the above questions.

Modelled estimates based on current prices and on expected use of both diagnostic tests and antimalarial medicines show that cost savings can be achieved through reduced drug consumption. The more expensive the ACTs and the greater the proportion of malaria over-diagnosis detected by parasitological means, the higher could be the cost savings – assuming that patients with negative results will not be treated.

A field-based pilot study in Senegal *(24)* showed that the direct costs of treating slide-proven malaria with artesunate-amodiaquine was 53% lower than that of treating clinically diagnosed patients with chloroquine or intramuscular quinine in accordance with current policy.

6. Large-scale experience of RDT use

- RDTs can be used effectively by trained health workers, including community health workers
- Large-scale confirmation of clinical malaria by RDTs is feasible, but requirements for distribution, transport and storage need to be addressed.
- Monitoring and supervision are essential if community-based use of RDTs is implemented. RDTs are being widely used in the private sector, particularly in urban areas. The public sector should provide guidelines, training and quality assurance for the private sector.

RDTs have been used experimentally for many years, initially in Thailand *(25, 26)* and the United Republic of Tanzania *(17)*. The first experimental RDT was an HRP2-detecting test (for more information, contact: mal-rdt@wpro.who.int). This product is no longer on the market; it has been supplanted by many new HRP2 tests, including those incorporating monoclonals to pLDH and aldolase to detect pan-malaria antigens or *P. vivax* infections.

Since the introduction of RDTs to the open market, several national malaria control programmes in south America and Asia have started using them as the definitive diagnostic tool where there is no microscopy. In southern Africa (Botswana, Mozambique, South Africa and Swaziland), the use of RDTs or microscopy is promoted in the public sector for routine confirmation of suspected malaria cases. In these countries, however, most RDTs are used in the formal private health sector.

The Cambodian experience

In 1996, an HRP2-detecting test, was introduced in one province where multidrug-resistant malaria was steadily increasing in public health centres without microscopy facilities (for more information, contact: mal-rdt@wpro.who.int). In 2001, the Ministry of Health began introducing RDTs and blister-packed ACTs nationwide. The products are distributed through the general health services, through outreach services provided by volunteer malaria workers, and through the private sector (through Population Services International, Cambodia). This programme is targeting 1.6 million people – mainly ethnic minorities living in forest and hilly areas – who are at high risk of malaria.

In the public health sector, RDT or microscopy confirmation of clinically diagnosed malaria cases is provided free. If an RDT result is positive, artesunate plus mefloquine is given free to the patient, reserving chloroquine for use when an RDT result is negative but there is clinical suspicion of *P. vivax* or

P. malariae infection. Similarly, after a positive blood slide result, artesunate plus mefloquine is given free to those with *P. falciparum* or mixed infections, and chloroquine is offered to those with *P. vivax* and/or *P. malariae*. If a blood slide is negative, a differential diagnosis of fever is made and, in the absence of other causes of fever, chloroquine is provided.

The private sector (Population Services International) uses only an HRP2-detecting RDT for malaria diagnosis (for more information, contact: mal-rdt@wpro.who.int). If a test result is positive, Malarine[5] is offered at a subsidized price (US$ 0.61); if the result is negative, the patient is referred to the nearest health facility. The price of treatment, and of an RDT (US$ 0.30/test), is based on a "willingness to pay" study carried out in 2003.[6] From a pilot phase in 2001 the programme has been expanded to 10 provinces, involving between 75 and 100 shops per province in 2004.

Four provinces have started implementing outreach services through village malaria workers (VMW) in 300 villages, with two VMWs per village. Each VMW was trained to use RDTs and received prepacked antimalarials for use in their communities. Funding for both the RDTs and the ACTs, as well as for monitoring and evaluation to measure the success of the system, was provided by GFATM.

The following *advantages* of using HRP2 RDTs were identified in Cambodia:

- no need for laboratory facilities,
- simplicity and rapidity of the tests ,
- no need for electricity or laboratory equipment,
- minimal requirements for training (basic skills acquired in 1 day),
- acceptable levels of sensitivity and specificity, and
- feedback on conditions of use can be provided to manufacturers.

The following *disadvantages* were identified:

- more expensive then microscopy,
- limitation in species identification, and
- persistent positivity of HRP2 tests after effective treatment.

In addition, the following *operational problems* were encountered:

- supply delays, resulting in stock-outs,
- delayed disbursements and limitations of funds (for procurement),
- delays between peripheral and central levels in re-ordering supplies,
- transport/distribution problems (no temperature control for RDTs and no fuel for vehicles),
- poor storage conditions at the peripheral levels,

[5] Malarine is the commercial name of artesunate–mefloquine co-blister for distribution in the private sector. The Ministry of Health supplies the same product under the name "A+M".

[6] The cost of Malarine is US$ 2.80–3.50 per dose, depending on age/weight group, and the cost of a HRP2-based RDT is US$ 0.65 in Cambodia.

– difficulty in ordering specific products, and
– consumer pressure for treatment despite a negative RDT result.

The following aspects were investigated through *operational research*:
– quality of RDTs and ACT blister packaging,
– sensitivity and specificity of RDTs before and after distribution to health facilities at the periphery,
– monitoring of RDT storage temperatures, and
– development of a national quality assurance system for RDTs.

Although identified in Cambodia, the advantages, disadvantages, and operational problems listed above are considered to be applicable to many malaria-endemic countries worldwide.

A pLDH-based RDT was evaluated in Cambodia. After 3 months' storage under field conditions, its sensitivity was reduced to 79% of the initial value. Monitoring has shown that storage temperatures frequently exceed 30 °C. To reduce exposure to such temperatures, a low-cost cooler box was developed, based on the principle of evaporation of water to reduce the internal temperature. This experimental system substantially reduced the internal temperature of the box, keeping it below 30 °C for a period of 3 months, even though the external temperature exceeded 35 °C on approximately 75% of the study days. An improved storage cooler box is currently under development.

The MSF experience

Médecins Sans Frontières (MSF) deploy ACTs in all their field stations in malarious areas. They have a policy of confirming clinically diagnosed malaria with microscopy or RDTs, and use 3–4 million HRP2-detecting RDTs annually (for more information contact: mal-rdt@wpro.who.int). The HRP2-detecting RDT was selected after the evaluation of five different HRP2-detecting tests *(11)*. In their experience, routine use of RDTs is feasible and facilitates the delivery of better-quality care for patients in areas of intense transmission.

In some missions (e.g. Sudan), RDTs are being stored at temperatures above the recommended limit of 40 °C – sometimes at over 50 °C. The effects of these extreme storage conditions have not been systematically examined.

In Sierra Leone, MSF have compared clinical diagnosis followed by treatment with clinical diagnosis, RDT confirmation and treatment. When the treatment cost is US$ 1.20, a break-even point is reached when the real prevalence of malaria infection among sick patients is 40–50%. If the positivity rate rises above this threshold, the RDT diagnostic strategy becomes more expensive than clinical diagnosis alone. However, if the individual treatment costs are calculated at US$ 2.00, the break-even point is reached at a real positivity rate of almost 70%.

Concluding remarks on large-scale experience with RDTs

Both MSF experience and that from Cambodia indicate that large-scale confirmation of clinical malaria by RDT is feasible, although difficulties of procurement, distribution, transport and storage need to be addressed.

Properly trained health workers, including community health workers, can effectively use RDTs. In hospital settings, RDTs could be usefully employed in outpatient and emergency clinics (because of time saving and accuracy compared with clinical diagnosis), with microscopy being employed for inpatient diagnosis of malaria. In peripheral health facilities, the use of either RDTs or microscopy is recommended. At the community level, with well-trained community resource persons, the use of RDTs to guide good case management should be considered; however, monitoring and supervision are essential. RDTs are being widely used in the private sector, particularly in urban areas. The public sector should provide guidelines, training and quality assurance for the private sector.

7. Risks in the use of parasitological diagnosis for malaria

- Health worker disbelief/non-compliance with negative results.
- Misdiagnosis – particularly false-negative results.
- Over-reliance on positive malaria results (demonstration of parasites may divert attention from concomitant diseases).
- Over-reliance on RDTs as screening tests, rather than to support clinical diagnosis in symptomatic patients.
- Using tests that have expired or become unreliable as a result of adverse transport and storage conditions.

It is expected that there will be risks involved with the introduction of RDTs into any area for the first time. It may be difficult to persuade health workers that RDTs are more reliable than clinical diagnosis and that treatment should be given according to test results. After years of relying on clinical diagnosis alone, it is likely that some health workers will not believe negative results and will prescribe antimalarial treatment regardless of test results.

In addition, health workers may be over-reliant on positive malaria results (the demonstration of parasites may divert attention from concomitant diseases) and fail to recognize the presence of a concomitant illness in an asymptomatic, but positive, malaria patient.

Exposure to high temperatures and humidity will have a negative impact on the sensitivity of tests, so that poor storage and distribution conditions are likely to affect the performance of even the best tests, leading to false-negative results. Moreover, the suboptimal sensitivity of some RDT products will give rise to misdiagnosis of malaria. False-negative results – that is, negative RDT results in patients who have malaria parasites – are of particular concern, especially in those individuals who are most vulnerable to severe malaria and death.

Health workers may use the RDTs as screening tools (testing every patient "just to be sure") rather than to support clinical diagnosis in symptomatic patients.

With supply systems, particularly to the periphery, varying in reliability, out-of-date stocks may be used. Expired tests are likely to give unreliable results.

8. Operational requirements for the use of RDTs

- Quality control of batches through central/regional quality assurance systems.
- Correct estimations of requirements for both RDTs and ACTs.
- Coping with stock-outs in the supply/distribution systems.
- Disposal of used sharps and used RDTs (biohazard).
- Maintenance of cool chain for transport and storage (to protect RDTs from excessive heat and humidity).
- Training and supervision, especially in remote peripheral areas.
- Need for behaviour change to counter health workers' non-reliance on laboratory results for malaria diagnosis.
- Need to change public perceptions of the parasitological diagnosis of malaria.

Lot [7] and product [8] variation

Product modification is common in the commercial sector. Moreover, manufacturing problems may give rise to lot–lot variation in some products, which will affect RDT sensitivity. Lot testing is therefore essential and must be standardized, repeatable, simple, inexpensive, and based on parasites derived from the region where the product will be used *(27)*.

Quality assurance systems should be established at all levels of RDT implementation. At the international (manufacturing) level there must be lot and product testing and standardization. At the national level, lot testing, storage and distribution systems should be monitored to ensure that RDTs are maintained within recommended guidelines for product stability and to prevent stock-outs. At the local level, the reliability of the product and the users must be monitored in line with quality assurance guidelines, and the interpretation of results must be compared with microscopy as reference standard.

In the current absence of quality control during the manufacturing and shipping processes, field experience has shown that some batches do not perform properly. These substandard RDTs must be identified and discarded immediately and the manufacturer must be informed. In addition, it is reasonable to assume that RDTs will deteriorate over time, and testing throughout the recommended shelf-life is therefore strongly recommended.

[7] *Lot* – Defined as one manufacturing run of a product, using the same source and concentration of monoclonal antibodies, same signal reagent, same buffer, same source of nitrocellulose and same consistency in all constituents of the RDT that may have an impact on accuracy.

[8] *Product* – Defining malaria RDT "product" for the purposes of a product testing and pre-qualification scheme is difficult. It should be based on consistency in overall design and in major constituents of the RDT that are likely to have a significant impact on the stability or accuracy of the test.

Stability and storage

Proteins are denatured by heat, and RDTs are thus susceptible to being inactivated through exposure to excessive heat. Apparently, the HRP2-detecting RDTs currently available can withstand higher temperatures than pLDH-detecting RDTs.

Exposing RDTs to temperatures of 0 °C and below may also cause damage. This can happen during shipment from the country of manufacture, as well as during transport in unpressurized cargo aircraft.

High humidity will also adversely affects RDTs. The nitrocellulose layer is disrupted and the RDT cannot function. Extreme care should be taken in storing boxes of RDTs.

Because they are likely to be bought in bulk, RDTs are unlikely to be used immediately. It is therefore important that RDTs have a reasonably long shelf-life: WHO recommends a minimum shelf-life of 18 months to 2 years for RDTs destined for use in remote areas.

It is important to ensure the highest possible quality of packaging. Tests should be individually packaged in sachets comprising two layers of foil – a single foil wrapping is absorbent. All RDTs should remain sealed until immediately before use. If stored in a cool environment, they should be allowed to reach room temperature before being opened to avoid condensation forming on the strip.

Transportation

Environmental conditions during transportation can be extreme, and every precaution should be taken to avoid RDTs being kept in conditions of excessive heat or humidity (e.g. in vehicles; in aircraft on the ground; in buildings such as customs sheds that lack air-conditioning). Similarly, storage and transport in cold and humid containers should be avoided, and RDTs should not be transported at high altitude in unheated aircraft cargo holds.

Systems for distribution and safe disposal

It is critical that sufficient quantities of RDTs and ACTs are always available at the points of delivery. It is therefore essential to develop a system that will ensure re-supply before stocks are exhausted. Frequent stock-outs result in loss of public confidence and are discouraging for health providers. Adherence to the following points should ensure a reliable supply management system:

- correct estimates of requirements for both RDTs and ACTs at all points of delivery,
- a stock recording system,
- consumption data, based on usage and supply times, as the basis for ordering and distribution, and
- feedback from delivery points to ensure efficient procurement.

The national, regional and district medical stores should be able to prevent stock-outs in the supply/distribution systems, by implementing a stock control system.

The incorrect disposal of any product that has been contaminated with blood is a biohazard yet many health facilities lack safe disposal systems. It may be necessary to introduce and maintain appropriate disposal systems for used sharps and used RDTs.

Ease of use

One of the prerequisites for the acceptability of RDTs for use in the field is ease of use, and most current RDTs are designed to ensure this.

Concern has been expressed about the ability of some groups of RDT users (principally health volunteers) to read accurately, to discriminate and interpret the intensity of test lines, or to differentiate between *P. falciparum* and "all species" lines on the pan-malaria tests *(8, 28)*. With adequate training, however, village malaria workers and health volunteers, medical assistants and nurse aides can use RDTs effectively, achieving similar acceptably high sensitivity and specificity. Indeed, the widespread use of RDTs is dependent on the possibility of unskilled health persons routinely carrying out the tests. Three trials – one in the United Republic of Tanzania *(29)*, one in Uganda *(28)*, and the third in the Lao People's Democratic Republic *(30)* – reported on the results of training village health workers, district health workers and village health volunteers respectively. The HRP2-detecting RDT was used in the United Republic of Tanzania and Uganda; in the Lao People's Democratic Republic, HRP2-detecting RDT and pLDH-detecting RDTs were compared (for more information contact: mal-rdt@wpro.who.int).

In the United Republic of Tanzania, the sensitivity and specificity of the RDTs were both almost 90% when compared with the results obtained by an experienced microscopist. In Uganda, only 38 (3.1%) of 1226 RDT readings failed to agree with results obtained by experienced laboratory staff – and of these 38, 29 were categorized as false-positive readings. After 1 hour of training, 64 village health volunteers from rural areas of Lao People's Democratic Republic, with no previous laboratory experience, performed the two RDTs accurately, achieving 94% and 90% sensitivity and specificity, respectively, for detecting falciparum malaria with an HRP2-detecting RDT and 89% and 94% with a pLDH-detecting RDT (for more information, contact: mal-rdt@wpro.who.int).

It seems clear that it is possible to train unskilled – but willing – individuals to become reliably competent in the use of RDTs. The outcome of any training session will depend on the motivation of the trainees and the subsequent supervision of and interest in their performance. In the three experiences quoted, the training took different forms and was of different duration, indicating that it is the quality of training that is more important than the content and duration of the course.

Reading after the correct time

All RDTs work on the lateral-flow principle, the buffer "flowing" along a nitrocellulose strip, collecting the blood, and passing over the capture and control lines. The time taken for this to happen and for any reaction to take place is the minimum time that should elapse before the result is read. However, if the capture and control lines show positive responses (i.e. a positive result) before this time, that reading is acceptable. If the strip is left too long before reading, there is the possibility of a "back-flow" of blood and buffer appearing as a positive line and indicating a positive result on previously negative strips. These late results should be ignored. All RDTs must be read within the time period specified by the manufacturers.

Training and supervision

The training and supervision of health facility staff who will use RDTs are vitally important to the deployment of these tests but can be problematic, especially in remote peripheral areas. Nevertheless, continuous monitoring is essential to ensure the correct performance and interpretation of RDTs.

In many areas, resources for training will be limited, leaving just two alternatives – centrally managed training workshops or reliance on health workers' capacity to decipher the manufacturers' instructions. The former approach often cannot serve all health workers, especially in peripheral health care settings, and the latter approach has been shown not to work. However, appropriate training activities are essential if health providers are to learn the correct test procedures. There is now a third, intermediate, approach – the development and promotion of "job aids", using simplified words and pictures on a card to explain each step in the correct application of the test (*31*; see also: www.wpro.who.int/rdt/link11.asp). Simplified instruction cards can be used for self-learning by health workers as well as to transfer skills from trained health workers to other staff. Initial developmental work showed that even qualified personnel failed to wait the recommended time before reading RDTs, and this finding has led to further refinement of job aids, emphasizing the time requirements of each test. The importance of using such job aids cannot be overstated, as RDTs will ultimately be used by untrained peripheral health workers in both the public and private sectors.

Health workers used to diagnosing malaria clinically may have difficulties adapting to, and relying on, the results of parasite-based diagnoses. Adequate training is needed to ensure that such staff accept that a patient with a negative parasitological test result should not be given antimalarials but be evaluated for non-malarial illnesses and then treated appropriately.

There is a similar need to change public perceptions of the parasitological diagnosis of malaria. Most people living in rural areas will have had their "malaria" treated on the basis of clinical diagnosis, often through "self-treatment". These people need to be taught about the benefits of disease management guided by parasitological examination.

Cost

The total cost of RDTs varies with the original prices set by manufacturers, transport costs, the application of importation and other taxes and tariffs, and the retail price to the consumer based on "what the market will bear". Bulk procurement and negotiations with manufacturers may result in "discounted" prices. At present, the cost of most RDTs on the international market is between US$ 0.65 and US$ 2.50 per test.

Comparison of RDTs with routine microscopy for malaria diagnosis

The *advantages* of RDTs when compared with microscopy are:

- RDTs are simpler to perform,
- RDTs are faster (15–20 minutes),
- RDTs have low subjectivity, i.e. results show little variation between users,
- RDTs do not require electricity (but do benefit from being stored in a cool-box),
- RDT use can be learned in a few hours by health providers, including community health workers.

The *disadvantages* are the following:

- RDTs are vulnerable to extremes of temperature (becoming inoperable when stored above 30–35 °C or at or below 0 °C),
- the functioning of RDTs is adversely affected by high humidity,
- RDTs provide no quantification of parasite density.

9. Research priorities

Based on the review of the current evidence and operational experience with RDT use, especially in areas of intense malaria transmission, the following priorities for operational research were identified:

- What happens to patients with false-negative RDT results (false-negative when compared with good-quality microscopy), especially in vulnerable population groups?
- What are the main causes of fever not attributable to malaria, and the best patient management and follow-up of these patients in different epidemiological settings?
- What proportion of adults and older children with parasitaemia have sub-clinical/asymptomatic malaria, while their symptoms are caused by a concomitant disease?
- What are the main determinants of therapeutic decisions taken after the RDT results and what are their clinical outcomes?
- Are RDT results affected by severe malnutrition?
- How does malaria/HIV co-morbidity affect the results of RDTs?
- What are the potential clinical benefits to pregnant women if RDTs are used when a blood film is negative on microscopic examination?
- What is the impact of parasite variability on the performance of the various antigen-detection systems used by RDTs?
- Is there a potential for higher sensitivity of RDTs that can detect both falciparum and non-falciparum malaria?
- How can the stability of RDTs be improved under the expected different field and operational conditions of use?
- What are the effects of RDT use on treatment-seeking practices?
- What effect does parasitological diagnosis have on patient compliance with drug regimens?
- What is the evidence that parasitological diagnosis could reduce the risk of adverse drug reactions?
- What economic benefits can be documented after large-scale deployment of RDTs in areas of intense transmission?
- How can the public sector promote best practices for parasitological diagnosis of malaria in the private sector?

Conclusions

The Technical Consultation reached the following conclusions:

- Prompt and accurate diagnosis of malaria is the key to effective disease management, guiding the management of febrile patients and reducing the unnecessary use of antimalarial drugs. High sensitivity of malaria diagnosis is important in all settings, and essential for correct management of the most vulnerable population groups in which malaria infection produces an acute illness that can rapidly progress to death.
- Microscopy and RDTs are the methods currently recommended for parasitological confirmation of malaria. In all settings, laboratory services that provide malaria microscopy should be strengthened. Where microscopy is not possible, RDTs should be introduced. Carefully conducted field studies and large-scale operational experience have shown that RDTs can be used effectively by trained health workers at the periphery, including community health workers. To ensure reliable results, appropriate systems for quality assurance of microscopy and RDTs should be established and maintained.
- In settings of intense transmission, there is insufficient evidence to recommend withholding antimalarial treatment of children under 5 years of age with clinical presentation of malaria but negative parasitological results (microscopic examination or RDT). The risk of mortality due to a missed malaria diagnosis (false-negative result) may outweigh the risks and costs associated with over-treatment in these children. More research is needed to define the role of RDTs in this vulnerable age group.
- In areas of intense transmission, malaria diagnosis in children aged 5 years and above, adolescents and adults, in whom the prevalence of infection is lower, should be confirmed by parasitological means. Treatment in these population groups should be prescribed only if a parasitological test yields a positive result. This will reduce both the over-diagnosis of malaria that is a consequence of case management being based only on clinical diagnosis and the resulting high consumption of antimalarial drugs and high treatment costs in these population groups.
- Parasitological diagnosis by microscopy or RDT in pregnant women should also be promoted as part of good clinical practice, to improve the differential diagnosis of fever. This may reduce the unnecessary use of antimalarial drugs, some of which have questionable safety in pregnancy.

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