



Parasitological confirmation of malaria diagnosis

Report of a WHO technical consultation
GENEVA, 6–8 October 2009



World Health
Organization



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Abbreviations

ACT	artemisinin-based combination therapies
AMRO	WHO Regional Office for the Americas
CDC	Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
ELISA	enzyme-linked immunosorbent assay
FIND	Foundation for Innovative New Diagnostics
GMP	WHO Global Malaria Programme
HRP-2	histidine-rich protein 2
JSI	John Snow, Inc.
MSF	Médecins Sans Frontières
OR	odds ratio
PCR	polymerase chain reaction
pLDH	<i>Plasmodium</i> lactate dehydrogenase
RDT	rapid diagnostic test
TDR	Unicef/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
WHO	World Health Organization
WPRO	WHO Regional Office for the Western Pacific

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The meeting was chaired by Dr B. Nahlen, US Government President's Malaria Initiative, and co-chaired by Dr K. Mendis, WHO/Global Malaria Programme. The full list of participants, observers and WHO secretariat attending the meeting is given in the annex to this report.

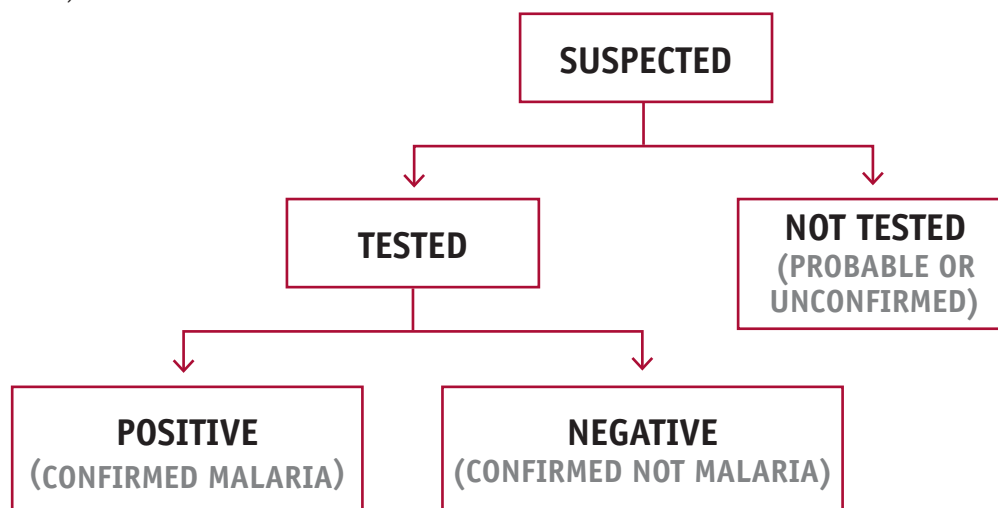
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Explanation of terms

Relationship between suspected, not tested (unconfirmed) and positive (confirmed malaria) cases.



Malaria infection: presence of *Plasmodium* parasites in blood or tissues, confirmed by the presence of parasites in peripheral blood by microscopy, malaria antigenaemia by a rapid diagnostic test (RDT) or parasite DNA or RNA by polymerase chain reaction (PCR)

Malaria (malaria disease): disease caused by infection of red blood cells with *Plasmodium* parasites, with fever as the commonest presenting sign

Parasite density: number of parasites per microlitre of blood, detected by microscopic examination of peripheral blood films; depends on *Plasmodium* species, host genetic and immunological factors, duration of infection and effectiveness of treatment

Parasite density and malaria disease: parasite densities at all levels can lead to clinical illness and contribute to transmission; particularly in areas of high transmission, febrile patients with parasitaemia may have other, concomitant causes of fever, which also require appropriate diagnosis and treatment. It is difficult to determine whether malaria parasitaemia is the primary cause of illness or incidental to another disease.

***Plasmodium* spp. antigen concentrations:** antigens produced by malaria parasites detected with RDTs; antigen concentration depends on parasite density in peripheral blood and total parasite load (including sequestered parasites); may also vary with parasite species, stage of parasite life-cycle, duration of infection, host immunity and other factors

Parasite prevalence and intensity of transmission: while the entomological inoculation rate remains the gold standard for measuring malaria transmission, it lacks precision at low transmission and is difficult to measure. Parasite prevalence in children is often used as a proxy.

High transmission area: hyperendemic or holoendemic area in which the prevalence rate of malaria is over 50% most of the year among children aged 2–9 years.¹ In these areas, virtually all exposed individuals have been infected by late infancy or early childhood.

Moderate transmission area: mesoendemic area in which the prevalence rate of malaria is 11–50% during most of the year among children aged 2–9 years.¹ The maximum prevalence of malaria occurs in childhood and adolescence, although it is not unusual for adulthood to be attained before an infection is acquired.

Low transmission area: hypoendemic area in which the prevalence rate of malaria is 10% or less during most of the year among children aged 2–9 years.¹ Malaria infection and disease may occur at a similarly low frequency at any age, as little immunity develops and people may go through life without being infected.

The relations between entomological inoculation rate, spleen rate and parasite rate, and classical endemicity level are shown in **Table 1**.

TABLE 1. Spleen rate, parasite rate, entomological inoculation rate and classical endemicity levels

Spleen rate ¹ (% children aged 2–9 years with palpable spleen)	Parasite rate ¹ (% children aged 2–9 years with parasitaemia)	Entomological inoculation rate ² (infective bites per person per year)	Endemicity
1–10	1–10	< 1	Hypo
10–50	10–50	1–10	Meso
50–75	50–75	11–100	Hyper
> 75	> 75	> 100	Holo

1. Garnham PCC. *Malaria parasites and other haemosporidia*. Oxford, Blackwell, 1966.

2. Beier JC, Killeen GF, Githure JJ. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *American Journal of Tropical Medicine and Hygiene*, 1999, 61:109–113.

¹. *Terminology of malaria and of malaria eradication*. Geneva, World Health Organization, 1963.

Background

The *WHO guidelines for the treatment of malaria*² strongly recommend confirmation of a diagnosis of malaria in all suspected cases before administration of treatment. This new recommendations emphasizes the importance of high-quality microscopy or, where not available, quality-assured RDTs. It recognizes the latter as a valid alternative to microscopy for the diagnosis of falciparum malaria infection.

Much progress has recently been made in quality assessment and quality assurance of diagnostic tools for malaria. The WHO/TDR/FIND Malaria RDT Evaluation Programme, jointly coordinated with the United States CDC, completed the first round of product-testing in 2009. The published results allow comparative assessment of RDTs in relation to parasite density thresholds for detection, stability, false-positivity rate, invalid test results and ease of use. The optimal parasite density threshold and optimal standards for other performance criteria may, however, differ according to the malaria situation and patient age group. The implications of using these thresholds for disease management and disease outcomes were still unclear at the time this report was published. Product-testing and lot-testing allow procurement agencies to make informed decisions; other tools will, in the near future, allow standardized lot-testing at national level and will facilitate standardized testing of the quality of RDTs at clinic level. The WHO guidelines for quality assurance of malaria microscopy,³ published in 2009, provide new, practical approaches for quality assurance in malaria microscopy, including methods for accreditation of national expert microscopists and routine validation of slide examination. Revised WHO malaria training manuals and bench aids⁴ with an accompanying CD-ROM, were published in early 2010.

Widescale introduction of expensive antimalarial medicines, decreasing trends in malaria morbidity in many countries due to effective control interventions and strong demand from countries to strengthen both microscopy and malaria RDTs indicate the importance of reviewing international quality assurance systems to improve clinical management of malaria in different epidemiological settings. The programme elements for strengthening laboratory diagnosis of malaria must be defined for countries undertaking malaria control, elimination and intensified surveillance, including defined thresholds for the sensitivity and specificity of diagnostic tools to ensure successful management of febrile illness.

². *Guidelines for the treatment of malaria*, 2nd edition. Geneva, World Health Organization, 2010.

³. *Malaria microscopy quality assurance manual*. Manila, WHO Western Pacific Regional Office, 2009

⁴. *Basic Malaria Microscopy*, 2nd edition. Geneva, World Health Organization, 2010.

I. Introduction

The adoption and use of expensive artemisinin-based antimalarial therapies in the past few years is unprecedented but has not been matched by a similar increase in parasitological confirmation of malaria diagnoses. Targeted treatment is important, not only to limit unnecessary dispensing of antimalarial treatment but also to allow judicious use of these precious, life-saving medicines, for which the supply of raw materials is decreasing because of reduced cultivation of *Artemisia annua*.

In introductory remarks to the meeting on which this report is based, it was noted that the malaria burden is decreasing in several countries, thus raising the demand for malaria surveillance systems. By 2008, 29 countries outside Africa had reached the target of a 50% reduction in malaria in comparison with 2000; the target has also been achieved in at least nine countries in Africa, meeting the 2010 global targets. It is essential that parasitological confirmation of malaria diagnosis be universally accessible and that all febrile cases suspected of being malaria be confirmed by laboratory testing before malaria treatment is given. More effective approaches are also needed for investigating and managing fever that is not due to malaria, to maximize the benefit of parasitological diagnosis in fever management.

In view of the importance of clarifying standards for malaria diagnosis for case management and surveillance, WHO convened a technical consultation to review the evidence base for the thresholds required by current malaria diagnostic tools and to make recommendations for the essential elements of their use.

II. Objectives of the meeting

The aims of the technical consultation were:

- to review the results of the first round of WHO product-testing of malaria RDTs and of the WHO/FIND RDT lot-testing programme, with specific attention to the clinical significance of detection rates at different parasite densities, in order to provide the best advice to national and international procurement agencies and to guide the future activities of the product-testing programme;
- to review the clinical implications of different parasite densities in *P. falciparum* and *P. vivax* malaria (from household surveys and health facility data, in different age groups and in areas with different intensities of malaria transmission) and to assess the risks of missing low-parasite density infections with use of routine field microscopy and most RDTs on the market;
- to identify key programme elements from a review of country experiences in order to strengthen laboratory diagnosis of malaria in countries undertaking malaria control, elimination and intensified surveillance; to determine the requirements for effective integration of laboratory services with other public health and disease control programmes; and to define the performance requirements for future quality control of malaria RDTs; and
- to identify future malaria diagnostic needs and product specifications.

This document reports the findings presented and the discussions held at the meeting.

Section III covers the current status and expected developments in international quality support systems for malaria diagnosis.

In **Section IV**, studies on the distribution of parasite densities, the effect of using current diagnostic tools on case management, and use of other detection methods for specific purposes are reviewed.

The implications of specific parasite density detection thresholds are discussed in **Section V**, with emphasis on the requirements for product-testing, lot-testing and preparation of positive control wells.

The operational experiences of countries in which programmes to strengthen parasitological confirmation of diagnosis have been implemented are presented in **Section VI**.

The report concludes with recommendations for future diagnostic development and use.

III. International quality assessment programmes for rapid diagnostic tests and microscopy

1. Standardized, repeatable quality control (lot-testing): A. Albertini, P. Jorgensen, D. Bell

In the WHO/FIND programme for the evaluation of malaria RDTs, lots of RDTs are tested before their use in the field. A manual of standard operating procedures is available,⁵ which is used as a guide by other lot-testing laboratories. The programme involves an independent, standardized assessment of malaria RDTs to guide lot procurement. It is based on an algorithm derived from appropriate testing of lots during procurement and leads to improved RDTs at points of use.

Lots should be tested at purchase because:

- lots of most products vary;
- manufacturers are likely to submit unrepresentative, good products for testing;
- clinicians, users and regulatory authorities must be convinced that the tests work;
- it is important to ensure that no damage has occurred during transport to a country (post-purchase testing).

The ideal system will be:

- representative of parasites found in the field;
- highly standardized, and readily transportable;
- inexpensive (e.g. < 1% of RDT procurement costs);
- available to manufacturers to test RDTs before releasing them; and
- available to procurement agencies and programmes to test RDTs before using them.

⁵. *Methods manual for laboratory quality control testing of malaria rapid diagnostic tests*. Manila, WHO Western Pacific Regional Office, 2008. http://www.wpro.who.int/NR/rdonlyres/A31142C7-CA46-465F-83D7-776C691C79BA/0/RDTLaboratoryQCtestingMethManuV5_a_WEBVERSIONAug2008.pdf

Panels used for lot-testing

Currently, there are two types of panels. Pre- and post-purchase lot-testing is performed with standardized, diluted wild-type parasites which are subsets of wild types used in the product-testing programme. “Manufacturer panels” are diluted cultured *P. falciparum*, which are subsets of the culture panel used for product-testing at the CDC, derived from different geographical areas: one Asian, three African and one South American. This panel includes one type C, three type B and one type A HRP-2 structural variants. Its availability is limited, but it is used by some manufacturers.

The problems of panel distribution include their limited availability, due to the fact that they are expensive to collect and transport, are difficult to standardize fully and are limited to a few laboratories in order to maintain quality. Wild parasites rather than cultured parasites are used for preparing quality control samples for lot-testing, as the relation between cultured parasite density and antigen concentration in the culture medium is significantly different from that expected in vivo.

Parasite quality control samples are derived from fresh blood and prepared and stored in a manner designed to minimize loss of antigen and other changes that may affect RDT performance.⁶ RDTs are tested against quality control panels diluted at the lowest concentration of 200 parasites per microlitre. This parasite density was chosen instead of 100 parasites per microlitre to prevent incorrect rejection of good-quality tests due to limited microscopy accuracy, inaccurate dilution, loss of antigen during preparation and storage, and the natural variation in the ratio of parasite density to antigen concentration.

Procedures for lot-testing

A request for lot-testing is sent to WHO or FIND, and the devices are submitted by courier. The results are available within 5 days of receipt of the tests.

For *P. falciparum*-only RDTs, lot-testing involves initial testing of 22 RDTs and repeated testing of batteries of eight tests at intervals of 3 months, six times, up to the expiry date.

For *P. falciparum* and pan- or *P. vivax* combination RDTs, lot-testing involves initial testing of 34 RDTs and repeated testing of batteries of 14 tests at intervals of 3 months, six times, up to the expiry date. As spare tests are retained for repeated testing and for sending to confirmation laboratories, 125 RDTs per lot are required for *P. falciparum*-only RDT testing and 175 RDTs per lot for *P. falciparum* and *P. vivax* combination RDTs.

⁶ *Methods Manual for Laboratory Quality Control Testing of malaria Rapid Diagnostic Tests (2008)*. http://www.wpro.who.int/NR/rdonlyres/A31142C7-CA46-465F-83D7-776C691C79BA/0/RDTLaboratoryQCtestingMethManuV5_a_WEBVERSIONAug2008.pdf

P. falciparum-only RDTs are tested against four different quality control panels and 10 different negative quality control samples. For each of the four quality control samples, two RDTs are tested at an aliquot of 200 parasites per microlitre, and one RDT is tested at an aliquot of 2000 parasites per microlitre. One RDT is tested with each of the 10 negative quality control samples.

P. falciparum and pan- or *P. vivax* combination RDTs are tested against four different *P. falciparum* quality control panels, four *P. vivax* quality control samples and 10 negative quality control samples. For each of the four *P. falciparum* quality control samples, two RDTs are tested at an aliquot of 200 parasites per microlitre and one is tested at an aliquot of 2000 parasites per microlitre. For each of the four *P. vivax* quality control samples, two RDTs are tested at an aliquot of 200 parasites per microlitre, and one is tested at an aliquot of 2000 parasites per microlitre. If the RDT fails to detect *P. vivax* at 200 parasites per microlitre, it is re-tested with a *P. vivax* sample diluted at 500 parasites per microlitre. One RDT is performed for each of the 10 negative quality control samples.

Between 2005 and August 2009, 176 lots were received and tested at the three lot-testing sites, corresponding to 6.7 million tests and to approximately 10% of RDTs procured for the public sector. Eleven to twelve types of RDT have been tested every year since 2007, reflecting the product choices of the agencies that are demanding pre-purchase lot-testing; the proportion of failed lots has dropped progressively over the years. It is estimated that only 10–15% of public-sector RDT lots are tested, implying that many unevaluated lots and products are used in the field.

FIND and WHO are collaborating with the CDC, the Hospital for Tropical Diseases (United Kingdom) and other partners to develop, evaluate and test recombinant antigen-based lot-testing panels to allow standardized lot-testing at national programme level.

2.Round 1 WHO product-testing of malaria rapid diagnostic tests: J. Cunningham

The first round of testing of malaria RDTs was conducted at the CDC in 2008 to evaluate the performance of RDTs against panels of culture-derived, patient-derived (wild-type) and parasite-negative samples. Twenty-one manufacturers submitted 41 products. Parasite-positive samples were used at dilutions of 200, 2000 or 5000 parasites. The full protocols are available elsewhere.⁷

In phase 1, detection of *P. falciparum* was assessed from a panel of 20 parasite cultures from various locations, diluted at 200, 2000 or 5000 parasites per microlitre. Protein content (for the antigen concentration of HRP-2), *plasmodium* lactate dehydrogenase (pLDH) and aldolase were determined by enzyme-linked immunosorbent assay (ELISA) in cultures of *P. falciparum*.

In phase 2, a worldwide laboratory network was set up for collecting and shipping standardized blood samples, characterized for antigen concentration, species and HRP-2 sequence at the CDC, the Hospital for Tropical Diseases (London) and the Queensland Institute of Medical Research (Brisbane). ELISA results for HRP-2 and pLDH showed wide variation for the same level of parasite density, corresponding to a wide range of antigen concentrations, which determines the intensity of the colour of lines in positive tests.

Detection of *P. falciparum* against a panel of wild-type parasites was assessed against 79 samples, each derived from a falciparum malaria case. Detection of *P. vivax* against a panel of wild-type parasites was assessed against 20 samples, each derived from vivax malaria cases from nine collection sites in Asia, Africa and South America. Assessment was made after dilution to a low parasite density (200 per microlitre) and to higher parasite densities (2000 or 5000 per microlitre). The primary structure of HRP-2 sequences in *P. falciparum* samples were defined in phase 2, and the antigen protein content was determined by ELISA to determine the concentrations of HRP-2, pLDH and aldolase from wild-type *P. falciparum* and *P. vivax* parasites.

The negative panel for testing the false-positivity rate was derived from 90 samples negative for *Plasmodium* spp. from donor blood banks in non-endemic and endemic areas and from commercial and non-commercial sources of diseases or blood factors known to elicit false-positive results in parasite-negative panels.

Test performance was evaluated by two readers, with two lots (1100 tests of each of two lots were submitted for evaluation), two tests per lot at 200 parasites per microlitre and one test per lot at 2000 parasites per microlitre. The detection rate is the percentage of malaria samples in the panel against which positive test results were obtained from two of two RDTs per lot (four positive readings) at the lower parasite density, and a positive

⁷ *Methods manual for product testing of malaria rapid diagnostic tests*. Manila, WHO Western Pacific Regional Office, 2009. http://www.wpro.who.int/NR/rdonlyres/FAF25E46-D009-4B96-BAE3-A0A937EA08D7/0/METHODSMANUALMalRDTPT_FINAL_Version2a.pdf.

test result from one RDT per lot (two positive readings) at the higher parasite density. The performance is not a measure of the clinical sensitivity of the RDT or the positivity rate against the panel but a combined measure of positivity rate and inter-test and inter-lot consistency.

Heat stability for *P. falciparum* detection was assessed with a single culture strain before and after 2 months' storage at 4 °C, 35 °C and 45 °C. Ten RDTs per lot were evaluated at each temperature.

The performance evaluation also included a semiquantitative assessment of ease of use, specifically with regard to blood safety, the quality of instructions, number of timed steps, time to results and other aspects of performance. The tests were prepared in accordance with the manufacturer's instructions and the results recorded at the minimum specified reading time. During the evaluation, each test was read against a standard colour chart, and the band intensity was graded as 0 (no band), 1, 2, 3 or 4. Tests with high detection rates had good band intensity at 200 parasites per microlitre. The detection rate was high for most RDTs assessed at 2000 or 5000 parasites per microlitre, indicating that this parasite density has limited value as a threshold for discriminating between RDTs that perform well and those that perform poorly. Good discrimination was seen at 200 parasites per microlitre. Thus, several RDTs with high detection rates at a low parasite density (200 parasites per microlitre) have low false-positive rates, are stable at tropical temperatures, easy to use and can detect *P. falciparum*, *P. vivax* or both (but with limited selection).

P. falciparum tests for HRP-2 have higher detection rates, and some tests targeting pLDH also have high detection rates. Some of the 22 combination tests detected both *P. falciparum* and *P. vivax* consistently at a low parasite density (200 parasites per microlitre), and both sensitivity and specificity were good.

Performance varied among lots and among products, confirming the need for lot-testing. The recommended reading time for several tests was too early, and delayed reading resulted in an increased detection rate.

The full report of the first round of malaria RDT product-testing, with the results for the 41 RDTs evaluated, is available at: <http://www.finddiagnostics.org/export/sites/default/media/press/pdf/Full-report-malaria-RDTs.pdf>.

FIND has posted a web-based interactive guide to guide selection of RDTs on the basis of target malaria species, minimum detection rate⁸ for both *P. falciparum* and *P. vivax* at 200 parasites per microlitre, invalid rate⁹ (proportion of tests without a visible control band) and test format. The guide is available at: http://www.finddiagnostics.org/programs/malaria/find_activities/product_testing/malaria-rdt-product-testing.

⁸ The term "detection rate" is a composite index of test positivity as well as of inter-test and inter-lot consistency in performance. Detection rate is not equivalent to sensitivity - see page 12 of the full report of the Round 1 of WHO/TDR/FIND/CDC malaria RDT product testing for a detailed description of this parameter.

⁹ Proportion of tests deemed invalid, i.e. without visible control band.

3. Influence of results of round 1 product-testing on procurement of malaria rapid diagnostic tests

WHO procurement criteria (2009): A. Bosman

The selection criteria for procuring RDTs established as a result of the first round of product-testing, for both *P. falciparum* and *P. vivax*, as part of the latest “request for proposal” issued in July 2009, are a false-positive rate < 10% and an invalid rate < 5%.

For RDTs targeting *P. falciparum* only, the detection rate must be $\geq 50\%$ against wild-type *P. falciparum* at 200 parasites per microlitre. For RDTs targeting both *P. falciparum* and non-falciparum species, the detection rate must be $\geq 50\%$ against wild-type *P. falciparum* and $\geq 25\%$ against wild-type *P. vivax* at 200 parasites per microlitre.

On the basis of these minimal performance criteria, 21 RDTs are eligible for WHO procurement. Accordingly, no new orders were placed for malaria RDTs that did not reach these standards, and procurement of some products has been discontinued by WHO, posing operational challenges to national malaria control programmes that have trained health workers in use of these products.

United States President's Malaria Initiative: L. Barat

The President's Malaria Initiative has been procuring RDTs since 2006, first through UNICEF and then mainly through JSI/Deliver. JSI/Deliver, in consultation with CDC and WHO, established technical criteria and issued two “requests for expressions of interest”. Seven manufacturers were selected.

A standard operating procedure for quality assurance has been defined for pre-shipment testing of all lots before each consignment is deployed to the field. Field samples of lots from certain countries were also re-tested after exposure to field conditions. All 11 lots from mainland United Republic of Tanzania and from Zanzibar, tested at 200 parasites per microlitre, passed.

Desk audit reviews and on-site inspections were performed at the two main suppliers. A review of the technical selection criteria is planned. New requests for expressions of interest will be issued, and pre-shipping lot-testing and periodic reviews of the manufacturing process of large-volume suppliers will be conducted.

Médecins Sans Frontières: D. Orozco

Médecins Sans Frontières (MSF) has used approximately 3 million RDTs per year in the past few years, predominantly a single product. On the basis of an analysis of the results of round 1 of product-testing and in response to experience in the field, MSF plans to diversify the products used and to concentrate on those shown to have better detection rates. The products used currently will continue to be used, with attention to suboptimal detection rates for groups such as infants, pregnant women, immunosuppressed individuals and non-immune patients with suspected malaria.

MSF has identified four additional manufacturers eligible for procurement and will consider procuring combination tests rather than *P. falciparum*-only RDTs. MSF will continue quality control of all lots as part of procurement and will also conduct more frequent audits and inspections of suppliers and manufacturers.

Key points

- In addition to the recommendations in the first-round WHO/FIND/CDC evaluation report (primarily based on detection rates, false-positive rates, ease of use and heat stability), MSF considers it important to visit manufacturers to evaluate the reliability of production, supply chains and the internal quality control system and to discuss the prices of products. Lot-testing will continue and will be part of pre-purchasing agreements with manufacturers.
- MSF has identified two manufactures and four products that include both HRP-2 and pan-malaria pLDH-based tests.
- New RDTs will be used in certain field projects, and performance in the field will be closely monitored by systematic assessments of ease of use and by supervisory checklists.

The Global Fund: S. Logez

The principles for procurement and quality assurance of pharmaceuticals adopted by the Board in 2002 apply to diagnostics and other non-pharmaceuticals. A “principal recipient” is responsible for procurement and is required to conduct competitive purchasing in order to obtain the lowest possible price for products of assured quality. For non-durable products, the principles established for pharmaceuticals should be followed, i.e. a “principal recipient” is required to select them from lists of prequalified products, if they exist, or to select products accepted by stringent regulatory agencies or

by national standards. For durable products, the lowest possible price should take into account the “total cost of ownership”.

At its 18th meeting, in November 2008, the Board requested the Secretariat to “review the current status of quality assurance for diagnostic products and to make recommendations”. An expert technical advisory group recommended that the general principles for establishing a new quality assurance policy on diagnostic products for the Global Fund should be: that the quality of diagnostics cannot be compromised, quality assurance is required at all levels of the supply chain, and the legal requirements of the countries of manufacture and use must be observed.

The technical advisory group also proposed that the framework for designing a quality assurance policy should consist of:

- defining minimum standards;
- promoting adherence to guidelines and protocols of recognized international technical agencies;
- building on principles of good procurement practices and the concept of “total cost of ownership”;
- defining a phased approach for implementation of the policy; and
- establishing a mechanism for monitoring and evaluating implementation of the policy.

The Secretariat of the Fund is preparing a quality assurance policy, in collaboration with the expert technical advisory group and consultations with partners, which will be proposed for recommendation to the Board at its 2010 meeting.

4. HRP-2 antigen polymorphism, including gene deletion: effects on performance and product- and lot-testing of rapid diagnostic tests: Q. Cheng

The results of RDTs depend not only on the quality of the RDT itself but also on the affinity of the antibodies on the device to the target antigen. Significant structural diversity has been observed in the antigen most commonly targeted by *P. falciparum*, HRP-2, which may affect affinity.

Sequence repeats and HRP-2 classification

The primary structure of HRP-2 is classified according to a value calculated from the numbers of common type 2 and type 7 amino acid-repeat sequences in the protein, into type A (≥ 100 repeats), type B (50–100 repeats) and type C (≤ 50 repeats). The primary structures of aldolase and pLDH have been shown to vary minimally within species.

No clear correlation was found between HRP-2 structure and detection rate in the 41 products and 79 wild-type *P. falciparum* isolates used in round 1 product-testing. Only the level of antigen was clearly correlated with the positivity or negativity of RDT results. This may be due in part to the effect of structural variation itself on antigen quantification by ELISA.

P. falciparum and HRP-2 gene deletion

A large proportion of isolates collected in Peru from patients for the WHO/FIND/CDC specimen bank and from patients in a retrospective study lacked HRP-2 (or HRP-3) genes; however, this phenomenon has not been seen outside South America. Therefore, HRP-2-specific RDTs may be unsatisfactory for South America, as they may generate false-negative results. While more sampling should be done elsewhere, especially in the Indian subcontinent, to rule out the presence of HRP-2 deletions, only in South America does the prevalence of such gene deletions appear to be significant, with none seen in 450 samples collected elsewhere.

Key points

- While aldolase and pLDH are highly conserved within species, significant structural diversity was observed in parasite HRP-2 in different *P. falciparum* isolates.

- The results of round 1 product-testing revealed no clear correlation between HRP-2 structure and detection rate, but showed a clear correlation between antigen level and the positivity or negativity of RDT results.
- A large proportion of *P. falciparum* isolates in the Amazon region of Peru were found to lack HRP-2 or HRP-3 genes, indicating that HRP-2-specific RDTs are not reliable for detecting *P. falciparum* infections in the region.

5. Antigen quantification and concentration: implications for product- and lot-testing of rapid diagnostic tests: P. Chiodini, S. Jones

The concentrations of the three target antigens (HRP-2, pLDH and aldolase) were analysed by quantitative ELISA in both the phase-I (culture) panel and the phase-II (wild-type) panel (Tables 2–4). The wild-type product-testing panels shown in Table 3 exclude samples at the upper and lower 5 percentiles of antigen concentration. Future panels will be maintained with similar limits.

The cultured samples consist of *P. falciparum* samples, mostly from the African continent. Twenty of these samples comprise the phase-I challenge panel. They are cultured in O+ blood and diluted in O+ sera, and are made up in high (2000 or 5000 parasites per microlitre) and low (200 parasites per microlitre) dilutions.

Key points

- Patient-derived (wild-type) samples generally have higher antigen concentrations than culture-derived samples.
- The antigen concentration ranges widely at the same parasite density, more so with HRP-2 and pLDH, but all show high variability; aldolase levels remain consistently low.
- The average concentrations of pLDH and aldolase are higher in vivax than in falciparum samples.

TABLE 2. Antigen concentration (ng/ml) in the phase-I (culture-derived) panel at 200 parasites per microlitre

	pLDH	HRP-2	Aldolase
Mean	2.7	9.1	0.9
Median	2.6	4.8	0.7
Standard deviation	2.2	18.5	0.5
Minimum	0.0	0.0	0.2
Maximum	8.3	79.9	2.0

TABLE 3. Antigen concentration (ng/ml) of the phase-II (patient-derived or wild-type) *P. falciparum*-positive panel at 200 parasites per microlitre

	pLDH	HRP-2	Aldolase
Mean	15.3	15.3	2.0
Median	12.0	9.6	1.6
Standard deviation	11.3	17.0	1.7
Minimum	47.2	73.7	9.9
Maximum	0.7	0.8	0.1

TABLE 4. Antigen concentration (ng/ml) in the phase-II (patient-derived) *P. vivax*-positive panel at 200 parasites per microlitre

	pLDH	Aldolase
Mean	17.4	6.7
Median	12.7	6.3
Standard deviation	11.6	2.9
Minimum	5.1	3.2
Maximum	44.4	13.2

6. Positive control wells and target antigen detection levels: H. Hopkins, I.J. González

Positive control wells

Positive control wells are being developed to provide suitable, standardized references for assessing the performance of malaria RDTs in remote health-care settings. The product specifications are:

- plastic tube compatible with standard blood transfer devices;
- lyophilized HRP-2, pLDH and aldolase antigens;
- concentrations of antigens equivalent to 200 parasites per microlitre;
- mimics blood viscosity after reconstitution with any clean water;
- single use, disposable;
- shelf-life of 2 years in storage at 40 °C;
- not infectious or hazardous;
- low cost (< US\$ 1.00); and
- reconstituted with clean water.

Stability testing of antigens for use in prototype positive control wells at temperatures of 4 °C, 45 °C and 60 °C for 2 months demonstrated good stability but at higher antigen concentrations than required for the final product. The purity and concentrations of recombinant proteins are being evaluated to define the minimal concentrations of antigens detectable by RDTs. The results are expected to become available in 2010.

Evaluation of containers by health workers in Uganda and the Philippines is completed, and job aids have been developed. Further studies will be conducted to assess the acceptability, utility and stability of the positive control wells under field conditions, and integrated training materials for the management of febrile patients will be provided.

Panel of recombinant proteins for lot-testing at regional level

A panel of recombinant proteins is being prepared to evaluate the performance and stability of RDTs at regional or national level, to replace the current cumbersome process of collecting, characterizing and shipping patient samples to regional specimen banks.

The product specifications are being prepared. They will include a 96-well plate and lyophilized antigens, potentially including all or a combination of HRP-2, pLDH and aldolase. HRP-2 types A, B and C and HRP-3 (detected by anti-HRP-2 antibodies), pLDH and aldolase from *P. falciparum* and *P. vivax* and perhaps other species. No data are available about concentrations in other species.

The target product profile includes:

- a range of antigen concentrations equivalent to 200 parasites per microlitre;
- a shelf-life of 2 years in storage at 40 °C;
- not infectious or hazardous; and
- negative controls with appropriate viscosity and flow characteristics.

Current activities include evaluation of recombinant proteins, in relation to protein characterization (SDS-PAGE and ELISA), purity, concentration, detection of different protein concentrations in RDTs and thermostability.

Next steps

- Final selection of recombinants.
- Definition of protein concentrations.
- Evaluation of the panel in the product-testing programme.
- Manufacture of a prototype.
- Evaluation of the prototype at regional level from late 2010.

It is envisaged that recombinant antigen-based lot-testing panels will replace the current format of lot-testing based on frozen parasites and will be available at national reference laboratories and to manufacturers. Simple positive control wells are being prepared as controls suitable for field use in health facilities.

7. Quality assurance of malaria microscopy

Blood slide microscopy in routine care: experience in rural United Republic of Tanzania: H. Reyburn

Approximately 5.3 million blood slides from 12 million patients with suspected malaria are read annually in the United Republic of Tanzania. The average slide positive rate for Giemsa or Field-stained blood slides in this country was estimated by the national malaria control programme in 2004 to be 19.7%. The average cost is US\$ 0.5–1 per slide (including costs for consumables, microscopy, staff training and salaries). Introduction of an effective quality control system, with upgrading and retraining, could double this cost.

Several studies have indicated that routine blood slide results in Africa fail to reach the minimum standards of accuracy, whereas RDTs at the same sites have a specificity of 96% and a sensitivity approaching 95%. In one health centre in the United Republic of Tanzania, “microscopically confirmed malaria” was diagnosed in 37% of 239 patients, but, after expert reading of the same slides, only two were positive, one of which had been diagnosed as negative by the field microscopist.

Inaccuracy in slide reading appears to have been tolerated for many years, as health care providers tend to expect malaria to be the diagnosis for any non-specific febrile illness. The proportion of patients treated for malaria who are found to be slide-negative on expert reading was found to be high in the United Republic of Tanzania, as shown in a number of studies: 30% at high transmission in the integrated management of childhood illnesses¹⁰, 41% in Rufiji district¹¹, 62% in Kibaha district¹², 73% in Ifakara¹³, 95% in Dar es Salaam¹⁴, and 96% in the southern highlands¹⁵. There are no systematic published studies of the causes of poor microscopic results, but they are likely to include poor microscope quality, poor slide staining, unqualified staff, low motivation, high workloads and a desire to please clinicians, who are perceived to expect a diagnosis of malaria.

¹⁰ Tarimo DS, et al. (2001). Malaria diagnosis and treatment under the strategy of the integrated management of childhood illness (IMCI): relevance of laboratory support from the rapid immunochromatographic tests of ICT Malaria P.f/P.v and OptiMal. *Ann Trop Med Parasitol*; 95: 437–444.

¹¹ Rooth I, Björkman A. (1992). Fever episodes in a holoendemic malaria area of Tanzania: parasitological and clinical findings and diagnostic aspects related to malaria. *Trans R Soc Trop Med Hyg*; 86: 479–482.

¹² Nsimba SE, et al., (2002). Case management of malaria in under-fives at primary health care facilities in a Tanzanian district. *Trop Med Int Health*; 7: 201–209.

¹³ Schellenberg DM, et al., (2003). The incidence of clinical malaria detected by active case detection in children in Ifakara, southern Tanzania. *Trans R Soc Trop Med Hyg* 97: 647–654.

¹⁴ Wang SJ, et al., (2005). Rapid urban malaria appraisal (RUMA) in sub-Saharan Africa. *Malar J*. 4: 40.

¹⁵ Reyburn H, et al., (2006). The contribution of microscopy to targeting antimalarial treatment in a low transmission area of Tanzania. *Malar J*. 5: 4.

It is certainly possible to have skilled microscopists performing to high standards in Africa, where numerous research projects exceed 90% positive-negative agreement between first and second independent readers of the same blood slide, and most discordance is found at low density

Few studies have examined the possibility of using microscopy for RDT quality control. In the United Republic of Tanzania, one study of the results of 12 539 pairs of RDT Paracheck and blood slides showed that 17% were unreadable and, of the remainder, many were of poor quality. The overall sensitivity of microscopy was 65% (range: 19–86%) and the specificity was 88%. In a second study, for 3914 RDT–slide pairs stained and read by research staff, the sensitivity was 91%, and the specificity was 74%, after removal of 30% damaged or lost slides. The concordance between the first and the second reader was 78%.

Key points

- Accurate blood slide results are complementary to RDTs and essential for successful parasitological diagnosis of malaria.
- Microscopy in Africa does not reach accepted standards, in spite of written policies for quality assurance of slide reading in many African countries
- Introduction of functioning, effective quality assurance systems for blood slide reading at district level in Africa is a neglected but essential objective for parasitological diagnosis of malaria.

WHO quality assurance for malaria microscopy: experience in selected Asian countries: K. Lilley

A model for assessing the competence of malaria microscopists has been used successfully over the past 7 years by the WHO Western Pacific Regional Office (WPRO) in the Philippines and in some other countries in the South-East Asia, Western Pacific and African regions. Since 2005, WPRO and the South-East Asia Region, with the Asian Collaborative Training Network for Malaria, have collaborated to support competence assessment and quality assurance for malaria microscopy. In this scheme, training is given at national level for senior “national core group” microscopists in cooperation with national ministries of health.

At an informal consultation on quality assurance in microscopy, microscopists and slide validation schemes in Geneva in 2006, assessment methods and grading schemes were endorsed as the WHO model and were used in assessing competence. At a further meeting on quality assurance in malaria microscopy in Geneva in 2008, the assessment

model was revised. So far, 35 competence assessment courses have been conducted in 16 countries, involving over 400 microscopists. The accreditation course is run over 5 days and includes:

- a pre-course theory test (25 questions);
- a pre-course practical test (identification and counting on 14 slides);
- identification and counting on 55 test slides, with 10 min per slide;
- presentations on species identification and counting;
- sessions on counting techniques and accuracy;
- use of the WPRO slide bank, a box of 69 slides per participant; and
- grading according to WHO certification standard.

The accreditation course is based as closely as possible on the slide sets recommended by the 2008 WHO consultation:

Slide set 1 (40 slides): assessment of presence or absence of parasites and species identification; 10 min per slide:

- 20 negative “clean” (not “spiked”) slides
- 20 low-density positive slides (80–200 parasites per microlitre)
- 10 *P. falciparum* slides
- 4 mixed two-species slides (including *P. falciparum*), > 40 parasites of each species per microlitre, co-infecting species according to local prevalence
- 6 *P. malariae*, *P. vivax* or *P. ovale* slides, at least one of each species, ratio according to local prevalence

Slide set 2 (15 *P. falciparum*-positive slides): assessment of quantification; 10 min per slide:

- 3–5 with 200–500 parasites per microlitre
- 9–10 with 500–2000 parasites per microlitre
- 2 with > 100 000 parasites per microlitre

Slide set 3 is used for the pre-course practice test on day 1.

A subset is available, consisting of approximately 25% (14 slides) of the slides in sets 1 and 2. A slide bank prepared by WPRO and the Asian Collaborative Training Network for Malaria is now partly operational, and validation is under way.

Results

Significant improvements were seen during courses, with a considerable increase in scores for both parasite identification and quantification after the programme in most countries. The mean increase in parasite species identification was 11.1%, and the mean increase in accurate parasite counting was 11.5% (highly statistically significant).

Since this analysis was completed, 10 additional courses have been run. Evaluation of participants' performance during the first five courses showed increases in percentage identification of 36, 22, 24, 18 and 21 (mean, 24%) and increases in percentage counting of 27, 19, 17, 38 and 26 (mean, 25%).

Competence assessment is the main goal of the course, as its short duration limits the possibility of increasing competence. These workshops need the support of specific training programmes to further strengthen the competence of senior microscopists.

The future

- Increase the number and frequency of assessments (about every 3 years).
- Prepare the WHO slide sets and promote the capacity of countries to prepare their own.
- Harmonize the assessments with country training objectives and standard operating procedures (perhaps produce WHO standard operating procedures).
- Delete the reference to “expert” and refer only to levels 1–4.
- Use country budgets to support local costs of competence assessments (accreditation courses).
- Expand to other regions.
- Harmonize with activities of other groups.

Malaria microscopy accreditation course in the WHO African Region: J. Carter

Assessments in nine countries that benefited from a programme to improve malaria diagnostics indicate that laboratory staff face serious challenges in microscopy. A model for competence assessment of malaria microscopists was tested during the past 6 years by WPRO in certain Asian countries and, on the basis of this experience, by the WHO African Regional Office, with the African Medical and Research Foundation. Two courses have been completed. The participants in the first course consisted of five technicians and one doctor from the African Medical and Research Foundation, one doctor

from Medical Care Development International, two staff from Hydas World Health (Pennsylvania, United States), two staff from the Ethiopian Health and Nutrition Research Institute and one member of staff from University Cheikh Anta Diop in Dakar, Senegal. The participants in the second course were all technicians and comprised three from the African Medical and Research Foundation, two from the Ministry of Health of the United Republic of Tanzania, two from the Ministry of Health of Zanzibar, two from the Ministry of Health of Uganda, two from the Ministry of Health of Swaziland and one from the Ministry of Health of Liberia.

The standard 5-day course consists of:

- pre-workshop theory;
- pre-workshop practical slide reading test (14 slides);
- presentations and revision of all aspects of malaria microscope diagnosis and reporting;
- examination of 55 slides under test conditions, 10 min per slide;
- review of test slides throughout (with opportunity for discussion);
- presentations of action plans from each country; and
- provision of the WHO malaria microscopy quality assurance manual to all participants.

The workshops did not include “wet” practical sessions.

At evaluation, most of the microscopists were rated at level 3 or 4 (need retraining); none were at level 1, and only three participants in the two courses met level 2. Species identification and parasite counting were both problematic. The constraints identified were lack of availability of slide sets and deficiencies in the slide sets provided, including no *P. ovale* slides, an incorrect result on one slide, inadequate mixed-infection films (only one, with multiple copies), inadequate films with *P. falciparum* counts > 100 000 per microlitre (only one, with multiple copies), poor quality of some thin films, two coverslips mounted (three slides), slides labelled on the wrong side (three slides), crooked coverslip, with the air gap preventing focus (one slide), and wide variation in staining. Other constraints were microscopes of inadequate quality (x 100 lens), participants with varied backgrounds and no recent refresher training, and poor counting proficiency, which pulled down the final scores.

Way forward

The course could be improved by the production of African slide banks, and a proposal and budget are being prepared in collaboration with the WHO African Regional office, the Ethiopian Health and Nutrition Research Institute and the University Cheikh Anta Diop in Dakar.

Training courses should be offered in malaria microscopy, including wet practical sessions, and further accreditation courses should be offered at various sites in Africa. All participants with scores below level 1 should be encouraged to repeat the course annually. Revision of the marking scheme might be considered.

WHO and National Institute for Communicable Diseases (South Africa) external quality assessment programme for microbiology (2005 to the present)

Since 2005, WHO has collaborated with the National Institute for Communicable Diseases of South Africa in an external quality assessment programme, providing African laboratories at central level with external specimens for bacterial meningitis, enteric disease, plague, malaria and tuberculosis microscopy. The aims of this programme are to identify national diagnostic capability and areas of need and to promote laboratory networks. Each year, three surveys are performed with shipments by courier service of simulated specimens (cerebrospinal fluid, stools) and smears or films. As of December 2008, 10 surveys comprising 93 challenges had been conducted.

For malaria microscopy, 10 thick and thin slide sets per survey are provided to 67 national public health or hospital laboratories participating in the programme; return rates of 60–85% have been recorded. The malaria assessment includes the ability to identify malaria species accurately and to count malaria parasites (for *P. falciparum*) accurately and consistently. An agreement between counts of $\geq 75\%$ is considered acceptable.

Conclusions

- Most microscopists enrolled in the programme can detect malaria but have difficulty in species identification, particularly non-falciparum species.
- False-negative results are found more often with low parasite densities.
- False-positive results for “no parasite seen” are received from one to five participants per challenge.
- Parasite counts are performed poorly and are not consistent for identical challenges.
- Most participants now count parasites on a thick film in parasites per microlitre.
- No participants use the “plus” system, and some still do not perform parasite counts.

It is planned to extend the programme to the WHO Eastern Mediterranean Region.

East African regional external quality assessment scheme (2000 to the present)

The East African regional external quality assessment scheme was introduced to the ministries of health of Kenya, Uganda, the United Republic of Tanzania and Zanzibar between 2000 and 2003 by the African Medical and Research Foundation, with support from WHO. The scheme was endorsed at a consensus meeting of the ministries in 2003, which drew up resolutions that included establishing a national quality assurance advisory body in each country to support the national components of the scheme and establishing a regional quality assurance committee. It was agreed that all quality assurance materials and tools would be shared. The African Medical and Research Foundation was appointed as the interim coordinating centre. Progress of the scheme was to be reported regularly to the East African Community headquarters.

Between 2003 and 2006, four documents were produced by technical committees in each country to establish laboratory and clinical standards: standard operating procedures for essential laboratory tests; standard operating procedures for laboratory use by clinicians; standard operating procedures for care and maintenance of laboratory equipment; and a “quality manual”. At the first meeting of the regional quality assurance committee, reference laboratories for the production of pathological materials were selected (two laboratories per ministry); standard operating procedures for the preparation of pathological materials were drawn up; pilot districts and health facilities were selected; and plans were made for two surveys per year in the first 2 years. The pilot study involved 193 facilities (60 in Kenya, 60 in mainland United Republic of Tanzania; 53 in Uganda; and 20 in Zanzibar). Facilities were grouped within five to eight districts, comprising the district hospital, a sub-district hospital, two to three government health centres, one to two faith-based facilities and one to two private facilities. Sensitization workshops were held for all district clinical and laboratory supervisors to discuss the logistics of the scheme and to outline measures and approaches to remedial action.

The scheme covers integrated services at primary health care level and includes materials for haemoglobin estimation, serology for HIV and syphilis screening, blood films for malaria and other parasites, Ziehl Neelsen stain, Gram stain and stool helminths. The materials are accompanied by clinical case scenarios and laboratory, clinical and public health questions. Target values and results are set by requesting the five “best” laboratories in Nairobi to process the materials. Three surveys have been distributed and the results presented. The results are analysed for laboratory, clinical and public health performance and further analysed to determine the effect of numbers and qualifications of laboratory staff, microscope light source and methods for haemoglobin estimation on performance.

The challenges include slow turnaround times and distribution systems to remote areas of the region, decreasing response rates (even though it is a ministry of health, and therefore mandatory, activity) and poor response of clinicians to the questions. In

addition, difficulties have been encountered in setting up a database that can contain the various parameters of the survey design. Future plans include extending the scheme to cover more laboratories in each country and to other countries in the region, primarily Rwanda and Burundi; increasing the range of specimens in the testing panels; and eliciting financial support from the involved governments to ensure sustainability.

Key points

- Approved malaria microscopy accreditation programmes are essential for assessing the competences of national technical staff and provide a framework for strengthening malaria microscopy skills.
- External quality assessment schemes can be delivered effectively at all levels of the health-care system and can improve laboratory performance over time.
- External quality assessment schemes must be endorsed by ministries of health to ensure full participation and to ensure that the results are used to guide policy.

IV. Clinical significance of density of *P. vivax* and *P. falciparum*

1. Parasite density in sub-Saharan Africa, Asia and South America: M. Aidoo, M. Chang, Q. Cheng, O. Gaye, N. Karunaweera, J. Luchavez, P. Marchesini, I. Mueller, B. Ogutu

In order to better understand the clinical and programme implications of the variable sensitivity of RDTs at low parasite densities (<200 parasites per microlitre), parasite densities in diverse epidemiological settings were reviewed.

Thirty-three studies or surveillance databases were available. We excluded data sets that systematically included only samples above a certain parasite density, that aggregated data for all *Plasmodium* species, that aggregated parasite density measurements with different thresholds, that included asymptomatic people or that were conducted only in highland areas with a distinct malaria epidemiology. Eighteen studies were eligible for inclusion in the final review, of which six were in regions outside Africa (Cambodia, Papua New Guinea, Peru, the Philippines, Sri Lanka and Thailand) and 12 were conducted in sub-Saharan countries (Angola, Kenya, Mozambique, Senegal, Uganda and the United Republic of Tanzania). The aspects of the studies that were examined were: objective, sampling method, population included, period of data collection, laboratory method for slide preparation, including any quality assurance measures, method for calculating parasite density, and the *P. falciparum* and *P. vivax* (for non-African data) parasite densities. Information on local transmission intensity and coverage with malaria interventions at the study sites was also sought; as this information was frequently not available, it was not possible to stratify the studies according to local transmission data. Instead, the studies were grouped regionally, corresponding generally to malaria prevalence categories of low–moderate and moderate–high. The studies had different primary objectives: estimating malaria prevalence or incidence, assessing malaria diagnostics performance, monitoring trends in confirmed malaria cases or evaluating drug efficacy. The definition of “symptomatic” varied across the studies, but all included the symptom of fever or history of fever. For this review, in the household surveys, “symptomatic” was defined as “history of fever within the past 2 weeks” in order to compare data sets.

The studies in sub-Saharan Africa covered the years 2002–2009. The periods covered by the studies in the Pacific, Asia and South America were more heterogeneous: most studies covered 1999–2008, except for the data sources in Sri Lanka, which reported parasite densities for both 1990–1993 (Kataragama) and 1990–2009 (Colombo). Parasite density data for *P. falciparum* were reviewed for all countries; *P. vivax* was included for countries in the Pacific, Asia and South America.

The technique for slide preparation was similar in all studies: 2–10% Giemsa stain was used for a thick film with or without a thin film preparation. In most studies, a slide was classified as ‘negative’ if no parasites were found after examination of 100 high-power fields. The methods for estimating parasite densities were also similar in most studies: by counting parasites per 200 or 500 white blood cells and calculating the number of parasites per microlitre, on the assumption of 8000 white blood cells per microlitre. The method for calculating parasite density differed in Sri Lanka, where parasites were reported as a percentage of red blood cells; the conversion factor for parasites per microlitre was 4.3×10^6 . Quality control of microscopy readings varied; however, most studies included a quality control system of two independent expert readings for every slide or re-reading 10% of slides. There was no standard definition of “expert microscopist”.

In the 12 studies in countries with moderate-to-high malaria prevalence (Angola, Kenya, Mozambique, Papua New Guinea, Senegal, Uganda and the United Republic of Tanzania), the proportions of *P. falciparum* parasite densities < 200 parasites per microlitre ranged from 0% to 21%. The proportions of low parasite densities differed for patients seeking care at health facilities and those who were “symptomatic” (having a fever in the past 2 weeks in a household survey): the proportion of parasite densities < 200 ranged from 0% to 7%, with one outlying data point at 17%, in health facility studies and from 19% to 21% in household surveys. This difference may be because the nonspecific symptom of fever was due to a cause other than parasitaemia in the community or that the household surveys identified a higher proportion of early disease, with lower parasitaemia, before the time that a person is likely to seek care at a health facility. Thus, on average, the proportion of the population of all ages that seeks care at health facilities for malaria parasite densities < 200 is estimated as approximately 5% in countries with moderate-to-high malaria transmission. In the studies in sub-Saharan Africa that included parasite densities in sufficient numbers of children (aged < 5 and 1–10 years), a similar range of parasite densities < 200 was found (1.5–8.0%).

The studies in areas with low-to-moderate malaria transmission were more heterogeneous and fewer. Because the methods used for the studies in Peru and Thailand were consistent with the objectives of this review, the estimate of the parasite density threshold was based mainly on data from these studies. On average, the best estimate of the proportion of symptomatic people with parasite densities < 200 seeking care at a health facility was in the range 5–10% for *P. falciparum* and approximately 15% (range, 8–22%) for *P. vivax*.

Figures 1–3 show the cumulative proportions of parasite densities at thresholds of 100, 200 and 500 parasites per microlitre in the studies included in the final review. The study types are designated as household surveys, malaria indicator surveys, health facility cross-sectional studies and surveillance databases (health facilities), and cohort study at a health facility. The sample size (*n*) covers all study participants with parasitaemia and with clinical symptoms as defined in the study. If the study enrolled only children, the age ranges are indicated (< 5 or 1–10 years).

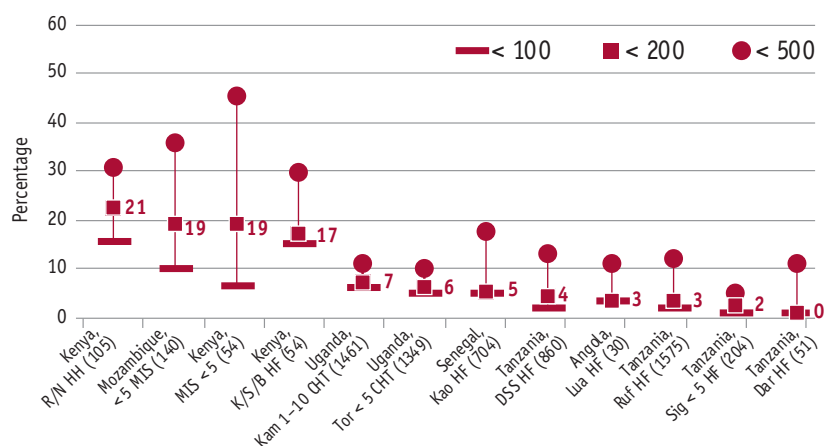


Figure 1

Cumulative proportions of symptomatic patients with *Plasmodium falciparum* parasite densities below 100, 200 and 500 parasites per microlitre: sub-Saharan Africa

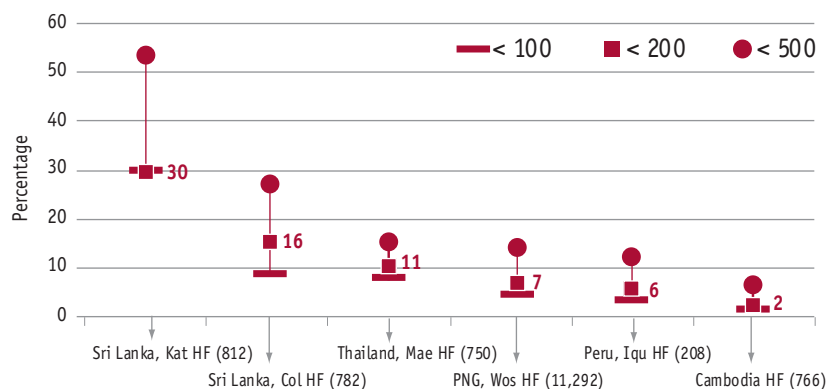


Figure 2

Cumulative proportions of symptomatic patients with *Plasmodium falciparum* parasite densities below 100, 200 and 500 parasites per microlitre: Pacific, South and South-East Asia, South America

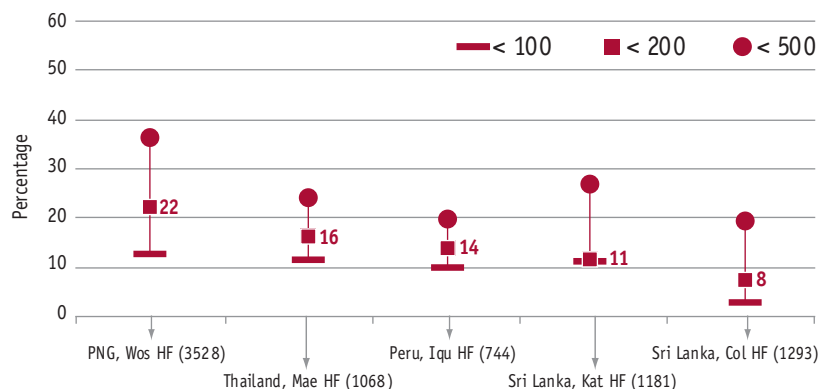


Figure 3

Cumulative proportions of symptomatic patients with *Plasmodium vivax* parasite densities below 100, 200 and 500 parasites per microlitre: Pacific, South and South-East Asia, South America

2. Desirable sensitivity of microscopy and rapid diagnostic tests for case management of falciparum malaria

Both microscopy and RDTs have limitations for detecting very low parasite densities. Their usefulness in case management therefore depends on the frequency and clinical significance of cases in which the parasite density is undetectable with these tests. This section covers data on the thresholds of parasite density at which malaria disease becomes significant.

P. falciparum pyrogenic threshold in naive individuals: Q. Cheng

There appears to be an association between *P. falciparum* parasite density and the threshold for onset of fever in malaria-naive patients. Studies of patients infected with *P. falciparum* as treatment for neurosyphilis, and of healthy volunteers, have shown that the pyrogenic threshold varies with the strain of *Plasmodium* and with host ethnicity; the level of parasitaemia that triggers fever appears to be lower in whites than in black Africans. Moreover, in untreated infections, the parasitaemia associated with the second fever episode is significantly higher than that which causes the first fever.¹⁶

Key points

- *P. falciparum* parasite triggers a fever episode when its density reaches the pyrogenic threshold.
- The pyrogenic threshold varies according to the strain of *Plasmodium*, host ethnicity and immune status.

Parasite density at community and health facility level in areas with low and moderate malaria transmission in the United Republic of Tanzania: V. d'Acremont

Studies have been conducted in areas of low and moderate malaria transmission, with entomological inoculation rates of 1.3 (< 5 parasite prevalence = 2%) and of about 100 (< 5 parasite prevalence = 16%), in both community- and health facility-based surveys.

¹⁶ Gatton M, Chang Q. Evaluation of the pyrogenic threshold for *P. falciparum* malaria in naive individuals. *American Journal of Tropical Medicine and Hygiene*, 2002, 66:467–473.

A community survey in Kilombero district of asymptomatic children under 5 years of age showed a parasite rate of 12.4% with RDTs and 9.1% with microscopy. In 9.4% of positive cases, the falciparum parasite density was < 200 per microlitre, and the median density was 720 parasites per microlitre. In children of the same age group presenting to a health facility with a history of fever, the positivity rate was 50.9% with RDTs and 30.4% with microscopy. In 1.5% of positive cases, the falciparum parasite density was < 200 per microlitre, and the median density was 37 760 parasites per microlitre. The same type of study performed in Dar es Salaam, an area of lower malaria transmission, showed a similar difference in median parasite densities between asymptomatic individuals (all age groups) and patients presenting in health facilities, but none of the positive cases in either group had a parasite density < 200 per microlitre.

Thus, when malaria transmission declines, the median parasite density in febrile patients decreases. When the population has no remaining immunity, the median parasite density in febrile patients is very low. These data did not allow determination of a parasite density threshold for WHO product-testing, as the RDT indicated malaria in many parasitaemic patients who were negative by microscopy. The sensitivity of the commonly used RDT has proven to be high enough for travellers in spite of the very low densities in this patient population. A strategy based on RDT as the sole test has shown to be safe. Microscopy (of high quality) has always been accepted as a safe strategy for patients, irrespective of the level of endemicity or immunity; therefore, RDTs should have a sensitivity that is not inferior to that of microscopy.

Key points

- When malaria transmission declines, the median parasite density in febrile patients decreases, to very low densities when there is no more immunity.
- The sensitivity of commonly used RDTs is high enough for travellers in spite of the very low densities in this patient population.
- For one century, high-quality microscopy has been accepted as a safe strategy for patients, irrespective of the level of endemicity or immunity. The sensitivity of RDTs should therefore be equivalent to that of microscopy.

Use of rapid diagnostic tests at primary care level in Zanzibar:

A. Bjorkman, A. Mårtensson, M. Msellem

A study was conducted in February–August 2005 to assess the effect of RDTs on treatment and health outcomes among 1887 febrile patients attending four primary health care facilities in Zanzibar. Patients of all ages with a history of fever within the past 48 h and symptoms compatible with malaria were randomly assigned to clinical

diagnosis or clinical diagnosis followed by parasitological confirmation with RDT, in a weekly cross-over design. The RDT used for the study was Paracheck *Pf* (Orchid BS, India), which is based on detection of HRP-2. In addition, blood slides were taken on days 0 and 14 of the study from all patients. The proportions of patients in whom malaria was diagnosed with the three methods are presented in **Table 5**.

TABLE 5. Proportions of febrile patients in whom malaria was diagnosed by clinical diagnosis (CD) plus rapid diagnostic test (RDT), clinical diagnosis alone and microscopy, by age group

Diagnostic test	< 5 years	5–15 years	> 15 years	Total
CD + RDT	228/544 (42%)	93/210 (44%)	40/251 (16%)	361/1005 (36%)
CD alone	423/503 (84%)	169/196 (86%)	160/183 (87%)	752/882 (85%)
Microscopy	347/1047 (33%)	128/406 (32%)	50/434 (12%)	552/1887 (29%)

Msellem MI, et al., (2009). Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar: a crossover validation study. *PLoS Med.* 6:e1000070.

The overall impact of different diagnostic approaches on medicine prescriptions (ACTs and antibiotics) on re-attendance at health facilities and on the overall cost per patient are presented in **Table 6**.

TABLE 6. Impact of diagnostic approach on medicine prescriptions, re-attendance at health facilities and overall cost per patient

	Clinical diagnosis + RDT (<i>n</i> = 1005)	Clinical diagnosis alone (<i>n</i> = 882)
ACT prescriptions	36%	85%
Antibiotic prescriptions	37%	27%
Re-attendance at health facilities	2.5%	4.9%
Cost per patient (US\$)	2.47	2.37

ACT, artemisinin-based combination therapies

No deaths or severe malaria were found among children followed for 2 weeks. Of the patients in the clinical diagnosis plus RDT group, 26 had been malaria-positive on day 0 by microscopy and not treated with ACTs (because they were RDT-negative). Of these, five patients (19%) had parasitaemia during follow-up. One spontaneously re-attended the health facility and was found to have 32 parasites per microlitre, while four patients were found positive at day 14 on a scheduled follow-up visit and had parasite densities of 32, 85, 360 and 47 000 per microlitre. Of the patients in the group assessed

by clinical diagnosis alone, only two were positive on day 0 by microscopy and not treated with ACTs (as they were considered not to have malaria on clinical diagnosis).

Thus, RDTs may be useful to improve the clinical management of patients presenting with fever in primary health care facilities, as they resulted in better health outcomes and unchanged costs per patient treated.

RDT use at community level in the United Republic of Tanzania: M. Mubi, A. Janson, M. Warsame, A. Mårtensson, M.G. Petzold, B. Ngasala, G. Maganga, K. Källander, L. Gustafsson, A. Massele, G. Tomson, Z. Premji, A. Björkman

A similar study was conducted with RDTs and ACTs by community health workers in five villages in Kibaba District in March–August 2006, in a weekly cross-over design to compare clinical diagnosis alone with clinical diagnosis followed by RDTs to confirm the diagnosis. Of 3005 febrile patients who consulted 22 community health workers, 2930 were enrolled and analysed on days 3 and 7 for health outcomes and were followed-up to day 28 to assess mortality. With RDT, 50.3% were positive, and 53.2% were treated with ACTs. The proportion considered to have malaria among those assessed by clinical diagnosis alone was 96.5%, and the proportion treated with ACTs was 98.5%. Only 33.2% were positive by microscopy; however, the reading was affected by staining problems, such as autofixation of thick films in the field.

The proportion of patients referred to the nearest health facility was higher among the patients assessed by RDTs (10%) than those assessed by clinical diagnosis alone (1.8%). The proportion of patients referred to the nearest health facility during the first week of follow-up was similar: 7.1% in the RDT group and 9.9% in the clinical diagnosis group.

Four deaths occurred in this study: two children with a diagnosis of malaria and treated with ACT, with 33 000 and 54 000 parasites per microlitre on day 0; and one child and one adult who were negative on both RDT and microscopy and therefore referred. Two of the patients not treated with ACTs had been RDT-positive on day 0. Another 32 patients had been RDT-negative, but blood slide-positive by microscopy. All these patients recovered, but two had to be treated with ACTs during follow-up.

The study suggests that RDTs in the hands of community health workers can lead to early, well-targeted ACT treatment at community level.

Key points

- At primary health care level, the use of RDTs improved the targeting of ACTs to true malaria patients and improved the health outcomes of children presenting with uncomplicated fever.
- Community health workers safely used RDTs to target ACT to malaria patients, and there was no evidence of adverse health outcomes in either study.
- The increased cost for RDTs was equal to the expense for unnecessary treatment with ACTs.

Safety of withdrawing antimalarials for febrile children with a negative rapid diagnostic test result in areas with moderate-to-high malaria transmission in the United Republic of Tanzania: V. d'Acremont

A study was conducted of children presenting for a first consultation at a health centre with a history of fever in the past 48 h in a moderately endemic area (Buguruni Health Centre, Ilala municipality, urban Dar es Salaam) and in a highly endemic area (Signal Dispensary, Kilombero district, Morogoro rural area). The characteristics of the study populations at the two sites are given in **Table 7**.

TABLE 7. Characteristics of study populations in urban and rural United Republic of Tanzania

Characteristic	Dar es Salaam	Signal
Total admitted	300	700
Girls/boys	143/157	341/359
Median age (months)	28	24
Mean axillary temperature (°C)	38.4	38.7
Positivity rate (%)	14	51

The consultation was performed routinely by clinicians at the two health facilities, the only request being not to prescribe an antimalarial agent if the RDT result was negative. On day 7, all patients were visited at home to assess their recovery. If they were negative on day 0 and still sick on day 7, the RDT was repeated, and the patients were visited again on day 14.

Two of the 603 children who were negative on day 0 died. Both were still negative by RDT and microscopy upon hospital admission and are therefore thought to have died from other causes. One child with an RDT-positive result on day 0 developed severe anaemia and was hospitalized on day 7. Four children who were RDT-negative on day 0 were hospitalized on days 2, 4, 7 and 7 for non-malaria illness (severe sepsis with exanthema, severe pneumonia, gastroenteritis with severe dehydration and severe anaemia without fever); they were all still negative by RDT and microscopy upon hospital admission. Three children became RDT-positive during follow-up, on days 2, 4 and 7; all lived in the Signal area. The RDT-positivity in these three cases after a negative result on day 0 was attributed to: *i*) low-density malaria infection fluctuating around the threshold of detection of RDTs, *ii*) symptoms and signs appearing before parasites can be detected in blood by conventional tests and *iii*) a “new” malaria infection. Interestingly, the rate of cases with an initial negative result that became positive later was similar to the rate found in a previous study based on microscopy¹⁷ and to the rate of new infections over this period predicted by passive case detection in another study in the area¹⁸ and therefore due to new infections rather than infections missed on initial testing.

There were no deaths or sequelae due to malaria among children with RDT-negative results on day 0, supporting the conclusion that the use of RDT in children under 5 years is safe, irrespective of the level of endemicity.

Key points

- In a prospective study to assess the safety of withholding antimalarials from febrile children with a negative RDT, no deaths or sequelae due to malaria occurred.
- The strategy of RDT diagnosis and treatment upon result is safe for children under 5 years, irrespective of the level of malaria endemicity.
- The amount of evidence required to change policy is arbitrary, but this study provides strong evidence, which adds to empirical observations that use of RDTs for children is safe.

¹⁷ Njama-Meya et al. Treatment of malaria restricted to laboratory-confirmed cases: a prospective cohort study in Ugandan children. *Malaria Journal*, 2007, 6:7.

¹⁸ Schellenberg et al. The changing epidemiology of malaria in Ifakara Town, southern Tanzania. *Tropical Medicine and International Health*, 2004, 9:68–76

3. Malaria screening methods for low parasite densities, detection of placental malaria and seroepidemiological surveys

Performance of rapid diagnostic tests, microscopy and polymerase chain reaction in screening for malaria in low-transmission areas in Cambodia: F. Arie

Malaria transmission in Cambodia is limited to the forest areas, and is controlled by an effective national malaria programme. Most areas are hypoendemic, but there is a long history of resistance to antimalarial drugs. In this context, several studies were conducted to compare the performance of microscopy with molecular diagnosis and RDTs with microscopy or PCR. The most sensitive method recommended for this context is molecular screening with mitochondrial gene amplification (>20 copies per parasite), with an expected sensitivity of one trophozoite per microlitre. Molecular diagnosis increases sensitivity, especially for *falciparum* infections.

In this study, RDT was less sensitive than microscopy (**Table 8**). Results obtained in the national malaria survey showed 2.5 times more positive samples with molecular biology than with microscopy, i.e. 217 microscope-positive and 538 PCR-positive slides (**Table 9**). In the same survey, molecular diagnosis of malaria allowed identification of 13 malaria endemic villages (of 76 sampled), which were not detected by RDTs and microscopy.

TABLE 8. Sensitivity and specificity of rapid diagnostic tests (RDTs) for detecting *P. falciparum*

	Number	Percentage (%)
Sensitivity		
Slide <i>P. falciparum</i> -positive	111	Gold standard
RDT positive	96	86.4
Specificity		
Slide <i>P. falciparum</i> -negative	521	Gold standard
RDT negative	513	98.5

A cross-sectional survey in Rattanakiri conducted in 2001 and 2002 in eight villages with the highest malaria endemicity in Cambodia showed many asymptomatic carriers, especially in older age groups.

TABLE 9. National malaria survey: samples tested by microscopy and by polymerase chain reaction (PCR)

Microscopy	PCR		Total
	Negative	Positive	
Negative	7307	332	7639
Positive	11	206	217
Total	7318	538	7856

The studies described above indicate that molecular diagnosis is the most sensitive technique, but its use should be restricted to large-scale studies. For the clinical management of malaria, RDTs and microscopy provide adequate sensitivity, but RDTs are more limited for detecting asymptomatic carriers, probably due to the low parasite densities in this population group.

Review of methods for screening blood banks for malaria: A. Moody

The following approaches are recommended to improve the safety of blood transfusions in developing countries with regard to infectious agents like HIV, hepatitis B and C viruses and malaria parasites:

- donor screening to eliminate high-risk donors,
- assumption of responsibility for blood safety by physician giving transfusion,
- adaptation of guidelines for blood administration to local needs,
- avoidance of unnecessary transfusions,
- cost-benefit analysis of blood safety procedures,
- monitoring of blood safety legislation,
- independent authority for blood bank licensing and monitoring and
- promotion of voluntary donation as a public responsibility.

The risks for malaria transmission through blood transfusion concern both *P. falciparum* and *P. vivax*. Transfusion malaria is transmitted by the merozoites, which do not enter the liver cells; for this reason, post-transfusion malaria is rapidly symptomatic, and treating the acute attack results in complete cure.

People living in areas of malaria transmission are semi-immune to malaria, and donors have low-level parasitaemia with no fever or other clinical manifestations. The infective dose of malaria required for transfusion infection is 1–10 parasites per microlitre in a unit of blood.

Various methods have been used to reduce the risk for transfusion-induced malaria in malaria-endemic countries:

- selective screening of donors during the rainy season;
- questionnaire as a preliminary screen to assess travel history, with time spent in a malarious area, time of return, area of residence in relation to malarious area, history of fever, previous blood donations, previous malaria events;
- basic laboratory screening only, by standard microscopic examination of thick and thin Giemsa-stained blood films, RDT (HRP-2 protein, pLDH, aldolase) or fluorescent microscopy (QBC® acridine orange), which can detect 10–100 parasites per microlitre at best;
- antibody detection: in regions of low endemicity and low population immunity, by serological assays to screen for infectious donors; in areas of high transmission, donors implicated in transfusion malaria are usually semi-immune, with low parasite numbers and high antibody levels.

In non-endemic areas, two antibody-detection methods have been used. A sensitive indirect immunofluorescence antibody technique with cultured malaria parasites with insufficient specificity could lead to unjustified loss of blood units. ELISA techniques with both *P. falciparum* and *P. vivax* antigens for antibody capture have proven successful in Europe. They include antibody ELISA kits with recombinant antigen and concentrated parasite extract.

In malaria-endemic areas where donors and recipients have some level of immunity to malaria, the strategies for blood transfusion screening are antigen detection, chemoprophylaxis of donor and recipient and avoiding transfusing patients from low-infective areas with blood of donors from high-infective areas. RDTs are not sufficiently sensitive for screening blood banks for malaria, and questionnaires and physical examination are recommended instead. Several studies have recommended the use of microscopy in combination with a specific ELISA for *P. falciparum* malaria. Real-time PCR can detect clinical cases with a sensitivity threshold of 0.5 parasites per microlitre and can detect asymptomatic infections. Addition of PCR to a standard questionnaire to identify donors with high risk factors is economically more attractive than standard screening procedures, as less donor blood will be inappropriately excluded from use.

Other possible approaches, including potential destruction of parasites in a blood bag with sulfadoxine-pyrimethamine or treating the recipient with sulfadoxine-pyrimethamine, have been evaluated, with variable cost-effectiveness when compared with other methods.

Performance of rapid diagnostic tests in detecting placental infection with *P. falciparum*: H. Tagbor

Diagnosis of placental malaria from a peripheral blood smear or with RDTs is a challenge because antenatal malaria infection is often asymptomatic and parasitized. Red blood cells may be sequestered in the placental microcirculation and not be detectable in peripheral blood. PCR may not be feasible in routine settings, and histology is sensitive but can only be used after delivery to assess the impact of preventive interventions.

Published studies on the performance of RDT in detecting placental malaria show varying performance levels, depending on the comparison, the antigen detected (pLDH, HRP-2, aldolase), the technique used and possibly also quality assurance. The results of different studies in terms of sensitivity and specificity are shown in **Table 10**.

More research is needed to determine whether RDTs in peripheral blood miss placental infections, by evaluating test performance under 'real life' conditions and determining the effect of persistence of HRP-2 and pLDH after treatment. In addition, standard guidelines are needed for systematic measurement of the performance of RDTs in detecting placental malaria infection.

TABLE 10. Sensitivity and specificity of rapid diagnostic tests (RDTs) in detecting placental malaria

Reference	Country	Parasite prevalence (%)			Diagnostic performance		
		Microscopy	RDT	PCR	Sensitivity (%)	Specificity (%)	Gold standard
Mockenhaupt et al. (2006)	Ghana	19	34	53	78	89	Placental smear
		35 P	41	39	56	98	Placental PCR
Mockenhaupt et al. (2002)	Ghana	32 P	38	56	81	97	Placental smear
Onyenekwe et al. (2002)	Nigeria	77	75	ND	ND		
Singer et al. (2004)	Burkina Faso	22.6 P	43.1	51	89	76	Placental PCR
Malhotra et al. (2005)	Kenya	9.4	7.3	37.9	15.3	85	Real-time PCR
		1.7 P	18.8	17.4	55	90	
		9.4	29.3	37.9	40	97	
Tagbor et al. (2008)	Ghana	22	21	ND	96.4	85.5	Peripheral blood smear
Vander Jagt et al. (2005)	Nigeria	7.5	1.1	6.7	ND		
Mankhambo et al. (2002)	Malawi	7.3	24	51.8	70.1	93.8	Peripheral blood smear
					38.4	90.8	Placental smear

 HRP2
  *Plasmodium* lactate dehydrogenase
  PCR, polymerase chain reaction; P, placental; ND, not determined

Review of methods for malaria seroepidemiological surveys: C. Drakeley

Antibodies are markers of exposure over time, acquired and boosted at each infection and gradually lost. Antibodies are species- and antigen-specific and can be detected at low concentrations in order to measure seroprevalence or the magnitude of the antibody response (titre). The two methods most commonly used at present are the indirect fluorescent antibody technique and ELISAs. The indirect fluorescent antibody technique is highly sensitive and multi-antigenic; it is, however, an irregular source of antigen, subject to cross-reactivity and subjective interpretation, and labour-intensive. ELISAs are highly specific to specific antigens, species-specific, rapid, reproducible and relatively cheap; however, they can be too specific, missing cases if there is antigenic variation in the local parasite population. Species-specific tests are probably best done with ELISA.

Seroepidemiology is used to complement other markers of malaria transmission, and the longevity of antibodies allows surveys not restricted to the malaria season. Measuring antibodies in populations can be useful to indicate the level of exposure and changes in malaria transmission, to identify risk groups (by age, travel history, residence) and to identify geographical foci of infection. Seroepidemiological methods are also used for measuring transmission, as the rate of seroconversion (how quickly antibodies are made) is linked to the entomological inoculation rate (number of infective bites per person over a certain period).

The technical limitations of serology include:

- lack of a widespread standardized assay, although several in-house options based on “favourite” antigens are in use;
- lack of convention for controls and defining positivity;
- difficulty in detecting *P. vivax* infections and transmission and hypnozoites;
- variability in the decay of antibody levels in the population, affecting their use to indicate a decline in transmission;
- lack of wide acceptability: interpretability with entomological inoculation rate and parasite rate, integration into the project for mapping malaria risk in Africa; and
- lack of well-defined survey and sample sizes needed for serology to confirm “malaria free status”.

V. Implications of parasite density thresholds for product-testing, lot-testing and positive control wells: J. Barnwell

The initial WHO recommendation made in 1999¹⁹ for RDTs was to promote the design of tests that could detect 100 parasites per microlitre with a sensitivity greater than 95%; the same recommendation was reiterated in 2003.²⁰ In view of the poor correlation between target antigens (HRP-2, pLDH and aldolase) and parasite density and the quantitative limitations of light microscopy for low parasite densities, with greater variability, a WHO consultation in 2004 set the target parasite density for assessment of RDT performance at 200 parasites per microlitre, which is probably equivalent to microscopic diagnostic ability in most places in the field. This reduces the probability of inappropriate rejection of adequately performing RDTs.

A review of the density of *P. falciparum* and *P. vivax* parasites in febrile malaria patients presenting at health facilities showed that 0.8–40% infections involved < 200 parasites per microlitre. The malaria-attributable fraction of such low parasite densities has been the subject of few studies, and so there is still no firm evidence that detecting such levels is critical to case management. The equivalent concentration of target antigens that correlates with the agreed thresholds of target parasite density is being assessed. On this basis, recombinant antigen panels are being prepared for use at a defined concentration for the preparation of positive control wells, which can be used for quality control by health workers in the field and to guide manufacturers in quality control of their own tests.

Cultured parasites tend to have lower antigen concentrations than panels derived from wild-type parasites, as shown in the example from the panel used in the first round of product-testing in **Table 11**.

The RDT product-testing programme is evaluating diagnostic performance against *P. falciparum* cultured parasites and panels of *P. falciparum* and *P. vivax* wild-type parasites diluted at 200 and 2000 parasites per microlitre.

¹⁹. *Malaria diagnosis: new perspectives*. Geneva, World Health Organization, 2000.

²⁰. *Malaria rapid diagnosis – making it work*. Geneva, World Health Organization, 2003

TABLE 11. Antigen concentrations in cultured parasites and in wild-type parasites, panel used in first round of product-testing

Parasite	Antigen	Mean concentration (ng/ml)	
		Cultured	Wild
<i>P. falciparum</i>	HRP2	9.07	18.38
	pLDH	3.26	20.30
	Aldolase	0.91	2.00
<i>P. vivax</i>	pLDH		27.65
	Aldolase		8.96

The evaluation at 200 (and not at 2000) parasites per microlitre allowed a clear discrimination between well-performing and poorly performing tests and should be maintained. As there is no evidence at present of the clinical need for RDTs to detect as few as 100 parasites per microlitre, the minimal threshold for product-testing should be maintained at 200 parasites per microlitre.

In most settings, RDTs should have a detection rate of at least 75% against *P. falciparum* and *P. vivax* wild-type parasites diluted at 200 parasites per microlitre, with “detection rate” defined as a composite index of test positivity, including consistency in inter-test and inter-lot performance. The term is often confused with sensitivity or positivity rate; for this reason, a detection rate of 50% is often interpreted to mean that the test would miss 50% of cases, reducing the confidence of clinicians and managers. In reality, the detection rate as defined in the current RDT product-testing programme is the proportion of times two tests from two different production lots provide four positive results against a target parasite density of 200 parasites per microlitre of panels of wild-type parasites, after testing against panels of *P. falciparum* and *P. vivax*.

Several published studies have shown that tests for *P. falciparum* with a detection rate > 50% in round 1 of the WHO RDT product-testing programme result in improved health outcomes and no risk for the safety of children in different epidemiological settings who are appropriately followed-up. Demanding higher detection rates as an immediate requirement might cause supply problems, as it is not clear that the few manufacturers that are producing RDTs with higher detection rates can supply all the tests required by national programmes in the short term. In order to minimize programme disruption, it is appropriate to continue to use RDTs with moderate performance (> 50% detection rate) in areas of high transmission, while ensuring wider lot-testing.

Lot-testing is still limited: only a small fraction of orders of a very few brands of RDTs procured by selected agencies are currently lot-tested. It will probably be appropriate to

raise the recommended threshold in the future, giving programmes and manufacturers time to adjust.

The use of RDTs with detection rates in the medium range (near 50%) against *P. falciparum* wild-type parasites diluted at 200 parasites per microlitre may not be safe in low-transmission settings. A detection rate of 25% is not sufficient for *P. vivax*, as a significant fraction of patients with *P. vivax* infection are symptomatic at a parasite density ≤ 200 parasites per microlitre. RDTs with higher detection rates should be selected for this malaria parasite species.

Manufacturers should be encouraged to improve the performance of their products progressively, without setting the requirements at too high a level from the start. Information from lot-testing and feedback from field evaluations should be made available to provide additional guidance on selection of RDT products by national programmes. Field testing is problematic, however, in terms of the complexity of trials and sampling and recruiting populations with suitable parasite densities, and lot-testing has limited power for detecting defective RDTs, in view of the limited number of tests assessed. With product-testing, an evaluation is performed on a significantly larger sample size and the system is more effective in discriminating poorly performing tests. In the future, better standardization of RDT evaluations and comparative data will become available from panels of positive control microwells with various concentrations of recombinant antigens.

Ideally, users should select two or three well-performing RDT products on the basis of the results of the WHO RDT product-testing programme and then field-test them to assess ease of use and training requirements under the conditions of intended use. Field performance should be assessed and continually monitored against microscopy, where possible, at sentinel sites. All products should be lot-tested before use. In addition, as part of the guidelines and training materials to be prepared, clinicians should be given appropriate guidelines on managing negative results. As for any other diagnostic test, users should also be informed about the possible limitations of RDTs and advice on how best to use them as a supportive tool for the clinical management of patients.

VI. Programme requirements for strengthening parasitological confirmation of malaria diagnoses

1. Experience in Ethiopia: A. Kebede

The Ethiopian strategy to strengthen laboratory confirmation of malaria diagnoses is part of a “roadmap” for strengthening the public health laboratory system in 2008–2012. This provides operational guidelines for public health and clinical laboratories, for health programmes that rely on the national laboratory system and for donor organizations looking for opportunities to fill resource gaps. It has five strategic objectives:

- to identify, plan and build capacity for integrated diagnostic laboratory services for multiple diseases;
- to integrate HIV diagnostics and monitoring into general laboratory services;
- to build the capacity of regional laboratories;
- to improve the procurement, supply and distribution of laboratory reagents and supplies; and
- to expand and strengthen the national laboratory quality system.

In the national malaria treatment guidelines for health workers in Ethiopia, the management of uncomplicated malaria is supported by clinical or RDT diagnosis at health posts and laboratory confirmation of malaria at health centres and hospitals. Guidelines for training in laboratory diagnosis, covering both microscopy and RDTs, have been distributed as the basis for refresher training at all levels.

Challenges still remain in:

- equipment maintenance,
- coordination and harmonization of laboratory support,
- harmonization of procurement for all diseases requiring laboratory support, and
- expansion of external quality assessment and quality assurance programmes to all laboratories.

Further challenges are due to:

- weak logistics and inventory control systems,
- inefficient budget use by regions and weak follow-up,
- weak referral links among the levels of the health-care system,
- high turnover of laboratory personnel, and
- lack of an effective laboratory monitoring and evaluation system.

Problems associated with the treatment of RDT-negative cases in Ethiopia by health extension workers are linked to the use of HRP-2-detecting RDTs and the relatively high proportion of *P. vivax* malaria. The planned change to combination RDTs to detect all parasite species is expected to change treatment behaviour.

2. Experience in Uganda: R. Ndyomugenyi

The national laboratory system in Uganda provides laboratory services to all health centres, from national referral to level-III health centres. As only 60% of level-III health centres have the capacity for laboratory diagnosis of malaria, due to lack of microscopes, personnel or infrastructure, RDTs are being introduced to allow parasitological confirmation of malaria diagnoses when microscopy is not feasible or non-functional.

The malaria diagnostic policy is part of the national malaria prevention and control policy, which includes all malaria intervention strategies. According to the national policy, all suspected cases of malaria should be confirmed by microscopy or RDT. Only at community level should malaria treatment be provided on the basis of clinical suspicion alone. Malaria diagnosis is provided free of charge in public health facilities.

Numerous in-country assessments of multiple RDT products were conducted; after national expert review, the Ministry of Health selected HRP-2-detecting RDTs. Training in RDT use has been provided to 794 health workers in six of 80 districts. RDT job aids and training manuals were adapted, pre-tested and adopted. Furthermore, 345 laboratory personnel were re-trained in malaria microscopy. The challenges met in training are shown in **Table 12**.

Supervision in all districts is conducted quarterly by the national control programmes and the Central Public Health Laboratory, while district laboratory personnel supervise the peripheral health units. Funding for supervisory visits is limited, and difficulties have

been found in harmonizing supervision by the Central Public Health Laboratory, the national malaria control programme and the tuberculosis programme.

TABLE 12. Difficulties encountered in training personnel in malaria diagnosis

Diagnostic	Training component	Challenges
Rapid diagnostic tests	WHO Paracheck job aides	Irregular supplies of tests
	Immediate follow-up supervision on site	Funding
	Health facility-based; back- to-back; cascade training	Training duration (2 days) Ratio of trainers:trainee (1:10)
Microscopy	Sentinel site microscopy for research	Poor reporting by civil society organizations

At present, microscopes are obtained by the Procurement and Disposal Unit of the Ministry of Health, and microscopy reagents and kits are procured from the National Medical Store; RDTs are currently being procured by WHO and are distributed in a “push” system for two procurement cycles. The procurement and supply management of RDTs is difficult, particularly in quantification at all levels, delays in processing procurement payment and late deliveries at district level and lower health-care units.

In order to monitor the quality of microscopy, a national external quality assurance system is in place, managed by the Central Public Health Laboratory. RDT quality is assured by post-purchase lot-testing.

Routine health information data from outpatient departments in Kisoro and Rukungiri districts, where malaria is hypoendemic (parasite rate, $\leq 10\%$), showed a large reduction in the proportion of cases in all age groups diagnosed as malaria after the introduction of RDTs. Only 11–12% of patients with negative RDT results were treated with anti-malarial medicines.

The challenges faced in strengthening laboratory confirmation of malaria diagnoses are sustained funding; frequent stock-outs of both laboratory supplies and RDTs; difficulties in training, especially in quality assurance and poor public–private diagnostic service linkage.

3. Experience in the Philippines: J. Luchavez

Policy and guidelines for malaria diagnosis were established by a technical working group subcommittee on diagnosis of the National Malaria Control Programme and were consolidated after consultation with programme implementers in the regions and provinces and with our partners: WHO, the Asian Collaborative Training Network for Malaria, the Research Institute for Tropical Medicine, representatives of nongovernmental organizations and academia.

All patients in endemic areas with fever or symptoms of malaria are tested in hospitals (public and private), rural health units (municipal level) and at village level, where there are either village health centres (*barangay*) with a trained microscopist or health workers trained in using RDTs.

RDTs were introduced in 2003 as part of Global Fund and WHO Roll Back Malaria projects in provinces with ≥ 1000 malaria cases per year and in areas where patients had > 2 hours travel (by any means of transport) to the nearest microscopy centre. Municipal health officials identified the number and location of sites for provision of RDTs. RDTs are also used in investigation and control of epidemics and as a temporary measure in selected hospitals with no trained microscopist.

In areas of stable and unstable transmission, both microscopy and RDT are used for diagnosis. If the transmission changes to sporadic low transmission or epidemic-prone, microscopy is the main diagnostic service recommended. In areas with very low malaria transmission that are progressing towards elimination, microscopy is used. In private health care centres, patients are charged a laboratory fee for both microscopy and RDTs. In public health facilities (hospitals and rural health centres), malaria diagnosis is recommended to be a free service, but sometimes a minimal user fee is collected.

Initially, the main RDTs procured were those targeting HRP-2 antigens, as the predominant species in the country is *P. falciparum*. After a relative increase in the proportion of cases due to *P. vivax*, because of the success of the programme in reducing cases of falciparum malaria, the Department of Health decided to procure combination RDTs. Each lot or batch purchased is tested at the Research Institute for Tropical Medicine, one of three global centres accredited by WHO for RDT lot-testing.

Medical technicians and community health workers have been trained in malaria microscopy, while midwives and village health workers were trained in RDT use. External evaluation of the effectiveness of training showed that the performance of the microscopists was generally good and that of village microscopists was comparable to or sometimes better than that of medical technicians. Training of *barangay* microscopists was a problem in view of the long duration of training (5 weeks) and subsequent attrition of trained personnel. Village midwives are responsible for supervising microscopists and

health workers performing RDTs, but this is not happening in most areas. Medical technicians in rural health centres and hospitals are supervised by the facility head. Regional and provincial validators provide on-site supervision at least once a year, using well-standardized supervisory checklists, for a maximum of 20 microscopists per validator.

RDTs are procured through WHO with funding from the Global Fund and distributed to project sites by courier service. The provinces then deliver them to rural health centres and hospitals. There is a limited budget for distribution only up to the municipal level, and distribution to communities depends on the schedule of the midwife, nurse or health worker assigned to the village.

A regular system for slide validation is in place, whereby 100–120 slides per year (20–30 slides per quarter) are submitted for cross-validation. The results are available to the microscopist and facility head or supervisor within 1 month of submission. In 2010, RDT performance will be monitored during supervisory visits, on a quarterly basis, as part of the the Global Fund project. In addition, RDT results obtained in the field will be evaluated by comparison with expert microscopy at sentinel sites.

4. Experience in Senegal: O. Gaye

The antimalarial treatment policy in Senegal was revised in 2004, resulting in recommendation of ACTs as first-line treatment and provision of prompt parasitological diagnosis. RDTs were introduced on a wider scale after a pilot study in 2006 of random introduction of RDTs in 10 districts at health post level, for use by nurses and community health workers. The proportion of malaria diagnoses was 10-fold lower in the intervention districts than in the 10 control districts.

The national malaria diagnostic policy recommends use of microscopy and RDT to confirm a diagnosis of malaria in any patient with malaria symptoms presenting to a hospital or health centre. In health posts and communities, use of RDTs only is recommended. Fees are levied for microscopy diagnosis, while testing with RDTs is free of charge.

After several comparisons of the specificity, sensitivity, stability, cost and ease of use of RDTs, one was selected after evaluation in the wide-scale pilot study in 10 districts.

A total of 2607 health workers (biologists, laboratory technicians, nurses) were trained in both microscopy (thick and thin film preparation, staining, counting, interpretation of results) and RDTs (preparation, interpretation of results), while 3300 community

health workers were trained in the use of RDTs. The challenges met during training included the short duration of training in view of the low educational level of the trainees and the limited availability of technicians to serve as trainers.

Two supervisions per year are planned, with a standardized checklist to assess the competence of technicians, the availability and quality of microscope slides and reagents, the quality of registers and working space. Common problems identified were limited availability of slides and RDTs for cross-checking and weaknesses in reporting.

RDTs are procured every 6 months, and each lot of RDTs undergoes quality control by the medical parasitology department of the University Cheikh Anta Diop in Dakar, which is certified by WHO/FIND for malaria RDT quality assurance. RDTs, supplies and equipment are distributed by car from the national procurement department to the regions and then to districts. A cold chain is present at central level and during transport but not at health centre level.

Quality assurance of microscopy is based on slide validation: 10 positive and negative slides are cross-checked at regional laboratories, with coordination with the medical parasitology department at the University. During the 6-monthly supervisory visits, 20 negative and positive slides are cross-checked by staff at the University and the national malaria control programme, and remedial action (retraining or replacement) is taken on the basis of the findings.

The performance of health workers in using RDTs is monitored by direct observation during supervision. The accuracy of RDT results is validated by comparing 20 positive and negative slides against the corresponding blood slides read by the supervisor.

After the introduction of RDTs, there was a large reduction in the consumption of anti-malarial medicines: the national malaria control programme reported consumption of 1 500 000 treatments in 2006 and 320 000 in 2008, with a similar decrease in malaria morbidity (**Figure 4**).

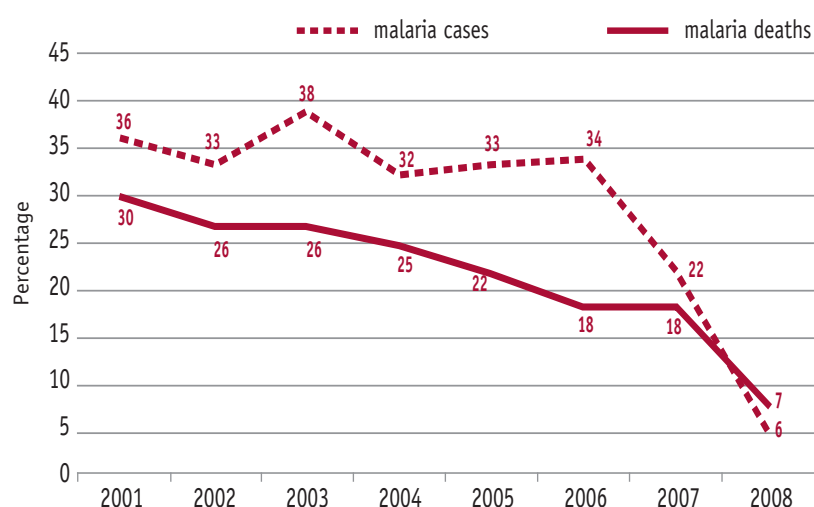


Figure 4

Changes in proportional malaria morbidity and mortality between 2001 and 2008, Senegal

Sources: RBMME/PNLP
Dec. 2008

5. Experience in Zambia: E. Chizema-Kawesha

Malaria is endemic throughout Zambia, but the number of cases has been reduced by targeted interventions such as indoor residual spraying, insecticide-treated nets and new diagnostic and treatment approaches. In 2009, a new diagnosis policy was introduced, which recommends parasitological confirmation of all suspected cases of malaria, without exception, and treatment according to the test results. The policy recommends use of both microscopy and RDT, depending on the circumstances, and free testing and treatment in rural facilities.

A nationwide inventory of laboratory facilities conducted in 2004 showed that 19% of health facilities had functional microscopy. RDTs were introduced in 2005 in places where microscopy is not available. The National Malaria Control Centre initially selected one RDT, but two brands are now in use, since a country evaluation of 10 brands.

All health workers and community health workers will be trained in a phased train-the-trainers approach, with RDT job aids, which have been translated into seven local languages. Quality assurance is being instituted with support from the United States President's Malaria Initiative and Improving Malaria Diagnostics. A national quality assurance coordinator is being recruited.

The numbers of both clinical and confirmed cases of malaria are being reduced progressively as a result of effective control interventions, and the reported number of outpatients with malaria dropped dramatically after introduction of a register of patients tested with RDTs.

Several challenges were faced during implementation of the RDT programme. As RDTs were first introduced during the low-transmission period, most results were negative, and, because of the resulting lack of confidence in RDTs, most health workers did not follow up the results. Furthermore, the training did not provide guidance on management of negative results. Compliance with the new diagnostic guidelines has been variable, and even high-level facilities are placing orders for RDTs. There is severe attrition of trained staff, so that repeated training courses are required. Delayed procurement due to delayed disbursement has resulted in stock-outs at central and peripheral levels. The funding and procurement policies of funding agencies have often countered the decisions of national health authorities, who are expected to procure the cheapest brand, even if it has been found to be unreliable locally. Some agencies require that field studies should be conducted to guide procurement, instead of relying on recent WHO product-testing.

Critical elements for strengthening malaria diagnosis in Zambia will include:

- regular review of diagnostic procedures and enforcement of policy,
- training and supervision of health workers,
- promotion of quality assurance of laboratory diagnoses and
- improved supply chain management.

6. Experience in Zanzibar: M. Msellem

Three main laboratory methods are in use Zanzibar for parasitological confirmation of malaria: standard optical microscopy, acridine orange fluorescence microscopy and RDTs. RDTs are being used in 109 of 138 public health facilities, standard optical microscopy is used in 24, acridine orange fluorescence microscopy in 5 and both types of microscopy in 4 facilities. All private dispensaries and hospitals use standard optical microscopy.

According to the national malaria diagnosis policy, all febrile patients, especially children under 5 years, should be tested before treatment. Primary health care units have RDTs, while district and referral hospitals are equipped with microscopes. Malaria testing is free in public health facilities, while in the private sector, laboratory diagnosis costs patients US\$ 0.4–1.

The Paracheck Pf device is widely used, and a well-conducted study supports the choice of this product in terms of sensitivity, specificity and health outcomes, including assessment of RDT-negative patients.

Training in the use of RDTs is given to the staff of primary health care facilities, and 243 out of 860 have been trained. Training is followed-up after a few months by national supervisors using a checklist for assessing performance. The Zanzibar Malaria Control Programme undertakes supervision every month in collaboration with laboratory technicians from district health management teams in their respective districts. During the supervisory visits, standardized checklists are used to monitor RDT supply and storage, the techniques and procedures used, recording and the test rate. Funds for sustaining RDT supervision are a major challenge.

At present, RDTs are procured by the President's Malaria Initiative through the procedures of the United States Agency for International Development. In the future, Ministry of Health procurement procedures will be used through funding from round 8 of the Global Fund. Preshipment lot-testing is conducted on all lots ordered from the Pasteur Institute of Cambodia and from the Bagamoyo Research and Training Unit in the United Republic of Tanzania. As for all laboratory consumables, RDTs are supplied quarterly by the Central Medical Store through a "push" system. Zanzibar has experienced stock-outs of RDTs and laboratory consumables due to delayed disbursements and shortages of funds.

Slides reading is validated by expert technicians at national level, who cross-check all positive slides and 10% of negative slides. A third reader is involved in cases of discordant results between health facilities and the Zanzibar Malaria Control Programme. No system is yet in place to assess RDT performance by health workers.

The results of RDTs and microscopic observations are incorporated into the malaria surveillance system. Increased use of antibiotic has been observed in health facilities, due to increased reporting of non-malaria febrile illnesses.

7. Experience in Oman: M.S. Al-Zedjal

A national malaria programme was launched in 1991 in order to interrupt malaria transmission. It was based on integrated vector control, early case detection and radical cure of cases. Since then, the Government policy requires that malaria cases be notified within 24 hours, all cases should be confirmed microscopically before treatment, management of malaria (both diagnosis and treatment) is free of charge, and antimalarial medicines are provided only in Government health institutes.

The quality control system for malaria microscopy is based on cross-checking slides, supervisory visits, feedback and training. Slide readings are validated monthly at regional and national malaria laboratories for all positive slides and 10% of negative slides. Feedback is provided immediately after notification of doubtful results, with regard to film preparation, staining quality, species diagnosis and parasite density.

Supervisory visits are made annually or biannually by the regional or national malaria office to all health institutes with malaria diagnostic services. The aspects evaluated during these visits are laboratory management, equipment and reagents, film preparation and staining and competence (rapid assessment).

Training is conducted both by training trainers in regional laboratories and by in-service refresher training. All newly graduated laboratory technicians receive 2-week mandatory training in malaria microscopy during their internship.

8. Programme requirements for strengthening laboratory diagnosis: L. Barat

Quality-assured malaria diagnosis requires:

- comprehensive policies;
- a diagnostic algorithm to ensure that the right people are tested;
- clinical guidelines for managing negative results;
- a highly accurate test;
- good-quality supplies and equipment;
- a reliable supply chain with temperature control;
- appropriate facilities and biosafety measures in place;

- biological waste disposal;
- standard operating procedures to ensure correct performance of tests;
- good training;
- real, regular supervision;
- quality assurance, including quality control; and
- behaviour change to ensure that prescription of treatment is based on results and the patient accepts and follows the prescribed treatment.

Common elements in countries that have been successful in strengthening parasitological confirmation of malaria include comprehensive policies for strengthening public health laboratories, which are integrated into national policies; training that has been shown to improve or develop skills; and evidence of an effect, i.e. a decrease in the number of reported cases and in ACT consumption.

Problems persist in supply chains, equipment maintenance, frequency of supervision and quality assurance. Quality assurance and quality control with adequate feedback to staff are lacking in almost all the countries. Requests for laboratory diagnosis should be monitored to avoid overloading staff, as most laboratories are understaffed even for their current case loads. The confidence of patients in the test results provided may be a significant barrier to appropriate treatment based on test results. The effect on the management of other diseases should also be considered, as the demand for products such as antibiotics, oral rehydration salts and zinc may increase dramatically.

Challenges will also be encountered in ensuring the quality of malaria diagnoses in the private sector, as feasible mechanisms for regular assessment of private sector providers are rarely available. New regulations may be required. The effect of subsidizing the cost of RDTs for clients in the private sector should be evaluated in relation to initiatives to subsidize ACTs (e.g. by the Affordable Medicine Facility for malaria), as lack of a harmonized approach to medicines and diagnostics could encourage less testing.

A number of gaps in knowledge should be addressed by operational research:

- the durability and stability of RDTs in peripheral settings;
- their use by clinicians with regard to who gets tested and whether the test result affects treatment decisions;
- the expectations of patients and caregivers and their acceptance of test results;
- best practices for training and supervision;
- the feasibility of quality assurance and quality control at facility and community level; and
- integration with the management of other febrile illnesses at community level and with strengthening laboratory testing for infections such as tuberculosis and HIV.

Key points

- Some countries have made progress in strengthening malaria diagnosis, including comprehensive national policies and training materials, distributing RDTs and laboratory supplies and providing training. Evidence of effect, such as reduced consumption of ACTs, has been documented in some countries.
- Numerous challenges were identified, including weak supply chains, limited resources to sustain supervision and quality assurance and insufficient human capacity to test all suspected cases of malaria.
- Further research is needed with regard to the durability of RDTs in peripheral settings; the use of malaria testing and adherence to results by clinicians; best practices for training, supervision, quality assurance and integration of malaria diagnostics into laboratory strengthening.

9.WHO draft operational manual for strengthening malaria diagnostic services: P. Olumese

Prompt, accurate diagnosis of malaria is an integral part of effective disease management. Diagnosis with high sensitivity and specificity improves the management of malaria, reduces unnecessary treatment with antimalarial medicines and improves differential diagnosis of febrile illnesses. A diagnosis of malaria is based on clinical criteria (clinical diagnosis) and detection of parasites in blood (parasitological or confirmatory diagnosis).

Clinical diagnosis of malaria is based on the presence or a recent history of fever. Clinical diagnosis alone is not specific, as the signs and symptoms of malaria are nonspecific. High transmission (stable) is indicated by fever or a history of fever in the past 24 h; low transmission (unstable) is indicated by fever or a history of fever in the past 72 h.

Prompt parasitological confirmation by microscopy or by RDTs is recommended in all cases of suspected malaria before treatment is started. Treatment solely on the basis of clinical suspicion can be considered when parasitological diagnosis is not available or is likely to delay treatment, particularly for high-risk groups, e.g. in severe malaria cases or in children under 5 years of age in areas of high transmission.

In several malaria-endemic countries, the quality, availability and use of both microscopy and RDTs remain problematic. These challenges can be addressed through a

comprehensive malaria diagnosis policy and the availability of high-quality diagnostic services to ensure that health workers and patients are confident in the test results. Therefore, national programmes should be established for setting up or strengthening a network of laboratories (diagnostic services), training staff at all levels, communicating and coordinating activities at all levels of the health-care system and ensuring the quality of diagnostic test performance in order to change health workers' practices so that greater reliance is placed on test results for treatment decisions.

The aim of the WHO draft guidelines is to provide operational managerial and technical advice to countries for strengthening or setting up of malaria diagnostic services in accordance with the current WHO guidelines. They are designed primarily for national, provincial and district health managers involved in strengthening malaria diagnosis services. They are not intended to serve as a manual for use by first-line health workers in diagnosing malaria, but rather as global guidance to be adapted to each country's needs.

The guidelines are part of national malaria treatment guidelines, clearly indicating the role of parasite-based diagnosis in malaria case management at each level of the health-care system. Malaria diagnosis should be harmonized with the national laboratory policy for the tests used, deployment of the tests in the health system, the competence required to perform the tests and the elements necessary to ensure the quality of service delivery. The elements of an effective malaria diagnosis policy include:

- harmonization and alignment with the national laboratory policy and other relevant policies for health service delivery;
- standard operating procedures and bench aids for proper diagnosis, including waste disposal and blood safety protection measures for both health workers and patients;
- the availability at all levels of personnel adequately trained to perform RDTs and microscopy;
- high-quality service delivery ensured by a robust, integrated system for quality control and quality assurance, supported by regular supervision;
- confidence of health workers in using laboratory test results to initiate treatment and in providing feedback for external quality control;
- advocacy and community sensitization about the need for a laboratory test before treatment; and
- good logistics for storage and distribution of equipment and supplies, consistent with the national supply management system.

10. Parasitological confirmation of malaria in integrated management of childhood illnesses: W. Were

The strategy for integrated management of childhood illnesses covers the major causes of child mortality in many developing countries: acute respiratory infections; diarrhoea, dehydration, persistent diarrhoea and dysentery; meningitis and sepsis; malaria; measles; ear infections; malnutrition and anaemia. In certain situations, through a country-specific adaptation, other diseases may be included, such as wheeze, dengue haemorrhagic fever, sore throat and HIV infection.

In this system, case management for all conditions includes identification and classification of patients as having either a severe illness that requires pre-referral treatment or immediate referral, a non-severe disease that can be treated in an outpatient department, or a condition that can be managed at home with advice for home care and no medicine. Health workers may send patients for pre-referral treatment, decide on appropriate referral and treatment for non-severe disease, or provide advice about home care and follow-up.

For children presenting with fever, the management principles are to:

- recognize fever associated with severe illness (severe malaria, meningitis, septicaemia, sepsis) from the general danger signs of convulsions, lethargy, unconsciousness, inability to drink or breastfeed or vomiting everything;
- assess all febrile illness from other main symptoms, which may include cough or difficulty in breathing (pneumonia), diarrhoea (dysentery), fever (malaria and measles), ear infection and malnutrition (anaemia);
- refer children who have had fever for more than 7 days for assessment, in order to differentiate it from simple viral fever and other diseases in which the only presenting symptom is fever, and to detect conditions with non-localizing signs that require diagnostic and therapeutic interventions, which cannot be distinguished at a first-level health facility without laboratory diagnostic capacity, such as urinary tract infections, typhoid, osteomyelitis, relapsing fever, tuberculosis and HIV/AIDS; and
- provide appropriate treatment and care, which may include pre-referral treatment, appropriate referral or treatment for non-severe disease at a health facility, advice on home care and follow-up.

The current guidelines for integrated management of childhood illnesses do not recommend parasitological confirmation of malaria with RDT or microscopy at a first-level health-care facility for febrile children presenting with a general danger sign or stiff neck, who need urgent referral. These children should be given pre-referral treatment

with antimalarial medicines to treat severe malaria and appropriate parenteral antibiotics and referred without delay.

For children with non-severe febrile illness, parasitological diagnosis is recommended before treatment if malaria is suspected. In areas of high malaria risk (where the slide positivity rate among febrile children under 5 years seen at a primary level health facility is $\geq 5\%$), parasitological confirmation is recommended for all febrile children, while in areas of low malaria risk (where the slide positivity rate is $< 5\%$), parasitological confirmation is performed only for children with no other obvious cause of fever.

Acute measles can be detected in febrile children presenting with generalized rash if they have red eyes, a runny nose or a cough. There are three major categories of children presenting with fever: 1) fever due to infection with localized signs; 2) fever due to infection with non-localized signs; and 3) fever with rash. The localizing signs that may support a differential diagnosis of febrile illness are listed in **Table 13**.

If the test for malaria is negative, the integrated management of childhood illnesses algorithm recommends treatment with paracetamol for high fever ($\geq 39.5^\circ\text{C}$) and advice to a parent or caregiver on when to return if the child is still sick. If the fever persists after 2 days, the child should be reassessed and the laboratory test repeated. The integrated management of childhood illnesses “fever box” is shown in **Figure 5**.

TABLE 13. Localizing signs for differential diagnosis of febrile illness in children

Diagnosis	Localizing sign
Meningitis	Positive on lumbar puncture Stiff neck Bulging fontanelle Meningococcal rash (petechial or purpuric)
Otitis media	Red, immobile eardrum on otoscopy Pus draining from ear Ear pain
Mastoiditis	Tender swelling above or behind ear
Osteomyelitis	Local tenderness Refusal to move the affected limb Refusal to bear weight on leg
Septic arthritis	Hot, tender, swollen joint
Skin and soft tissue infection	Cellulitis Boils Skin pustules Pyomyositis (purulent infection of muscles)
Pneumonia	Cough with rapid breathing Indrawing lower chest wall Fever Coarse crackles Nasal flaring Grunting
Upper respiratory viral infection	Symptoms of cough or cold No systemic upset
Throat abscess	Sore throat in older child Difficulty in swallowing, drooling of saliva Tender cervical nodes
Sinusitis	Facial tenderness on percussion over affected sinus Foul nasal discharge
Dengue	From epidemic area in at-risk season Joint and muscle pain

Adapted from: *Pocket book of hospital care for children*. Geneva, World Health Organization, 2005, p. 135

Does the child have fever?

(by history or feels hot or temperature 37.5 °C* or above)

If yes, decide malaria risk:	high or low
Then ask:	Look and feel:
• For how long?	• for stiff neck
• If more than 7 days, has fever been present every day?	• for runny nose
• Has the child had measles within the past 3 months?	Look for signs of local bacterial infection
Do malaria test	Test positive:
If no general danger sign	• <i>P. falciparum</i> present
	• <i>P. vivax</i> present
	Test negative

Classify for malaria

Any general danger sign	Very severe febrile disease	Give first antimalarial for severe malaria Give appropriate antibiotics for apparent bacterial cause of fever Treat the child to prevent low blood sugar Give one dose of paracetamol in clinic for high fever (38.5 °C or above) Reer URGENTLY to hospital
Malaria test positive**	Malaria	Give recommended oral antimalarial Give one dose of paracetamol in clinic for high fever (38.5 °C or above) Advise mother when to return immediately Follow up in 2 days if fever persists If fever is present every day for more than 7 days, refer for assessment
Malaria test negative	Fever, no malaria	Give one dose of paracetamol in clinic for high fever (38.5 °C or above) Advise mother when to return immediately
Runny nose present		Assess for other possible aetiological cause of fever***
Other cause of fever present		Follow up in 2 days if fever persists If fever is present every day for more than 7 days, refer for assessment
Any general danger sign, or Clouding of norena, or Deep or extensive mouth ulcers	Severe complicated measles**	Give vitamin A treatment Give first dose of an appropriate antibiotic If clouding of the cornea or pus draining from the eye, apply tetracycline eye ointment Refer urgently to hospital
Pus draining from the eye, or Mouth ulcers	Measles with eye or mouth complications****	Give vitamin A treatment If pus draining from the eye, treat eye infection with tetracycline eye ointment If mouth ulcers, treat with gentian violet Follow up in 2 days
Measles now or within the past 3 months	Measles	Give vitamin A treatment

If measles now or within the past 3 months, classify

If the child has measles now or within the past 3 months	Look for:
	• mouth ulcers; are they deep and extensive?
	• pus draining from the eye
	• clouding of the cornea

* These temperatures are based on axillary temperature. Rectal temperature readings are approximately 0.5 °C higher

** If no malaria test available, classify as malaria

*** Other possible causes of bacterial infection may include: urinary tract infection, typhoid, cellulitis and osteomyelitis

**** Other important complications of measles – pneumonia, stridor, diarrhoea, ear infection, malnutrition – are classified in other tables

Figure 5

Generic version of the proposed IMCI algorithm with inclusion of a malaria test in the assessment and classification of fever, in line with the new WHO recommendations on diagnosis and treatment of malaria

11. Targeting the use of parasitological confirmation of malaria in areas with decreasing transmission: V. d'Acremont

In areas where malaria transmission is decreasing, programmes increasingly need to limit use of malaria tests without missing any cases in health facilities. In order to restrict testing for malaria, clinical predictors of malaria could be used, such as high temperature of short duration, absence of cough, presence of splenomegaly, absence of rash and absence of abdominal pain. If clinical predictors are added to decision-making about testing, the programme must decide how many malaria cases it is prepared to miss for each added predictor and how many RDTs it is worth saving for a malaria case to be missed.

In a recent study of the causes of fever in Dar es Salaam, the predictors of malaria identified were: no runny nose (odds ratio [OR] = 2.7), high temperature (OR = 2.9), no cough (OR = 3.9) and no diarrhoea (OR = 6.0). On that basis, if a decision was taken to stop malaria testing for children who have diarrhoea (“no diarrhoea” was the most strongly negatively associated with malaria), 1.7% of malaria cases would be missed, but, as only 8% of children present with diarrhoea, this would result in a net saving of only 8% of malaria tests. In the same example, if “no running nose” was selected as the clinical predictor for not testing for malaria, there would be a net saving of 27% of tests, but 13% of malaria cases would be missed. These studies of predictors of clinical malaria indicate that no clinical predictor is good enough to restrict malaria testing at present. Universal clinical predictors that are easy to assess are difficult to identify.

Indicators used in some countries include the malaria transmission season (in countries with highly seasonal malaria and a long dry season) and recent history of travel to endemic areas (in non-malarious areas). When the prevalence of malaria is decreasing and approaching elimination, data from health facilities, such as positivity rates, are the most relevant for surveillance. It is at health facility level that an index case can trigger active case detection and intervention, to stop local foci of transmission or epidemics rapidly. In order not to lose the important role of parasitological confirmation of diagnosis in malaria surveillance, it is important to promote wide use of diagnostics, and it is not appropriate to restrict malaria testing too early.

Key points

- A study on the causes of fever in Dar es Salaam and previous studies on predictors of malaria indicate that no clinical predictor is sound enough to restrict malaria testing safely, even in low-endemic areas. Useful, universal clinical predictors that are easy to assess are difficult to find.
- As statistics from health facilities are the most relevant for surveillance when approaching elimination, it is not appropriate to restrict malaria testing too early.

Conclusions and main recommendations

A. Role of diagnosis in malaria control

- Parasite-based diagnosis and effective treatment should be available to all people at risk for malaria, irrespective of their age and the intensity of transmission in the area.²¹
- WHO recommends that a diagnosis of malaria be confirmed by quality-assured parasite-based diagnosis in all cases before treatment is begun.
- Treatment solely on the basis of clinical suspicion should be considered only when a parasitological diagnosis is not available or the results are not available within 2 hours of presentation of the patient.
- The reduction in the prevalence of malaria among cases of fever in many endemic areas associated with increased coverage by control interventions makes it imperative to ensure that diagnostic capacity is in place to guide appropriate treatment, improve fever management and provide the surveillance data necessary to manage programmes and monitor impact.
- It is therefore necessary to ensure access to high-quality diagnosis for patients with malaria-like symptoms seeking care at all levels of the health system, to distinguish malaria from other causes of febrile illnesses and treat the patients accordingly.
- Major reorientation of many malaria control programmes, health services (including laboratory services) and funding agencies is needed to ensure access to adequate malaria diagnosis and treatment. This should include support for the institution and maintenance of quality control, training, logistics, monitoring and other support systems necessary for the delivery of quality-assured diagnostic services.

B. Implications of parasite density for diagnosis of clinical malaria

Conclusions on the distribution of malaria parasite densities

- The frequency of low-density infections is determined by multiple factors, including host immunity, parasite factors, stage of illness and effectiveness of treatment.

²¹. WHO guidelines for the treatment of malaria. Geneva, World Health Organization, 2010.

- The frequency of parasite densities < 200 per microlitre in patients seeking treatment in health facilities varies with transmission intensity and parasite species.¹⁹
- In high-transmission areas, only about 5% of patients with *P. falciparum* malaria have parasite densities < 200 per microlitre.
- In low-to-moderate transmission areas, 5–10% of patients with *P. falciparum* malaria have parasite densities < 200 per microlitre.
- Patients with *P. vivax* malaria present with parasite densities < 200 per microlitre more commonly than those with *P. falciparum* malaria (~15%).
- The frequency of low parasite densities (< 200 per microlitre) is higher in population and household surveys than among symptomatic patients who present to health facilities for treatment.

Implications for use of current diagnostics

Use of diagnostic tests to support clinical management of malaria

- In health facilities, diagnostic tests are used to detect malaria infection among symptomatic patients. Data presented at the meeting indicate that high-quality microscopy and well-performing RDTs can reliably detect parasite densities \leq 200 per microlitre.
- The use of current quality assured diagnostics is safe. Limited evidence from studies conducted in controlled clinical settings indicates that withholding antimalarial treatment from RDT-negative febrile children in low-to-moderate transmission areas does not adversely affect their clinical outcomes when good follow-up is available. These patients rarely return with malaria during follow-up, suggesting that it is unlikely that a malaria infection has been missed. Providers and patients should be informed about the importance of clinical follow-up and counselling of patients with fever or other symptoms, regardless of the results of RDTs.

Use of malaria diagnostic tests in population surveys

- In population surveys, diagnostic tests are used to detect malaria infection in symptomatic and asymptomatic individuals. Because parasite densities are different in symptomatic and asymptomatic people, the minimum performance requirements of diagnostic tests for case management may be different from those for population surveys.
- Currently available good-quality microscopy and RDTs are adequate for surveys to monitor trends in malaria prevalence. Good-quality microscopy and RDTs have different roles in population-based surveys: RDTs enable rapid detection and treatment of infected people during the survey, and expert microscopy at a central laboratory is needed to estimate the prevalence of infection, including very low parasitaemia, after the field work has been completed.

Use of malaria diagnostic tests in population screening in malaria elimination and special containment projects

- The currently available RDTs and microscopy are likely to miss many cases of asymptomatic parasitaemia, and molecular diagnosis is more appropriate in population screening.

C. Selection of rapid diagnostic tests for malaria from the results of the first round of the WHO product-testing programme

WHO currently evaluates the performance of RDTs against two dilutions: 200 and 2000 or 5000 parasites per microlitre. The appropriate threshold for selecting RDTs for clinical management is 200 parasites per microlitre, and not 2000 or 5000 parasites per microlitre, for both *P. falciparum* and *P. vivax* malaria.

Interpretation of WHO product-testing results for procurement of RDTs

- For detecting *P. falciparum* malaria in low-to-moderate transmission areas, it is highly advisable to select RDTs that achieve well above 50% detection rate²² at 200 parasites per microlitre (e.g. 75%).
- For detecting *P. falciparum* malaria in high-transmission areas, the detection rate should be at least 50% at 200 parasites per microlitre. As the extent of such areas is likely to decrease with effective malaria control, detection rates well above this level (or the equivalent thereof) should become the basis for product selection in the future.
- The current WHO interim procurement criterion of a 25% detection rate at 200 parasites per microlitre (in round 1 product-testing) is inadequate to ensure adequate sensitivity for *P. vivax* case management. The selection criteria for *P. vivax*-detecting RDTs should be at least equivalent to those for *P. falciparum*-detecting RDTs.
- In view of the likelihood of inter-lot variation, all lots of RDTs should be tested with a standard equivalent to 200 parasites per microlitre, as part of good procurement practice.

²² The term “detection rate” as used in the WHO malaria RDT product testing programme is a composite index of test positivity and inter-test and inter-lot consistency in performance. It is not equivalent to sensitivity or positivity rate. (See p. 12 of the full report of the Round 1 of WHO/TDR/FIND/CDC malaria RDT product testing for a detailed description of this parameter.) In reporting the results of Round 2, this term will be replaced, but the criteria will be retained as the primary discriminator of test quality.

D. Manufacturing standards for rapid diagnostic tests

- Standards set for manufacturers for product development and parasite or antigen panels should be extended and made more widely available for quality control before lot release. The standards and panels should be equivalent to those used in the WHO product-testing programme.
- As malaria control improves, there will be greater demand for RDTs that consistently have detection rates of at least 75% at low densities (200 parasites per microlitre) of *P. falciparum* and *P. vivax* parasites.
- It will be an advantage for malaria programmes if RDT formats, blood transfer devices and procedures are standardized, to allow standardized health worker training.
- Manufacturers should design RDTs to detect parasite densities well below 200 per microlitre, especially for low-transmission areas where low-density parasitaemia is frequent; however, the clinical significance of specific cut-offs below this level is unclear.
- More combination tests that consistently detect low-density *P. vivax* infections are needed.

E. Implementation of good quality malaria diagnostic programmes

Conclusions on parasitological diagnosis

Microscopy

- There are examples of good quality of microscopy being maintained in malaria programmes with adequate training, supervision and quality control. There is also evidence that microscopy services of grossly inadequate quality are used for malaria diagnosis in the general health services in many countries.

RDTs

- RDTs are used effectively at health facility and community level when there is adequate training and supervision, with provision of job aids, and good-quality RDTs are used.
- Some of the RDTs currently on the market have high performance standards, while others are of unacceptably low standard.

- Most of the RDTs used in the public and private sectors are not checked systematically for quality before and after deployment to the field.
- Both microscopy and RDTs are necessary in most programmes, although the optimum mix will vary with the capacity of the programme. The main criteria for determining the method to be used should be the quality of the diagnosis that can be made at the site of use, the need for consistent detection of parasite densities ≤ 200 parasites per microlitre and the needs of the programme.

Recommendations on requirements to ensure the quality of diagnosis

Both RDT and microscopy programmes must include comprehensive quality assurance.

Microscopy

- Adequate equipment and good-quality supplies (especially staining solutions).
- Accreditation, external quality assessment and slide banks at regional level.
- Systematic training and re-training and structured slide validation and feedback of results to field microscopists at national level.
- Structured supervision, with standardized checklists, standard operating procedures and on-site slide re-checking with immediate feedback.

RDTs

- Good procurement (based on performance, e.g. WHO product-testing).
- Lot-testing before use.
- Appropriate training, instructions and job aids.
- Good supervision and monitoring of use.
- Appropriate transport and storage systems.
- Monitoring of test performance in the field.

Quality assurance

- Quality assurance is an essential component of all diagnostic programmes. It is essential that funding for microscopy services and RDT procurement be accompanied by funding for comprehensive quality assurance (at least 20% of cost of goods).
- Funding is necessary at supranational level for structures and services (external quality assessment, accreditation slide banks, performance testing) to support national diagnostic programmes. This may require reorientation of some funding mechanisms to allow increased funding of global and intercountry quality support programmes.

F. Research needs

- Further studies on the clinical outcomes of patients with suspected malaria with negative results on RDT or microscopy.
- Evaluation, in different transmission settings, of the epidemiological and clinical significance of parasite densities at the lower detection limits of high-performing microscopy or RDTs (e.g. 50 parasites per microlitre).
- Distribution of parasite densities in patients presenting to community health workers in areas of high transmission where community case management programmes are being implemented.
- Use of RDTs as part of a comprehensive diagnostic and therapeutic strategy for the management of childhood fevers.
- Assessment of methods to increase adherence of clinicians and patients to appropriate follow-up of test results.
- Safety and effectiveness of clinical algorithms for use of parasite-based diagnosis in areas with decreasing transmission.
- Evaluation of RDTs for detection of placental malaria infection.
- Evaluation of methods for blood bank screening for malaria parasites.
- Development or evaluation of reliable methods for detecting low-parasite density infections in population surveys.
- Assessment and identification of reliable methods for seroepidemiological surveys in countries approaching malaria elimination and to confirm interruption of transmission.

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