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Malaria Rapid Diagnosis
Making it Work

Meeting Report

20–23 January 2003

WORLD HEALTH ORGANIZATION
Regional Office for the Western Pacific

MALARIA RAPID DIAGNOSIS
Making it Work

Informal Consultation on Field Trials and Quality Assurance on Malaria Rapid Diagnostic Tests

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Acknowledgements

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Glossary/Acronyms

ELISA	Enzyme-linked Immuno-sorbent Assay
GMP	Good Manufacturing Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HRP2	Histidine-Rich Protein 2
ISO	International Standards Organization
Mab	Monoclonal antibody
MRDD	Malaria Rapid Diagnostic Device
QA	Quality Assurance
QC	Quality Control
pLDH	Parasite Lactate Dehydrogenase
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
RITM	Research Institute for Tropical Medicine, Alabang, Muntinlupa, Republic of the Philippines
SOP	Standard Operating Procedure
TDR	UNDP/WB/WHO Special Programme for Research and Training in Tropical Diseases
USAID	United States Agency for International Development
VVM	Vaccine Vial Monitor
WHO	World Health Organization

1 Executive Summary

Early, rapid diagnosis of malaria is gaining increasing importance in health programmes in endemic countries in response to increasing drug costs and recognition of the importance of early, correct treatment to the reduction in malaria morbidity and mortality. Blood-based diagnosis using lateral-flow immunochromatographic tests, commonly called rapid diagnostics tests (RDTs), offers great promise in extending rapid diagnosis to areas where traditional microscopy diagnosis is impractical. Since a previous World Health Organization (WHO) informal consultation reviewed this issue in 1999, there has been a rapid increase in both the use of RDTs and the number products available. Together with this has come the publication of a wide range of field and laboratory trials, often with conflicting or inconsistent results. Large-scale operational use has raised questions over the accuracy of current RDT technology in tropical conditions. As utilization of RDTs is likely to increase rapidly over the next few years, there is a clear and urgent need to address issues of quality of performance and appropriateness of use, particularly in remote endemic areas.

This report details the recommendations of a WHO informal consultation on malaria RDTs held in Manila in January 2003. The 2003 consultation reviewed progress in the development of malaria RDT quality assurance (QA) since the previous consultation in 1999, aimed to define an appropriate path for further QA development, and to clarify priorities for further research necessary to guide the large-scale use of RDTs.

The need for QA systems to maintain the quality of microscopy diagnosis of malaria is well established but the extent of implementation varies widely. Good QA processes for RDTs will greatly enhance their value to populations at risk and to health systems in endemic countries, providing the evidence necessary to permit greater reliance on RDT results as a guide to treatment.

Quality assurance process must become an integral part of RDT budgets and implementation plans. Responsibility for overseeing QA processes, extending from post-purchase testing of RDTs to training and supervision of users and control of storage and transport, should be clearly defined and coordinated from a central

level. A system of regional and referral laboratories, based on standard operating procedures currently being developed by WHO, should be developed. This would test RDTs after purchase and for the duration of shelf-life using quality control (QC) panels prepared from wild-type parasites. A model for this process has been defined. Further research and development are needed on quality control testing closer to the point of use of RDTs, possibly using antigen-containing wells, and on appropriate training and supervision systems for end-users. Affordable temperature monitoring is needed for transport and storage. Quality assurance processes must be transparent, and good communication with manufacturers and end-users during QA development is necessary.

There is also a significant need to improve the quality and flow of information on RDT testing and use and to develop a good evidence base to guide their introduction into health systems. Minimum standards for field trials of malaria RDTs were defined during the consultation and these should be disseminated to assist in the planning and interpretation of field and laboratory trials. There is a place for a large multi-centre phase three field trial to establish the capabilities of a range of RDT products targeted at endemic populations. Further studies are required on operational issues to improve blood-transfer methods and clarity of product instructions. Cost-benefit studies and assessment of the impact of RDTs on treatment seeking and treatment provision will guide implementation, but need to be tailored to local conditions. Guidelines for such assessments should be made available.

Adequate disclosure by manufacturers of technical capabilities, such as heat stability, is essential to guide purchasers in choosing products appropriate for use in remote tropical conditions. In turn, an improved method of disseminating information on available products and planned tendering processes is in the interests of all. A website under development by WHO should facilitate this information exchange and improve dissemination of information and guidelines on other aspects of RDT use.

Malaria RDTs have the potential to significantly improve the quality of malaria management as a complement to microscopy, providing accurate diagnosis in areas where this has been previously unavailable. Success in improving management will depend largely on the support systems set up to ensure continued RDT accuracy in the hands of end-users. There is a window of opportunity now to ensure that such support systems become embedded in routine practice.

2 | Introduction

Early diagnosis and treatment are key to addressing morbidity and mortality due to malaria [1-3]. The development of rapid diagnostic tests (RDTs) over the past decade has offered the potential for the extension of accurate diagnosis to remote and poorly-resourced areas that are beyond the reach of high quality microscopy services. Rising drug costs and recognition of the inaccuracy of clinical diagnosis [4-6] are increasing the demand for demonstration of parasitaemia prior to therapy.

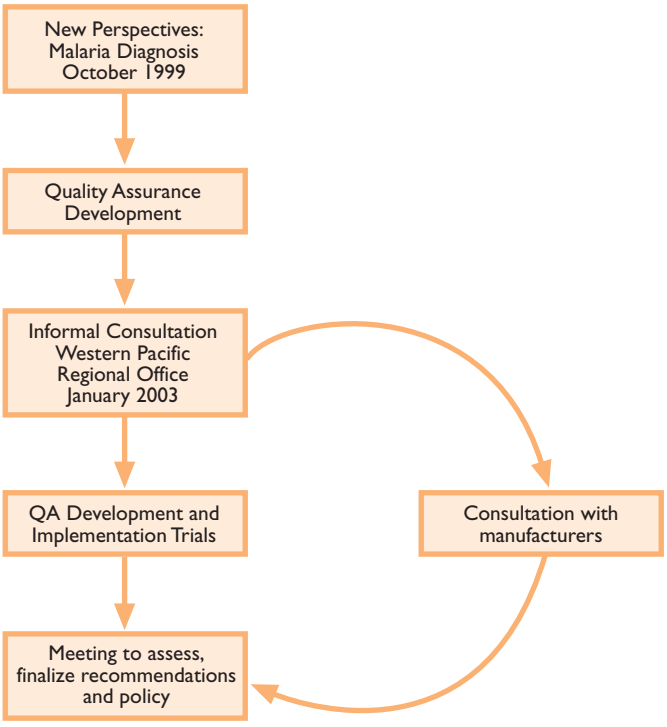
The World Health Organization (WHO), together with UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and Roll Back Malaria (RBM), began some years ago to develop policy on the use of RDTs in malaria diagnosis. The joint WHO/United States Agency for International Development (USAID) informal consultation *New Perspectives* in 1999 [7] outlined a general approach to these issues and defined certain research needs and standards for the wider implementation of RDTs in malaria management. Since that time there has been limited progress in addressing these goals and some confusion as to WHOs' position in addressing them. Various published trials have cast doubt on the accuracy of RDT-based diagnosis in remote areas, sometimes backed up by operational experience (see Section 5). There has also been a rapid expansion in the product range and the demise of some better-known products. There is a clear need to clarify the position of RDTs in malaria management and provide the support necessary for endemic countries to benefit from the potential they offer.

SCOPE OF THIS REPORT

RBM in partnership with TDR, through the Western Pacific Regional Office of WHO, commenced an initiative in 2001 to address the needs identified in the informal consultation of 1999 and develop policies defining the place of RDTs in malaria management and addressing the uncertainties which have arisen. This document reports on a RBM/TDR informal consultation held at WHO's Western Pacific Regional Office in January 2003 to assess progress thus far and map out a clear path for the future. Where possible, the consultation aimed to provide

specific guidance on issues raised by the 1999 informal consultation and it modifies some of the previous recommendations in the light of current experience. The recommendations of the consultation stated here are not necessarily WHO policy, but will form a basis for guiding WHO policy on RDTs. Comment on the document is welcomed by WHO.

WHO Policy Development for Malaria RDTs



3 | Objectives

The consultation brought together a small group of participants with a range of expertise in malaria RDT issues. It aimed to make a detailed assessment of the current status of RDTs in malaria control and progress since the previous WHO informal consultation in 1999 [7] and to define a firm pathway forward to address unresolved issues and impediments to the wide-scale use of RDTs by malaria control services. Three broad areas of policy were addressed:

1. **Quality assurance**
   - (a) Determine most appropriate methods / systems for maintaining and monitoring quality of RDTs before and during distribution and use.
   - (b) Discuss draft protocols for laboratory-based quality assurance of RDTs.
   - (c) Determine directions for future research on RDT QA systems.
2. **Field trials**
   - (a) Determine main issues of RDT use that need to be addressed by further research.
   - (b) Define minimum standards / guidelines appropriate for RDT field trials.
   - (c) Determine need for further large-scale phase three and four malaria RDT field trials and discuss contents of trial protocols.
3. **WHO policy on RDTs**
   - (a) Canvass opinions on future WHO policy on malaria RDT research and use.

4 | Malaria Rapid Diagnostic Tests

BACKGROUND

Malaria RDTs, sometimes called "dipsticks" or "malaria rapid diagnostic devices (MRDDs)," assist in the diagnosis of malaria by providing evidence of the presence of malaria parasites in human blood. RDTs have a place as an alternative to diagnosis based on clinical grounds or microscopy in some situations, particularly where good quality microscopy services cannot be readily provided.

RDTs are already widely used for diagnosis of malaria in many countries and have potential for improving the quality, and in some cases reducing the cost, of case management. Changes in treatment policies to more expensive multi-drug regimes are increasing the importance of obtaining an accurate diagnosis based on demonstration of parasitaemia prior to treatment. The infusion of funds into national malaria control programmes from the Global Fund for AIDS, Tuberculosis and Malaria in particular is likely to further increase the utilization of RDTs. There is some urgency for facilities to be put in place to allow endemic countries to choose the most appropriate diagnostic methods for their needs and to ensure that these methods work effectively.

MECHANISM OF ACTION

Variations occur among malaria RDT products, though the principles of the tests are similar. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites, which are present in the blood of infected or recently infected individuals (other 'RDTs' that detect antibodies are used for screening blood for evidence of recent infection, and are not discussed here). Some RDTs can detect only one species (*Plasmodium falciparum*), some detect one or more of the other three species of human malaria parasites *(P. vivax, P. malariae and P. ovale*). Blood for the test is commonly obtained from a finger-prick.

RDTs are lateral flow immunochromatographic antigen-detection tests (ICT), which rely on the capture of dye-labeled antibodies to produce a visible band on a strip of nitro-cellulose. In the case of malaria RDTs, the dye-labeled antibody

first binds to a parasite antigen and the resultant complex is captured on the strip by a band of bound antibody, forming a visible line (test line). A control line gives information on the integrity of the antibody-dye conjugate, but does not confirm that the RDT can detect parasite antigen. A detailed description of the mechanism of action is given in a previous report [7].

5 | Current Situation of RDT Use

5.1 Growing needs and changing products

RDTs have a number of potential applications in malaria control; the major application in terms of volume of tests and likely impact on disease is that of improving diagnostic accuracy in remote areas. Other applications include rapid investigation of outbreaks, disease surveys, 'after-hours' diagnosis in hospitals and clinics and self-diagnosis by travelers. The relative benefits of RDTs and microscopy are discussed elsewhere [7] and are dependent on the cost of both services, the existing microscopy network and ability to maintain it, the importance of maintaining microscopy in remote areas for diagnosis of other diseases, the quality of both microscopy and the RDTs, and the case load.

Growing *P. falciparum* resistance to anti-malarial drugs, necessitating the use of more expensive combination therapies in Asia, Africa, and South America, is increasing the need for accurate diagnosis. Evidence from several countries indicates that symptom-based diagnosis results in a high rate of unnecessary treatment. The availability of increased funding for malaria through the Global Fund for the AIDS, Tuberculosis and Malaria will increase the ability of health services in many endemic countries to purchase RDTs in large numbers. A challenge these countries will face is the building of a sustainable structure that can continue to support RDT use if the availability of funds is reduced in the future.

The available product range of malaria RDTs has changed and expanded rapidly since the WHO/USAID consultation in 1999 [7]. At least 25 branded products are now commercially available, the majority targeting *P. falciparum* alone. At least six products involved in one trial in 1998 [8] have left the market. Other products, some targeting different antigens and specific or non-falciparum species, are under development and the market is likely to expand further over the next few years. While expected with the advent of a new technology, this situation makes assessment of products by potential purchasers increasingly difficult.

5.2 Experiences with wide-scale implementation

PUBLIC SECTOR USE

RDTs have been used on a large scale in the public sector in parts of South America, Southern Africa and South-East Asia. This has predominantly involved areas without microscopy services, and they have been used successfully for prevalence surveys. RDTs have now been integrated into routine practice in several national malaria control programmes (e.g. Thailand, Cambodia, South Africa), though significant problems with sensitivity have been reported in both Asia and South America, involving a range of products and sometimes requiring replacement of product lots. This has resulted in considerable uncertainty as to the place of RDTs in the health systems of endemic countries (Section 5.3). However, many countries using RDTs have no mechanisms in place to monitor RDT accuracy or determine where problems causing loss of sensitivity lie. The effectiveness of implementation can therefore not be gauged.

PRIVATE SECTOR USE

Use of RDTs in the private health sector has expanded rapidly in many regions and this is likely to continue. Private sector use presents opportunities to increase the availability of accurate diagnosis, but also further problems for maintenance of diagnostic quality. Quality of both products and test performance will be difficult to monitor in this sector.

Private-sector distribution of RDTs in some countries, such as Guyana, appears to be successful and national social marketing of RDTs has been performed in Cambodia, but anecdotal reports from Asia indicate that misuse of tests and misinterpretation of results may be widespread in some areas. Public health services have cooperated with the private sector with some success distributing RDTs in endemic areas [9].

The remoteness of many endemic areas and the limited resources of endemic countries will make control of diagnostic quality in the private sector difficult. Many countries have no national regulations to control licensing or importation of diagnostic products.

5.3 Recent trials and interpretation of results

Early field trials of various RDT products demonstrated their potential for achieving high sensitivity and specificity [10-13]. Over the past few years several published trials have reported sensitivity and specificity for *P. falciparum* well below that

required for operational use [14-24]. Sensitivity for non-falciparum species is generally lower [15, 17, 25-27]. There is little consistency in the results obtained for individual products. Direct comparative studies often give widely conflicting results when testing the same products [14-17].

Recent poor RDT trial results may reflect increased either exposure of RDTs to the environment during transport and storage or poor quality RDTs, perhaps as a result of scaling-up to larger manufacturing lots. Researchers may now be more willing to publish poor results. The design and published details of trials are frequently inadequate to distinguish the possible causes of failure. While they serve as a useful warning that the accuracy of RDTs needs continued monitoring, they often do not provide the information needed to ensure performance is improved. Due to the delay in publication of results, many papers also report on products no longer on the market, further limiting their use in guiding purchasers.

6 | Quality Assurance of RDTs

INTRODUCTION

Variation in RDT accuracy in published trials and operational experience (Section 5.2 and 5.3) underline the need for an accurate, transparent system for monitoring the accuracy of RDTs after release by the manufacturer. The development of a comprehensive quality assurance scheme is essential to ensure that test quality is maintained, reducing the likelihood of misdiagnosis and maintaining confidence of health service providers and consumers. In time, such a scheme will provide standardized evidence of test performance to guide purchasing and development.

Malaria RDTs are affected by various conditions of manufacture, storage and use that can impair their accuracy and reliability. The global initiative to scale-up the introduction of RDTs to aid in the management of malaria, especially in locations where laboratory-based diagnosis is unavailable, therefore requires a system in place to assure that service quality is guaranteed.

Quality assurance (QA) is defined as a total process, both in and outside the laboratory, including performance standards, good laboratory practice (GLP) and management skills to achieve and maintain a quality service and provide for continuing improvement. The purpose of QA is to provide *reliable, relevant, timely* test results that are interpreted correctly thereby increasing efficiency, effectiveness, enhancing patient satisfaction and decreasing costs brought about by misdiagnosis. This is increasingly important with the advent of combination therapies and their higher associated costs. A QA process for malaria RDTs should aim to ensure high accuracy of tests in the hands of end-users. This will include both monitoring of the technical standard of the RDTs, processes to minimize environmental insult and training and monitoring of preparation and interpretation by end-users (Figure 6.1.1).

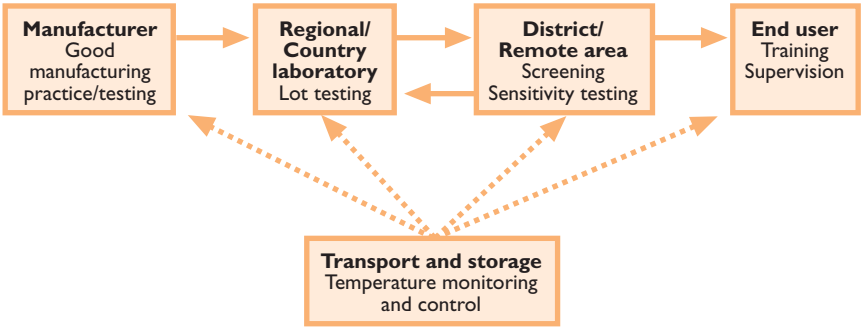
Quality control (QC) describes all the activities taken by a laboratory to monitor each stage of a test procedure to ensure that tests are performed correctly and are *accurate* and *precise*. Quality control must be practical, achievable and

affordable. A system for laboratory-based assessment of performance of RDTs throughout their shelf-life and the appropriate Standard Operating Procedures (SOPs) is described in Section 6.3.

6.1 Quality assurance for malaria RDT use in remote areas

The process should be overseen by a designated person in the regional laboratory and by a designated person in each country. This process must include feedback to people involved at each level and a clear policy of action on results coordinated by the designated person and known to all levels.

Figure 6.1.1



6.2 Quality control and RDT manufacturers

The number of manufacturers and rate of change of products, the diversity of national regulatory backgrounds and the potential costs make monitoring of manufacturing standards by WHO impractical at present. Approval of manufacturing facilities through a process similar to that used for drug manufacturing may be appropriate for review in the future. At present, it is wise for purchasers to consider asking for evidence of GMP/ISO certification prior to purchase.

Manufacturers of malaria RDTs currently use a number of standards during internal quality assurance processes. It would benefit manufacturers if a panel of quality control samples were available for testing products. These panels of parasite-based QC samples could be prepared by regional laboratories as part

of post-manufacturing quality control coordinated by WHO (see Section 6.3). Pre-release QC testing of RDT lots could then be performed using the same samples used for post-release testing by purchasers/users in QC laboratories (Section 6.3).

Real-time data should be available on RDT products as evidence of stability to the expiry date. Changes in the format or raw materials used in RDTs (e.g. different sources of monoclonal antibodies or nitrocellulose) should be indicated by appropriate changes in labelling to clarify that the product is different from previous versions.

While all products should have real-time stability data available, it is also desirable to have accelerated heat stability data available on each lot prior to release. Both real-time (product) and accelerated (lot) data should be requested as part of the tendering process. Accelerated data can be used to estimate real-time stability using the Arrenhius equation [28].

Some products have inadequate labelling, with absent or insufficient labels on individual RDT envelopes. This prevents identification if boxes are divided or tampered with. Product type, lot numbers and expiry dates should be clearly labelled on all packaging, including RDT envelopes, to allow identification of individual RDTs to QC results.

Main recommendations:

- Purchasers should request evidence of GMP/ISO certification, accelerated heat stability data on each lot, and real-time heat stability data on each product.
- Changes in raw materials and format of products should be clearly noted on the product label. Essential information (product, lot, expiry) should be included on product envelopes.
- Parasite-based QC panels used for WHO-coordinated QC should be available to manufacturers. These should be from geographically-diverse areas.

6.3 Quality control at regional and country levels

OUTLINE

There is a clear need for a system for accurate sensitivity testing of RDTs after purchase. Such a system needs to be transparent, standardized and of reasonably low technology if it is to have the confidence of manufacturers and purchasers, and be widely applicable.

Standard Operating Procedures (SOPs) were previously developed by WHO's Western Pacific Regional Office for preparation of samples for laboratory-based QC on consultation with various experts and manufacturers and through laboratory and field trials in the Philippines. The SOPs were discussed in detail during the consultation and various steps in sample preparation and use refined. A summary of the sections on QC sample preparation and storage is found in Appendix 1. The SOPs will be revised in early 2003 and distributed to laboratories involved in malaria RDT QC.

The method and organization of the proposed laboratory-based QC system is designed to ensure accuracy of assessment, transparency and applicability to all existing RDT formats. It is important that both malaria diagnostic services and manufacturers have access to results and documentation.

SELECTION OF QC STANDARDS

For the present, it is considered that stored dilutions of wild-type parasites form the best basis for QC testing of RDTs. Such samples are closest to the substrate with which malaria RDTs are designed to operate. They have the disadvantage of expense of transport of potentially-infectious material.

Cultures parasites are considered to have a number of disadvantages over wild-type isolates:

- varying gene expression and gene deletions;
- difference in supernatant target antigen concentration to that in human plasma;
- differing concentration of non-target antigens and metabolites;
- different nutritional state;
- synchronisation of parasites and choice of parasite stage; and
- lack of non-*falciparum* culture lines.

Development of antigen-based QC samples should be considered in the future, but limited pure sources of antigens are available at present and the range of antigens needed to test all RDTs is expected to increase in the near future with the development of new detection systems.

PRODUCTION AND STORAGE OF QC SAMPLES

Uniformity and reproducibility of QC testing of RDTs demands stability during storage and transport of samples for considerable periods. Unpublished reports indicate that both HRP2 and pLDH are stable in stored blood for up to six months at –20°C, HRP2 for considerably longer. Both are stable for at least two

to three days at 4°C. More data is needed on long-term stability in both situations and on stability of other target antigens. Repeated freeze-thawing of blood samples will result in premature lysis of red blood cells and parasites and may effect antigen availability and flow characteristics.

Recent use of anti-malarial drugs affects the relationship of parasite density to antigen concentration to varying extent with different antigens [29-31]. The concentration of pLDH closely mirrors microscopically-patent parasite density. It is, therefore, important that those receiving recent treatment be excluded when parasite donors are selected. This should be determined by careful history and a pLDH concentration appropriate for the parasite density (enzyme-linked immuno-sorbent assay [ELISA]) will confirm this. Further work is needed on the effects of mefloquine and artemesinin derivatives on antigen concentration.

Parasitised and non-parasitised blood for QC samples should therefore be prepared within two days while stored at 4°C and frozen at -20°C to -70°C. Samples should be transported on dry ice. On thawing, samples should be used within one hour and then discarded.

Prior to use, QC samples should be tested to ensure that target antigen concentration is in the correct range. ELISAs for some currently used antigens are available, others will be developed and curves of parasite density versus optical density coordinated by WHO. It is noted that the potential for variation in the relative concentration of antigens, and the effects of storage and thawing on blood flow, prevent QC samples developed and stored in this way from being used for direct comparison of the sensitivity of different products. They should only be used for assessment of sufficiency of sensitivity.

All QC samples should be screened for the Human immunodeficiency viruses (HIV) I and II, Hepatitis B virus (HBV) and Hepatitis C virus (HCV) prior to use. Universal blood safety procedures should be followed during use and disposal.

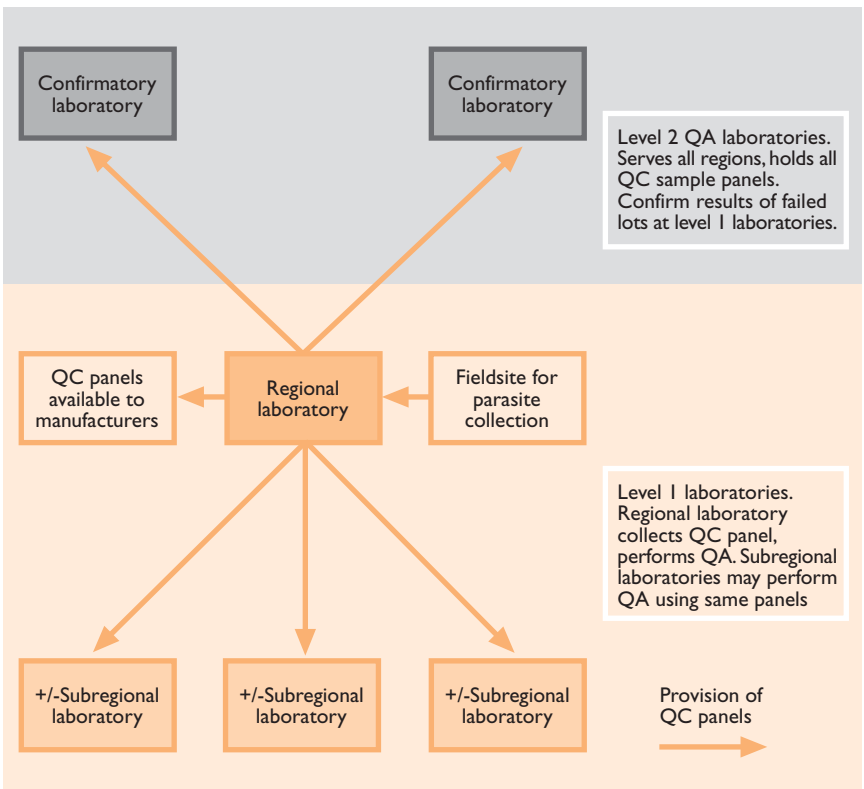
APPROPRIATE PARASITE DENSITY

The previous WHO informal consultation on RDTs [7] determined that RDT sensitivity should be close to 100% at 100 parasites/µl. This was confirmed by the present consultation. It is also important to ensure that RDTs are operating correctly at high parasite densities. Control lines can become faint if insufficient antibody-label conjugate is present when parasite density is high, and test lines may also decline in intensity ('prozone effect' (RITM, unpublished data). Levels for QC testing in laboratories should therefore be set at 100 parasites/µl and at least 5000 parasites/µl.

ORGANIZATION OF REGIONAL/COUNTRY-LEVEL QA

A provisional structure for the organization of quality assurance laboratories was mapped out at the consultation (Figure 6.3.1). The structure will be further refined by WHO. Preparation of QC samples should be performed in a small number of regional-level laboratories, while QC testing may be performed in some national laboratories if adequate facilities, staff and training and monitoring are available. Two expert laboratories should be retained for confirmatory testing of RDT lots that fail regional level testing. Manufacturers of such products should choose which of the two laboratories will be used for their product. Regional laboratories should prepare and retain their own QC sample panel, which will be distributed to sub-regional (national) laboratories. Confirmatory laboratories should hold a panel from each regional laboratory, and panels should also be available to manufacturers.

Figure 6.3.1
Suggested Organizational Structure of RDT QC Laboratories



Regional coordination should occur through or with the assistance of the regional WHO office. Health services using RDTs should designate personnel to oversee RDT QA activities at all levels, including the laboratory component.

TESTING THE RDTS

RDT lots at release from manufacturer are of uniform quality and RDTs within the lot will deteriorate at similar rates if kept in uniform conditions. It is therefore necessary to include only a small number for QC testing. Four RDTs (*number to be verified)*, including at least two boxes, tested at each parasite density on four QC samples, and a further RDT tested on a negative sample, are considered sufficient (Appendix 1). Laboratory QC testing should occur at least every three months on retained RDTs until three months before the expiry date. Reports should be sent to the purchaser and manufacturer. The RDTs should be stored at 2°C below the maximum storage temperature stated by the manufacturer.

Initial testing should occur prior to release of RDTs to the field. If RDTs are tested prior to transport of the remainder of the lot from manufacturer to the purchaser, it is mandatory that temperature be monitored prior to transport of the remainder to the purchaser. Transport to selected RDTs to the QC facility should occur at 4°C to ensure no deterioration in quality occurs.

MAINTAINING QUALITY OF QC LABORATORY FUNCTIONS

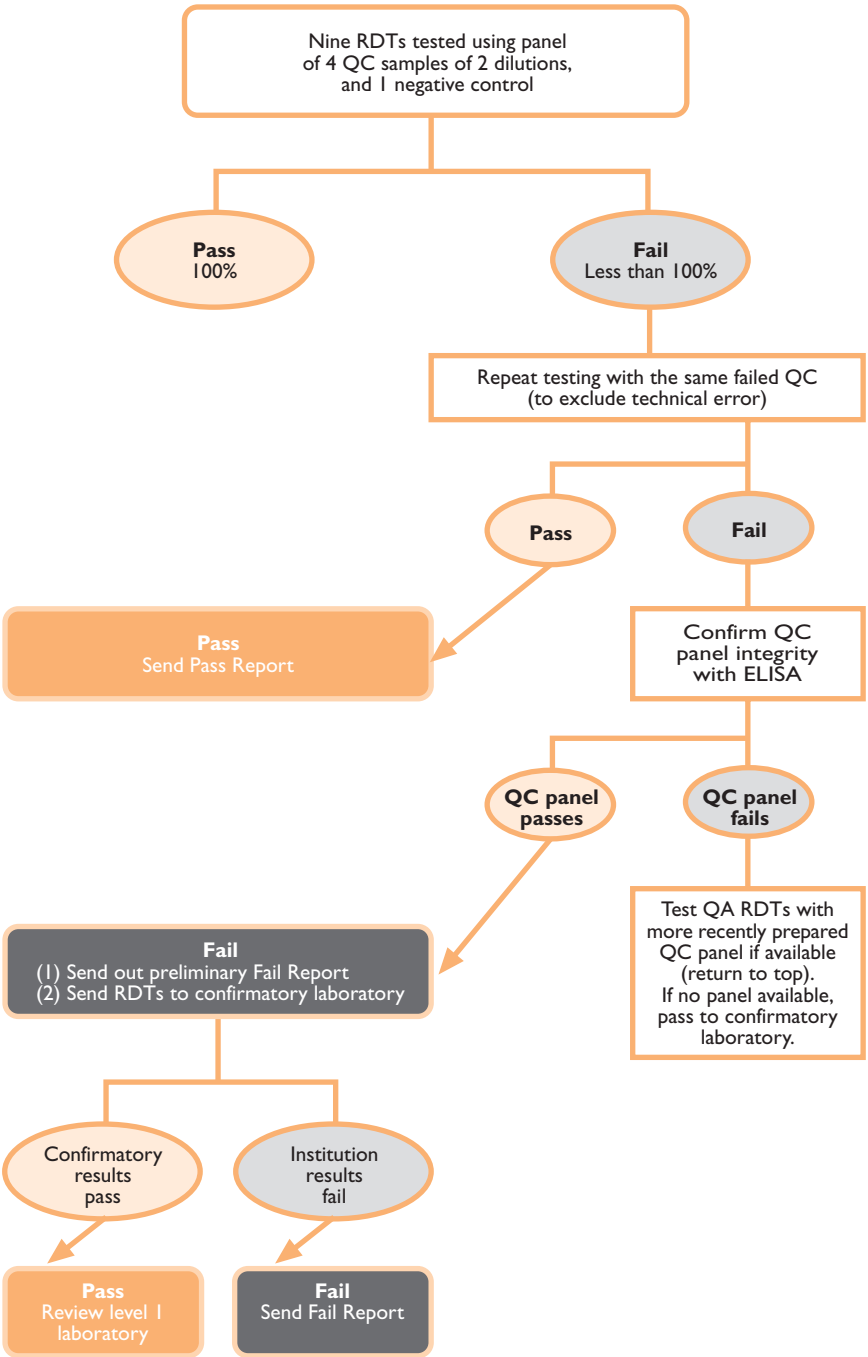
All laboratory QC activities should be performed according to the uniform SOPs. A training manual will be developed within these and direct training under WHO supervision should be provided prior to commencement of QA/QC activities. A mechanism for approval of QC laboratories, monitoring, withdrawal of QC laboratory status and dispute resolution must be developed. This could take the form of an annual inspection of laboratories by an independent evaluator and an ad hoc expert panel to oversee disputes. The details of passing/rejecting RDT lots are detailed in Figure 6.3.2.

FUNDING AND SUSTAINABILITY

At present, quality assurance activity is funded by WHO (RBM). A mechanism for funding and coordination, preferably embedded in existing WHO activities and with national support, needs to be addressed as a priority to ensure sustainability. At a national level, health/malaria services need to incorporate QA activity into standard practice for provision of malaria diagnostic services, as microscopy QA should be already, with appropriate provision for funding and personnel.

Figure 6.3.2

Flow Diagram of Proposed Laboratory-based Quality Control Testing of Malaria RDTs



6.4 Quality control in remote areas

The degree to which individual countries/malaria control programmes implement RDT QA schemes will depend on practical and organizational issues. Determinants to be considered are the percentage of the total product cost that will be devoted to QA, "permissible" wastage of tests and human resource costs.

Distribution of small numbers of RDTs to various health centres and health workers in remote areas from a district or municipality level makes it neither feasible nor practical for each testing centre to remove one to two tests per month for QC. Similarly, the transportation and storage of QC panels beyond the central level to district or peripheral locations, and maintenance of standards of use, would be highly problematic and is therefore not recommended.

Positive control wells, preferably of lyophilised recombinant antigen, could be used at the first referral level to periodically (one to two monthly) evaluate batch test performance over its shelf-life under local field and storage conditions. Alternatively, such antigen wells could be used on supervisory visits. Wells containing pLDH are commercially available and are being evaluated in Cambodia and the Philippines. The development of wells containing other target antigens should be considered. Antigen concentration should give a weak positive result on an adequately performing RDT when reconstituted with blood. Batches of RDTs failing this level of testing could be returned to a laboratory for QC testing using a parasite panel (see Section 6.3).

The QA focus at this level should concentrate on initial training, supervision and continuous education so that personnel working in remote areas achieve and retain competence and motivation. Training should not only include test procedure methodology but also trouble shooting guidelines, especially on how to suspect that the RDTs have failed, and operating procedures on reporting of suspected failed tests and recall of all proven failed batches of tests to the first referral level or distribution point.

6.5 Quality control and the end-user — training and supervision

The QA concept includes the notion that the personnel have sufficient education, training and experience to permit the proper performance of duties. More importantly they should be motivated to operate in accord with established guidelines, principles and defined standards. This latter concept becomes more difficult to implement at lower levels of the chain. A QA scheme is only as strong

as the weakest link in the chain, therefore everyone involved should receive appropriate and rapid feedback to maintain commitment to the programme. The importance of end-user performance to the accuracy of malaria RDTs is well documented. End-user supervision and training should be included routinely when budgeting for malaria RDTs.

The initial training, preferably standardized at a country level, is critical and must cover not only technical issues but also management aspects. Ideally training may consist of instructions received on the job as well as information gained in formal courses. The goal should be seen as the provision of adequate and timely corrective action in response to standards not being met. The provision of SOPs is one of the first steps, including clear pictorial instructions on RDT use.

The following should be emphasized during training and after implementation of such a scheme:

- exercising diligence in the performance of assigned duties;
- maintaining a required level of proficiency;
- exercising judgment /seeking advice in reacting to unforeseen circumstances;
- keeping accurate records;
- requesting necessary training;
- honesty; and
- motivation.

To maintain a quality service, the initial training must be supplemented by regular supervision and in-service courses. An organized system of supervision, that is periodic visits by experienced staff, especially to peripheral areas, will provide a mechanism by which problems are discussed and resolved and anomalies in reported results are investigated. Supervision provides not only information on technical performance, conditions of service, productivity and the use of SOPs but also a medium to motivate staff.

Supervisors themselves must be adequately trained so that supervision is carried out in a helpful, constructive way and is not just a critical analysis. Both parties must be aware of the importance of the work and be committed to improve performance.

Guidelines for training and instructions for RDT use require further development. WHO and other agencies should seek to develop generic guidelines that can readily be modified to suit local health service needs (see Section 10.2).

6.6 Transport and storage of RDTs

The susceptibility of immunochromatographic devices to degradation by heat and moisture mandates the need to avoid exposure to high temperature and damage to packaging during transport and storage. Freezing (e.g. during air transport) may also degrade some RDTs. Such environmental exposure may have been responsible for low sensitivity reported in field trials and operational use (Section 5.2 and 5.3). They should be treated with the care normally used for biological substances used for diagnostic and therapeutic purposes. This demands:

- careful coordination of transport to avoid unnecessary delays in customs, on airport tarmac or in non-air conditioned road transport;
- avoidance of exposure to direct sunlight;
- use of air-conditioned storage where possible (it is recognized that this is frequently impossible in peripheral areas, but distribution should be organized to minimize time in uncontrolled storage);
- temperature monitoring of storage facilities. Action will depend on temperature stability of individual products. Temperature monitoring may not be possible at peripheral levelsz; and
- rejection of RDTs where packaging is significantly damaged and it is likely that moisture-proofing of envelopes or canisters is lost.

Chemical-based temperature monitors, similar to vaccine vial monitors (VVMs) presently used from vaccine transport, could be developed for use with RDTs. VVMs may need to be designed specifically for different RDTs, depending on heat stability. It is recommended that manufacturers consider the inclusion of temperature-monitors in RDT boxes. WHO should consider this further and assess likely costs of inclusion.

The health service designated person responsible for overseeing QA of RDTs should coordinate the organization of 'cool chain' transport and storage. A draft information sheet for RDT transport and storage is included in Appendix 2. Integration of arrangements for RDT transport and storage with those in place for vaccines, where possible, will allow use of existing resources and personnel skilled in this area. Although longer storage times are required for RDTs at the end user, coordination of delivery to these areas with vaccination delivery should be possible.

6.7 Future directions for quality assurance

SUSTAINABILITY AND ORGANIZATION WITHIN THE HEALTH SERVICE

The initial implementation and maintenance of a QA scheme will involve considerable expense. This will include development and distribution of the necessary documentation, laboratory development and training, but may not differ significantly from well-run QA processes for microscopy-based diagnosis. Costs should decrease after establishment, but funding will be needed for laboratory maintenance and supplies, assessment of laboratories, periodic replenishment of QC panels, transport of QC panels, training of end-users and appropriate transport and storage of RDTs. These processes need more detailed costing. Country budgets for RDTs should routinely include QA costs, but some funding, at least initially, will be required from external sources. Cost-recovery may be possible to some extent for regional-level QC laboratories, both through provision of QC services to countries and through provision of QC panels to manufacturers.

One or two people should be designated to coordinate RDT QA at a national health service level, coordinating transport, storage, provision of samples for testing, and with oversight of training and monitoring of users. Feedback to other levels of the structure is vital to maintain the commitment and motivation necessary to ensure adequate time and effort is spent.

DEVELOPMENT OF NEW METHODS FOR QC TESTING

It is recommended at present that wild-type parasites form the basis of panels for QC testing. Methods using recombinant antigen should be considered for future development. These will have the advantage of ease of transport, consistency of quality, and ease of production.

At present, there is insufficient information on the relationship of antigen concentration to parasite density, and this may be subject to geographical variation. Curves of antigen concentration versus parasite density should be developed. Costs of development and production may be high, and the introduction of new RDTs targeting novel antigens would require the development of new QC samples.

Antigen-based QC has immediate application in development and use of positive control wells, for use in screening RDTs in more remote areas to determine the need for more detailed parasite-based laboratory testing (see Section 6-4).

7 | Field Trials

7.1 Minimum standard guidelines

The wide variation in results of clinical trials of RDTs and the lack of available data to assist in identifying the reasons for test failure (Section 5.3) underline the need for improved standardization and rigour in trial design. Field trials conducted in remote areas, in which RDTs are to be used are important, and it is difficult to tightly control the conduct of trial in such conditions. However, if trials are to be useful to malaria control programmes, then certain minimum criteria must be met in trial design and implementation. Similarly, publication must include certain minimum criteria if results are to be correctly interpreted. Guidelines of minimum trial standards approved by the consultation are included in Appendix 3 and will be disseminated by WHO.

7.2 Proposed WHO-coordinated multi-centre field trial

The need for a large, well-controlled Phase III trial of malaria RDTs was discussed in the previous report [7]. In view of the variable results of existing trials and the perceived confusion over RDT capabilities, a Phase III multi-centre clinical trial will be conducted under the auspice of WHO. This will aim to give purchasers a guide to the potential sensitivity of currently available products in a field setting and to give manufacturers the opportunity to demonstrate efficacy in a well-conducted independent trial.

Expressions of interest will be requested from companies marketing RDTs. Restrictions will include on-site manufacturing and a pre-qualification evaluation will be conducted at a well-established independent laboratory designated by WHO. Approximately five products representing both of the major assay types (pLDH and HRP2) will be selected based on efficacy in laboratory testing. Other selection criteria shall include cost (less than US $3.00/test), ease of use (e.g. provision of lancet, buffer and pictorial instruction, etc.), individual packaging of

the test and availability of stability data. Participating manufacturers will provide a defined number of tests from at least two different lots for the pre-qualifying phase.

Protocol for the trial were developed during the informal consultation and will be finalized by WHO in consultation with members of this group, other experts in the field and manufacturers. It is anticipated to include three field sites, each representing a major endemic region and including Asia, Africa and South America. The selected sites must have adequate infrastructure for slide preparation, feasibility of transportation to a laboratory with freezer facilities on a daily/weekly basis , presence of both *P. falciparum* and non-*falciparum* species at adequate levels and sufficient parasite prevalence to facilitate completion of data collection within one to two months. Results of the trial will be submitted for publication in a peer-reviewed journal and disseminated by WHO. A draft protocol is included in Appendix 4.

8 | Other Priorities for Research and RDT Development

The escalating use of combination therapies and the increasing importance of demonstration of parasitaemia prior to treatment have increased the need for operational research to guide large-scale implementation of RDT-based diagnosis. Since the informal consultation in 1999 [7], published research on RDTs has focused predominantly on test performance in laboratory and field conditions. The development of guidelines for minimum standards for field trials will improve the ability to compare results of different trials and their value to consumers and manufacturers. Field efficacy studies and development for quality assurance are discussed earlier.

There are several areas in which basic research on RDT development is still needed, though priorities need reassessment in light of the major foreseeable applications of RDTs in the field.

Recommendations for research raised in the informal consultation in 1999 that are no longer considered priorities:

1. *Development of methods that permit quantification of parasite density with RDTs.* As RDTs are likely to be used predominantly in remote areas where sophisticated follow–up of patients is not possible, the ability to estimate the total parasite load will be of little clinical importance.
2. *Development of tests that detect asexual parasitemia only.* It is rare to have gametocytes only in circulation except immediately after treatment. RDTs detecting pLDH closely reflect viable parasitaemia [30, 41, 42] and are better predictors of treatment failure than HRP2-detecting RDTs in the absence of gametocytes [17, 43]. However, refinement to distinguish asexual forms from gametocytes will have limited clinical value.

Ongoing needs for RDT development include:

1. *New target antigens.* Presently marketed RDTs target one or a combination of P. falciparum-specific pLDH, pan-specific pLDH, HRP2, or pan-specific aldolase. It would be clinically useful to target antigens which discriminate

more clearly between the non-falciparum species and P. falciparum. Development of tests speciating between P. vivax, P. ovale and P. malariae is not considered a high priority, but may have increasing application in the future if drug resistance of non-falciparum species becomes more widespread. Application will be dependent on the production costs of including further antibodies.

2. Improvement in current test performance characteristics:
   - A sensitivity threshold of greater than 95% and 100 parasites /il of blood in all four species is still considered ideal [WHO 2000]. Sensitivity for non-falciparum species in particular requires further improvements. Specificity is less important, but should be of a level to direct therapy with reasonable accuracy.
   - Reduction of time-critical steps, especially in the reading of the test results within a specified time would improve reliability especially in remote areas.
3. Improvement of existing blood transfer devices. The volume of blood transferred to the strip is a critical step that can lead to false results. Improving accuracy and ease of use of blood transfer devices will lead to improved test performance.
4. Development of a bank of reagents and a network of testing sites. Parasite-based QC panels should be developed and made available to manufacturers. They should be sourced from geographically-diverse areas, to allow for possible regional variations in antigenicity. Published and unpublished research indicates that sensitivity of some tests (HRP2-based) may vary in geographically distinct areas (University of the Philippines, unpublished data) [44]. Further work is needed to clarify this.

Specific operational research needs:

1. Evaluate the ease of use and accuracy of existing blood transfer devices and buffer solution dispensers in the hands of the likely end-users. Blood volume transferral is critical for test performance. Finger-prick technique and blood transfer methods are critical to the protection of health workers from blood-borne infection.
2. Evaluate the impact of package insert formats. Design of instructions can have a significant influence on operational sensitivity of the RDT [32]. Improved insert formats including diagrams and text adaptable to different cultural backgrounds, should be developed.

3. Evaluate the impact of the wide-scale introduction of RDTs at a national level on diagnosis and treatment of malaria and on the disease. Research questions include:
   - Impact of RDTs on quality of clinical care?
   - Cost-effectiveness and savings in drug costs?
   - Impact of correct diagnosis or improved patient management on patient satisfaction and treatment compliance?
   - The effect of RDT diagnosis on the health provider and treatment practices?
4. Assess through qualitative and quantitative research data what factors affect the choice of a diagnostic test, its implementation or expansion of usage at a national level.
5. Assess the use and application of RDTs in selected situations such as isolated communities, private health providers, home care management and travelers. Studies should include economic analysis, behavioural studies, monitoring and quality control and measurement of outcome.
6. Assess the impact of the introduction of a quality assurance scheme for RDTs on health service delivery including the quality of training materials.
7. Assess different RDT distribution systems to ensure a regular supply of RDTs to remote areas within their expiry date and in condition for use, including temperature monitoring and evaluation of the feasibility of developing low-cost temperature monitors for packaging with RDTs.
8. Investigate the practicality of using positive-control wells at a provincial or district level for quality control. Wells containing pLDH are currently available; the feasibility of developing wells containing other antigens should be assessed.
9. Assess the cost-effectiveness and cost-benefit of RDTs and diagnostic alternatives. Cost-benefit and cost-effectiveness will vary widely depending on epidemiology, treatment costs, availability of alternatives, case load, the aims of the intervention, and the cost of the RDT itself. While not the sole criteria for assessing RDT suitability, guidelines on basic cost-effectiveness /cost-benefit analysis will facilitate rapid assessment of the suitability of RDTs for an area proposed for use.
10. Assessment is needed of the current use of RDTs in the private sector, the potential for expansion, and likely regulatory problems. Monitoring the private sector will be complex and assessment will guide development of policy to encourage responsible use.

9 Developing Guidelines for Purchasing RDTs

9.1 Technical requirements of RDTs

Preferred characteristics of malaria RDTs were specified at the previous consultation in 1999 [7], including near 100% sensitivity at 100 parasites/µl for detection of *P. falciparum*, and a shelf-life of 2 years at 40°C to 50°C. These remain ideal specifications but it is likely that such performance will not be achievable in the near future.

The required sensitivity for operational should remain similar to previously stated. However, variation in the ratio of peripheral parasite density to antigen concentration, and possible variations in gene expression, make 100% sensitivity at low parasite densities an unrealistic target. Also, it may not be feasible to maintain long shelf-lives at the stated temperatures. While the clinical significance of this parasite density can vary among age groups and regions, the required sensitivity should apply irrespective of region in view of the potential consequences of missing infections in individuals with low immunity. The clinical situation should determine whether an RDT is used or not (i.e. whether parasitaemia should be treated).

Detection of non-falciparum parasitaemia by RDTs employing pan-specific antibodies is generally less sensitive than that for *P. falciparum*. While sensitivity close to that required for detection of *P. falciparum* may be obtained for *P. vivax* , this is currently not attainable for *P. ovale* or *P. malariae*. The target of near 100% sensitivity for the four non-falciparum species recommended in the previous report [7] is not likely to be obtained using currently available antibodies and RDT formats.

SENSITIVITY AND SPECIFICITY

Sensitivity should be near 95% at 100 parasites/µl for detection of *P. falciparum*. Sensitivity should be higher at higher parasite densities. Treatment algorithms should allow treatment cases with high suspicion of severe malaria to be treated whilst excluding other causes (Section 9). Lower sensitivity is acceptable for *P. vivax* in RDTs detecting pan-specific antigens.

Specificity should be close to 90% for detection of malaria. The necessity for speciation between *P. falciparum* and other parasite species will depend on the epidemiological situation and drug policies. Distinguishing between non-falciparum species has little clinical advantage at present. In the future, emerging resistance to non-falciparum parasites may increase the need to distinguish between them.

STABILITY AND SHELF-LIFE

A minimum shelf-life of 18 months to two years is appropriate for most remote-area applications. Temperature specifications should depend on the region of use and likely storage conditions, and a system for checking sensitivity throughout the shelf-life should be mandatory (see Sections 6.1 – 6.5). Close attention should be paid to temperature exposure during transport and storage (see Section 6.6). In most remote endemic areas, storage at temperatures above 30C will be unavoidable and this should be taken into account when choosing appropriate products for use.

Temperature exposure studies should be conducted to define the risk points in transport and storage and to determine the conditions RDTs need to withstand in the region of intended use. The addition of chemical temperature sensors in boxes of RDTs should be considered.

PACKAGING

RDTs for field use in tropical conditions should be individually packaged in moisture-proof envelopes, which should remain sealed until immediately prior to use.

USER-FRIENDLINESS, BLOOD TRANSFER, INSTRUCTIONS

The sensitivity and specificity of RDTs depends significantly on the way in which the test is performed, and on the interpretation of results [32-37]. Manufacturer instructions should be accurately followed. This is dependent on clarity of instructions and the ability of the user to interpret them. It is also dependent on the accuracy and ease of use of blood collection devices (lancets and tubes or loops).

Manufacturers should ensure that instructions on use are clear and concise with diagrams to assist interpretation across language barriers. Health services should ensure that instructions are appropriate to the end-users.

WHO should coordinate assessment of accuracy and ease of use of blood collection devices, and production of a common pictorial instruction format that

can be adapted by manufacturers and purchasers to assist in crossing language and literacy barriers.

STABILITY OF RESULTS ON TEST LINES

Several hours after test preparation of some products, back-flow of blood and buffer on the nitrocellulose strip may appear due to accumulation of colloidal gold or blood debris on the test line. This results in apparent positive results on strips that were negative on earlier reading. Such late results should be ignored and RDTs should always be read early per manufacturers instructions. While it is desirable to have stable results for purposes of checking reader accuracy at a later time, late result changes well beyond the specified reading time should not be seen as a reason to reject RDTs and are not indicative of poor accuracy.

POST-TREATMENT MONITORING

The close correlation between pLDH detection and viable *P. falciparum* parasitaemia makes this more useful for treatment monitoring than HRP2 [17, 30, 41-43], but potential detection of pLDH from gametocytes after elimination of asexual stages and inability to detect very low parasite densities limits the applicability of RDTs for these purposes. They may have a useful place in screening for possible treatment failure. However, the expense and logistical difficulties of performing multiple tests means that this requirement will not be a major factor in determining the appropriateness of RDTs in most circumstances.

QUANTITATION OF PARASITE DENSITY

Measuring the parasite density will not change clinical management in most potential areas of application of malaria RDTs; treatment is dependent on clinical assessment and the presence or absence of parasitaemia. Quantitation may sometimes have a place in monitoring treatment (see above), but is not a major priority in determining RDT appropriateness.

ACCURACY OF LABELLING/NOTIFYING CHANGES IN PRODUCT

Labelling and product inserts should accurately reflect the specifications of the product. Limitations in sensitivity and instability at environmental extremes should therefore be clear. Significant changes in technical aspects of the product that may change these should also be clear (Section 6.2).

9.2

Tendering and the availability of product information

In addition to considerations of the sensitivity, species of parasite detected and cost, it is helpful for potential purchasers of RDTs to have an idea of the quality of manufacturing processes (e.g. ISO certification, GMP procedures). The long-term viability of a company will determine the ability to replace product should the received lot fail and to provide long-term supply of a product to minimize the need to re-train end-users. Differences in time-zones can result in problems gaining technical support if specific provision is not made for this.

Apart from consideration of the technical aspects of RDTs discussed in Section 8.1, purchasers should consider requesting the following information from manufacturers during the tendering process:

1. Real-time temperature stability data on the product and accelerated data on the lot.[1]
2. Evidence of successful operational use or good quality field trial data on the product.
3. Long-term viability of manufacturer (staff size, financial statement and/or track record).
4. Evidence of GMP systems / ISO certification.
5. Availability of product support (24 hour help-line and web page).
6. Provision of sample products for assessment and testing for ease of use.
7. Agreement for replacement of products which fail agreed QC procedures (see Section 6.1 – 6.6).
8. Box sizes appropriate to the rate of use of tests in the intended area and to minimize uncontrolled storage and the need to split boxes.

Points three and four imply that the place of manufacture of RDTs should be disclosed to the purchaser in the case of relabelled RDTs.

In turn, it is in the interests of purchasers to have adequate systems for informing all manufacturers of impending tender specifications sufficiently in advance of the tender date.

[1] The relationship between accelerated stability testing and real time stability is approximated by the Arrenhius equation [28] Curves developed from this equation to predict likely shelf-life at various temperatures are being prepared for publication on the planned WHO RDT website (Section 11).

9.3 Return of failed RDT lots

A proposed system for QC testing of RDTs is outlined in Section 6.3. It is recommended that tendering requirements should include return of lots that have failed QC testing and replacement by the manufacturer. It follows that QC testing should follow strict and transparent protocols, including storage of RDTs destined for QC testing within manufacturer guidelines.

9.4 Packaging

Immunochromatographic tests degrade rapidly on exposure to moisture in warm conditions. Packaging should protect the RDT from environmental degradation due to moisture and provide adequate information for safe use of the test and identification of product and lot. Individual packaging, including buffer, has the advantage of preventing tampering with buffer solution (e.g. through dilution).

- RDTs intended for use in the field (outside of air-conditioned settings) in tropical areas should be individually packaged in moisture-proof sachets to minimize exposure to humidity prior to use. If stored cool, it is imperative that they reach room temperature prior to opening to avoid condensation on the strip.
- Boxes should be clearly labelled, including brand name and manufacturer, expiry date and date of manufacture, lot number, and required storage conditions. When significant changes in manufacture or source of raw materials (e.g. antibodies, nitrocellulose) occur, the change from previous versions of the product should be clearly apparent from the label.
- Individual sachets, and reagent bottles should be labelled with at least brand name, date of expiry and lot number.
- Boxes should include clear instructions on use, including pictorial descriptions of test preparation. WHO should undertake to coordinate development of appropriate generic diagrams, which could then be modified to a culturally/linguistically appropriate format, to assist manufacturers.
- Consideration should be given to inclusion of temperature monitors in boxes, designed for the stability of the product. WHO should assist in sourcing affordable monitors.

Packaging of an RDT with a first-line drugs is not recommended as this is likely to encourage misusage of drugs or lead to drug wastage.

10 | Action on RDT Outcomes

It is clear that the current RDT assays cannot always deliver an accurate diagnosis in field conditions. Published studies often provide conflicting evidence (see Section 5.3) and there is a danger that health services may formulate policies on RDT use based on insufficient grounds. At present, RDTs should be seen by health services as an adjunct to diagnosis where good quality microscopy is impractical, adequate training and supervision of RDT use is available and symptom-based diagnosis is considered inadequate for case management. Clinical signs and symptoms must always be taken into consideration in making a therapeutic decision. The attached diagram (Appendix 5) shows an example of an appropriate decision-making algorithm. Given the current sensitivity of most assays and their known limitations (see Section 4), some false negative results may be expected. In preparing treatment guidelines, the following should be borne in mind:

- RDTs should not be used if there is damage to moisture-proof packaging or other reason for suspicion that the test is degraded.
- Appropriate anti-malarial therapy should be initiated in any clinically severe patient with high index of suspicion for malaria but with negative RDT test result while a search for other possible causes continues.
- In non-severe cases, it is appropriate to re-test patients with negative results after one to two days if clinically indicated (consistent with malaria and other cause not found).
- Although false-positive results can occur, they are not common in the absence of recent effective treatment and positive RDT results should always be treated as parasitaemic, while bearing in mind the possibility of co-existent illness.

11 | Dissemination of Information (website)

WHO/TDR has commenced development of a website to assist in the flow of information on malaria RDTs. This will include guidelines for use and for testing of RDTs. This should include information to assist in communication between manufacturers and end-users for tendering purposes through advice on tendering specifications and notification to manufacturers of tendering processes.

12 | Conclusion

RDT use in malaria diagnosis is rapidly gaining in importance and will expand greatly in both volume and area of use over the next few years. The main application in terms of volume of tests is expected to be in remote-area diagnosis in endemic countries. There is a need for further development of malaria RDTs, particularly in improving sensitivity to non-falciparum species, in improving stability of tests to maintain adequate sensitivity for *P. falciparum* and in reducing cost. While WHO should take a role in facilitating such development, present emphasis should be concentrated on ensuring the technology currently available is used to its potential.

The main focus of work to achieve this goal should be developing and disseminating post-manufacture quality assurance, including testing of RDTs and guidance on transport, storage, and use. This should include guidelines to standardize and improve the quality of RDT trials. Better methods need to be developed for remote-area quality control and laboratory-based quality control methods should be available to all endemic regions.

There is a place for a well-conducted multi-centre trial of malaria RDTs to establish a number of products with high potential and set a standard for conduct of field trials. Immediate priorities for operational research include establishment of the reasons for RDT failure, the effect of RDT use on seeking and delivering treatment and improving the quality of test preparation and reading by end-users.

Appendices

Appendix 1

Summary of Methods for Preparation and Use of QC Samples Based on Wild-type Parasites, and Use of QC Samples

Summary of Proposed Revision of abbreviated SOPs 4.2, 5.1, 5.2 for Malaria RDT Laboratory QC Manual

Procedure for setting up dilutions

1. Parasite-free blood for dilutions
   - 1.1. Potential donors are screened for Hep B & C, HIV 1 & 2.
   - 1.2. Use O-neg or O-pos blood. Work out volume beforehand (e.g. 30 ml). Blood should be fresh, less than 48 hours old, stored at 4°C in EDTA. (citrated blood stains poorly on subsequent malaria thick blood films).
   - 1.3. Make thick, thin blood film with fresh blood. Exclude parasiteamia, morphological abnormalities.
   - 1.4. Perform pLDH and HRP2 assay with RDT (to exclude false-positive reactions).
   - 1.5. Replace plasma: centrifuge (2500g x 20 min), remove plasma, replace with equal volume of AB pos FFP, mix on rocker tray for 30 min.
   - 1.6. Store at 4°C.
2. Parasitised blood
   - 2.1. Screen potential donors in field:
     - ~> 7 years of age
     - No anti-malarial drug history within 4 weeks
     - Strong positive RDT result

     Urine screening for drugs can be considered, but methods are not readily available for several drugs.
   - 2.2. Obtain fresh parasitised blood by venipuncture, for thick & thin film, EDTA tube (e.g. 10 ml), plain tube (viral serology), drop on filter paper (PCR).
   - 2.3. Store blood at 4°C.
   - 2.4. Microscopy: 2 blinded readings of thick blood film by Earle-Perez method. If parasite density from each reader is within ~30% then take mean as parasite density. If >20% difference, further readings should be performed. A microscopy protocol will be refined by an expert group.

2.5. If no mixed infection, and RDT was positive, then prepare dilutions in the non-parasitised stock blood using gentle reverse-pipetting, discarding tip (?wide-bore) in glass tubes. Dilutions = 100 para per ìl, and 5000 or 10000 parasites.

2.6. Mix gently on rocking tray at 4°C for 30 min (prev. 4 hours).

2.7. Prepare thin blood film and check for absence of RBC clumping, and thick-thin blood film to dry for later confirmation of parasite density. If no clumping…

2.8. Dispense aliquots into cryotubes. Aliquot of 0.25 ml will suffice for testing 20-40 RDTs.

2.9. Freeze blood at –20°C to –70°C.
Thaw and check one aliquot of all dilutions against known good RDT(s).

Procedure for testing RDTs using QC samples

1. Follow the procedure for receipt of RDTs (SOP 4.1).
2. Use QC samples from 2 different sources to test RDTs. Select the following samples; 100 and 5000 parasites/ml and a negative control (0 parasites/ml).
3. Use the QC samples (100 and 5000 parasites/ml) from each patient to test duplicate RDTs ie. 4 RDTs will be tested with a QC samples containing 100 parasites/ml.
4. Use the negative control to test only one RDT i.e. only 1 RDT will be tested.
5. One hour before RDT testing, thaw a 50ml aliquot of the required QC samples. Refer to SOP 5.3 for thawing procedure.
6. From the RDT lot remove a total of 9 RDTs, using at least 2 different boxes at each dilution. RDTs should be brought to room temperature BEFORE OPENING the package for testing.
7. Check integrity of RDT packaging when opening, ensure no signs of moisture (e.g. silica gel desiccant, if present, should be blue). If signs of moisture DO NOT use the RDT.
8. Test the RDTs with the above QC samples.
9. Perform RDT testing as per manufacturer instructions. Transfer the blood to the RDT by pipette (See SOP 5.4).
10. Record the results on the QA result sheet (Appendix 8.5).

Note: Do not re-use frozen aliquots of QC samples — ONE USE ONLY.

Appendix 2

RDT "Cool Chain" Guidelines on Transport and Storage of Malaria Rapid Diagnostic Tests

Malaria rapid diagnostic tests are biological tests that can be rapidly degraded by exposure to high temperatures, exposure to moisture, and freezing. Most manufacturers recommend controlled-temperature storage between 2°C and 30°C. Expiry dates are generally set according to these conditions. RDTs stored at temperatures exceeding the recommended limits are likely to have a reduced shelf-life. Real-time temperature stability data and accelerated data on the current lot should be obtained from manufacturers prior to purchase.

In light of the above, a "cool chain" should be developed for shipment and storage of RDTs. This should commence at the site of manufacture, and extend as far as possible to the end-user. Where recommended storage conditions are breached, procedures should be in place to monitor sensitivity or replace the suspect tests. Health services using RDTs should have a designated person (e.g. at national level) with responsibility for overseeing all aspects of RDT transport, storage, and quality assurance.

The following procedures should be in place:

1. Before shipping from manufacturer: The manufacturer contacts consignees with details of air waybill numbers, airline carrier, flight number, numbers of containers, expected arrival time.
2. The shipper (courier) is notified of temperature storage requirements by the manufacturer in writing and by clear markings on cartons and related documents. (Storage of the shipment close to the skin of the aircraft in transit may result in freezing.)
3. The manufacturer initiates shipment only when the consignee confirms the shipping notification is received. Shipping times should be arranged to avoid delays due to weekends/public holidays.
4. Consignees or couriers arrange to have customs agents or other personnel on site to receive materials – avoiding delay on airport tarmacs, in customs sheds or in vehicles parked in the sun.
5. Shipments are moved immediately to moderate temperature storage (less than 30°C if possible, 4°C is ideal, do not freeze) .
6. Ground transportation during any stage of delivery is carried out without delay and with attention to temperature within the vehicle at all times, including when parked.

7. Storage at central and final field facilities at 30° C or less wherever possible.
8. RDTs with moisture-proof packaging damaged in transit should be discarded.

Transport and storage at temperatures above 30°C is sometimes unavoidable, as in many remote locations where RDTs are intended for use. In such cases, storage conditions should be as cool as possible and exposure to direct sunlight or storage under hot roofing avoided. Major storage facilities should have temperature monitoring and recording. All RDT lots should have regular monitoring of sensitivity while in use (this can be coordinated through WHO). Procedures should be in place to heighten monitoring or replace lots when the specified storage conditions are breached.

For further information, contact:

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Appendix 3

Minimum Trial Standards For Malaria Rapid Diagnostic Test Field Trials

Draft Guidelines

AIMS AND SCOPE

To define minimum standards for field trials of malaria RDTs. These will allow identification of the likely reasons for variation in RDT accuracy, allowing trial results to be used in determining suitability of RDTs for operational use. Some issues common to the conduct of all clinical trials are not included, and can be found in reviews elsewhere.[1]

POTENTIAL PROBLEMS/PITFALLS AND GENERAL SUGGESTIONS FOR RDT TRIALS

Transport/storage of RDTs

Most RDT products state that storage should be at 4- 30°C, which is difficult in a field trial in the tropics and impossible for many end-user health workers who will be using the products operationally. RDTs are sensitive to moisture (humidity) and high temperatures. It is therefore essential that storage and transport be carefully controlled and documented. Particular attention is needed during transport; RDTs baked in non-airconditioned vehicles or under tin roofs may rapidly lose sensitivity. Protection from mechanical damage and minimization of time from package opening to use will reduce exposure to humidity. At the end of the study, it may be useful to test RDTs taken to the study area against samples stored under controlled temperature. This will allow documentation of any deterioration in sensitivity under field conditions.

Local epidemiology

Sensitivity and specificity are dependent on the parasite density of cases, and predictive values are dependent on the parasite prevalence in the group recruited to the study. The study population therefore needs careful definition (recruitment criteria). Recruitment will depend on the aims of the study, and could range from patients fitting defined clinical criteria to recruitment of known parasitaemic patients and non-parasitaemic controls. It is preferablefor parasite density to be recorded,

[1] Bossuyt, P. M., J. B. Reitsma, et al. (2003). "Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative." Bmj 326(7379): 41-4.

if this can be done accurately, and sensitivity and specificity expressed in terms of density. At a minimum, a summary of local epidemiology of the area (previous surveys, local treatment practices etc) must be given.

Test Preparation and Reading

Various studies have documented significant variation between technicians in both RDT preparation and interpretation. RDT sensitivity is also directly proportional to the blood volume used when parasite density is low (up to the manufacturers' specified volume). Accuracy may also vary with the volume of reagent added to the test strip or well. Therefore, use of separate technicians for different products in comparative RDT studies may bias results. Multiple blinded readings and/or rotation of technicians will reduce this. Timing of readings needs to be documented (e.g. "Strictly according to manufacturer recommendations" or otherwise stated). Comment on later changes in results may be useful, though late readings should not be used in analysis. Any technical problems encountered in preparation of the tests should be noted.

Trials aimed at assessing local suitability of products need to address the suitability of the instructions and the technical demands of the products themselves to the proposed end-users (health workers) and the patients. Given the dependence of test sensitivity on user technique, this information may be the most relevant in assessing RDT suitability. The quality of product instructions and training can be documented both quantitatively (sensitivity, specificity, proportion of observed mistakes) and qualitatively (preferences of end-users).

Deterioration with time

RDTs have a limited shelf-life at room temperature. This may cause variation in the sensitivity of the RDT over the duration of the study, particularly at low parasite densities. Designs for prospective studies need to control for this.

Standards for comparison

Most studies employ microscopy as a 'gold standard'. PCR is often more sensitive for detection and species identification, but subject to its own limitations and not generally accepted as a primary means of malaria diagnosis. Comparison with both can allow estimation of relative benefits of RDT compared to present microscopy-based diagnosis. Microscopy of a single blood sample has reduced ability to detect fluctuating parasitaemia (as does an RDT based on a rapidly-cleared antigen), while microscopy, PCR and rapidly-cleared antigens respond more quickly to treatment-related parasite death (in the absence of gametocytes). Microscopy is highly technician-dependent, requiring blinded confirmatory readings.

Recent treatment

Recent treatment may reduce true specificity (persistent antigen after parasite death), or apparent specificity with regard to standard microscopy (reduction of parasite density below microscopy threshold but antigen still detectable). Parasites seen on microscopy post-treatment may not be viable. Documentation of recent treatment must be made, or these cases excluded from the study. Note on the availability of anti-malarial drugs near the study site assists interpretation of results.

SUGGESTED MINIMUM STANDARDS FOR EFFICACY TRIALS OF MALARIA RAPID DIAGNOSTIC TESTS

The following should be identifiable to all individual cases:

1. Details of RDT kits used:
   - 1.1. manufacturer (company name, actual site of manufacture)
   - 1.2. batch number (includes strip, reagents, wells)
   - 1.3. date of manufacture
   - 1.4. date of expiry
   - 1.5. Whether product is under trial or commercially available
2. Record general description of test kits:
   - 2.1. packaging type (sealed individually, multiple strips in same canister etc.)
   - 2.2. state and type of packaging, and whether canisters of test strips have been opened previous to the first patient seen. (RDTs in damaged packaging should not be used)
   - 2.3. inclusion of desiccant with strips
   - 2.4. inclusion of lancets/ capillary tubes etc needed to perform the test (or otherwise note the items used).
3. Description of previous storage /transport conditions since manufacture:
   - 3.1. duration of storage
   - 3.2. general temperature and humidity at storage (monitoring of temperature and humidity if available). RDTs should be stored away from direct sunlight.
   - 3.3. time to complete use from opening of canister (when this packaging is used).
4. Description of trial site:
   - 4.1. climatic conditions (mean local temperature and humidity).
   - 4.2. workplace conditions (type of facility, lighting used for reading RDTs.)
   - 4.3. local malaria situation

5. Description of trial subjects:
   5.1. criteria for patient selection (symptoms and signs, relation to normal selection for treatment, exclusion criteria).
   5.2. demographics (age, sex)
   5.3. recent anti-malarial therapy
6. Description of technique used:
   6.1. time of strip package opening to time of use
   6.2. blood extraction (venous or capillary)
   6.3. blood transfer to strip (device provided by manufacturer or pipette etc)
   6.4. time taken to obtain reading (per manufacturer guidelines, or reason if longer).
7. Record each line on strip separately, including control. Record of intensity is not necessary.
   8. Record organization of RDT readers /technicians
   8.1. one or multiple readers
   8.2. blinding to microscopy, other RDT readers, and preferably to clinical presentation (latter may not be possible in some circumstances).
   8.3. same technician/reader per RDT type, or alternating
   8.4. if possible, identify technicians/readers for later comparison.
   8.5. training/experience of technicians in this RDT use (including recency of training, validation of quality of training).
   8.6. any significant/recurrent problems encountered in kit preparation (including opening of packaging, obtaining blood etc.).
   8.7. record any variation from the exact RDT preparation technique detailed in the manufacturers insert.
9. Consider formal independent qualitative appraisal of 'ease of use' of product by each technician.
10. Microscopy:
   10.1. Reagents used.
   10.2. Time from preparation to staining.
   10.3. pre-qualification and training of microscopists
   10.4. blinding
   10.5. Criteria for counting parasites and assessment of slide negativity, parasite density.
11. Consider collecting dried blood on filter paper or EDTA samples etc. to allow for later clarification through microscopy /PCR. The criteria for

settling discordant results (e.g. PCR, ELISA, independent microscopist) should be formulated beforehand and clearly stated.

12. Sampling size, data analysis. Results should include sensitivity, specificity, positive predictive value, negative predictive value. The kappa statistic can give a useful guide to agreement between readers.
13. Ethical considerations and approval, including treatment guidelines and informed consent.

Notes on Guidelines

1. Note should be made in trial records of whether lot numbers, dates and product labels are on box, or individual test canisters, sachets, cards etc. It must be possible to trace aberrant results back to details of manufacture.

2, 3, 4, 6. RDTs are sensitive to humidity and temperature. The quality of the result may be affected by capillary tubes, pipettes etc. used to collect blood through variation in the droplet placed on the kit. Opening of sachets at a significant time prior to RDT preparation can reduce the result quality of some RDTs.

11. It can be useful to have samples for confirmation of discordant results at a later date. This applies particularly to samples negative by RDT, positive by microscopy, and so would entail collecting samples at the time of initial sampling on all patients. This may not always be possible, particularly if capillary blood (finger-prick) is used, but a drop of blood dried on filter paper and stored with desiccant (obtained from the RDT kits) in a sealed container is often sufficient. Cross-contamination between dried blood samples needs to be avoided.

Note on blinding. RDT strips need to be read independent of microscopy result, and vice versa. Microscopist needs to record on separate sheet without any knowledge of individual test strip results. It is preferable that the microscopist is also ignorant of the overall RDT results. Any repeat microscopy must be done blinded to RDT results and first microscopy.

Preparation and reading of RDTs. RDT results may be sensitive to any deviation from the manufacturers instructions including the timing of addition of buffer and blood, the volumes added, the angle at which dipsticks are placed in wells, and the timing of reading of results. If RDT results are checked later, the time delay between first and second readings should be noted. False-positive results may occur on late reading of some products. Any deviation from the manufacturers instructions must be clearly stated.

Appendix 4

Proposed Phase 3 Trial Protocol

(Draft protocol for proposed multi-centre phase 3 trial of malaria RDTs. WHO Informal Consultation on RDT, WPRO, January 2003.)

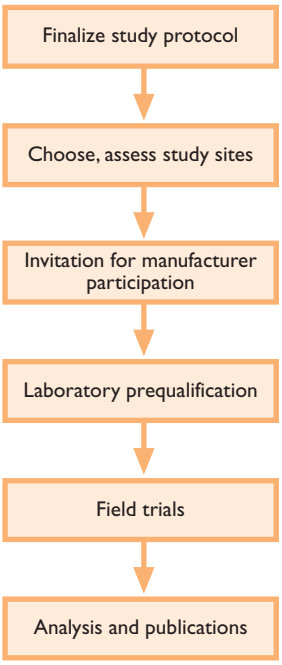
The following protocol is devised as an example of a large field trial consistent. Performance of a field trial based on these methods are under consideration by WHO.

Aim of Trial

Primary: Assess the potential accuracy of RDTs in the field.

Secondary: Measure ease of use of the RDTs.

The aim is to the ability of a range of RDTs to perform with high accuracy, not to rank these in order of accuracy.



Expected Outcomes

Efficacy of 5 RDTs in ideal field conditions in 3 separate sites.

Qualitative comment on the ease of use of the RDTs using the kit supplied by the manufacturer.

Guidance to potential purchasers-users on product quality, and potential sensitivity of products.

Overview of Study Design

It is proposed that the trial involve three field sites, one in each continent with endemic malaria.

The trial will be coordinated centrally (WPRO), but involve responsible regional WHO offices. Field work will be performed by collaborating institutions. The number of RDTs involved would be restricted to maintain quality of the trial and keep the trial manageable logistically.

Entry will depend on the willingness of the manufacturer to participate, satisfaction of a number of specific entry criteria, and passage through a laboratory-based assessment.

Results will be disseminated in brief form by WHO as soon as analysis is complete, and published in detail in a peer-reviewed journal.

Organization and Coordination

Draft protocols for the trial, including criteria for site selection, will be developed by WPRO and circulated to appropriate experts, manufacturers, WHO personnel for comment.

Work outside WPRO will be conducted with collaboration of appropriate focus of the regional WHO office.

Collaborating institutions will be visited before and during the trial by a designated WHO person/consultant.

RDTs will be provided free of charge by manufacturers, and storage and transport to study sites coordinated through WPRO.

A panel of 3 independent experts in the field will be designated to oversee problems that arise during field work resulting from, or requiring departure from, the protocol.

Results will be disseminated to WHO country offices, participating manufacturers and the WHO RDT website as soon as they are confirmed by the review panel and WHO. Agreement will be sought with a peer-reviewed journal prior to the panel to publish the detailed methodology and results. However, release of main outcomes for each product by WHO should not be delayed by a formal publication process.

RDT Qualification for Study, and Pre-Qualification Laboratory Trial

Criteria for qualification of RDTs for the field trial are aimed at ensuring the RDTs reach certain basic standards of sensitivity and specificity, that products detecting the main target antigens are represented, that only one of each production line is represented (i.e. not re-branded products), that numbers are manageable in the field, and that the products are suitable for use at lower levels of health services in malaria-endemic countries. A laboratory independent of study-design development and commercial interest will be selected for the pre-qualifying trial. A panel of experts selected by WHO will finalise criteria for qualification and trial design. It is anticipated that up to 5 products will be taken to the field.

Draft criteria for acceptance for pre-qualifying trial

1. Commercially available for under US$3.00 to endemic areas in large lot numbers.
2. RDTs individually packaged in moisture-proof envelopes.

3. Basic equipment to perform test included in box (*lancet*, swab, buffer, pictorial instructions).
4. Generic: manufactured on-site and not re-branded product of other manufacturer.
5. Temperature-stability data available from manufacturers to purchasers.
6. Willingness to provide sufficient RDTs for trial on time, from 2 lots, at no charge (excluding transport).
7. Some evidence of commercial stability, likelihood of remaining in the market place in the foreseeable future.

Draft criteria for passing pre-qualifying laboratory trial

- Attain pre-determined level of sensitivity and specificity for *P. falciparum* against panel of wild-type parasites and endemic negatives in laboratory testing (e.g. sensitivity 90% at 100 - 200 parasite / ìl, specificity 85%).
- For RDTs included for ability to target non-P. falciparum parasites, attain pre-determined level of sensitivity and specificity against *P. vivax* (lower than *P. falciparum* requirement).
- It is anticipated that 5 tests will be taken to the field. If more than the allowable number pass, the best 5 may be chosen (*the sample size to allow likelihood of distinguishing statistical difference will need to be determined).* Providing minimum criteria are achieved, this 5 should include:
  - A pLDH-detecting test
  - An HRP2-detecting test
  - A non-pLDH, non-*P. falciparum* test

At least two (2) lot numbers should be included in the pre-qualifying trial. The products submitted to the trial, and the results, should remain strictly confidential and not be included in the final publication of results. This will make it easier for manufacturers to submit products. The collaborating laboratory would have to agree to such a confidentiality agreement.

Field Trials

It is anticipated that field trials will take place at 3 sites/areas, one in Asia, Africa, and South America. Potential field sites will be identified in collaboration with regional WHO offices, after a call for interest from appropriate institutions. Potential sites will be short-listed according to pre-determined criteria, and assessed through a site visit to confirm suitability. A 'site' may include a small number of alternative points/clinics to allow for local fluctuations in malaria

prevalence. Each site will be visited during the trial to confirm compliance with the study protocol, and a panel of experts will oversee any necessary deviation from the protocol (e.g. time extension). The trial will be coordinated from WPRO.

Draft criteria for field site include

- Ability to perform the trial strictly to the protocol (see below). Includes track record.
- Microscopists of adequate standard and able to follow microscopy protocol
- Adequate parasite prevalence
  - Overall prevalence of >20%, <80% in symptomatic cases
  - 30%, <70% non-falciparum infections if possible (may be lower in Africa)
  - Sufficient cases to complete study in 2 months field work
- Acceptable budget
- Local ethics approval
- Willingness to comply with confidentiality agreements etc.

Sample population

- Eligibility for blood sampling will include:
- informed consent
- age (venipuncture is necessary, so children excluded)
- symptoms (fever within 3 days, or headache consistent with malaria)

Recent anti-malarial drug intake is not a criteria for exclusion, but will be noted for analysis.

Exclusion criteria include severe symptoms requiring hospitalisation.

Sample size

Sample size will be calculated according to the expected parasite prevelence of the field sites to give adequate statistical definition to the detection of *P. falciparum* in the lower and middle of 3 parasite density ranges (?200, 500 – 2000, and >2000 parasite /il) Numbers at each field site will be equal (i.e. site which requires most RDTS will determine numbers for each).

Transport and storage of RDTs

Transport and storage of RDTs will be coordinated from WPRO. RDTs will be stored and transported with temperature monitoring devices, and stored centrally at controlled temperature until shortly before use.

Field protocol

In general, the field protocol will follow that in the guidelines for minimum trial standards (Appendix 2). The major modifications are to microscopy standards. The protocol will be finalised prior to the call for expressions of interest.

The study will be independent of clinical management. If this is normally based microscopy, this will involve a separate slide and a microscopist unrelated to the study. Study microscopists and RDT technicians should be blinded to clinical management.

Specific points

- Patients will be coded with derived codes for samples/RDTs from each patient.
- Blood sampling will be by venipuncture, and blood stored at 4°C in EDTA immediately.
- Two (2) thick and thin blood films will be made immediately with fresh blood.
- Two (2) drops of blood will be dried on filter paper for storage.
- Stored blood will be aliquoted into separate coded samples to ensure blinding of each RDT reader.
- RDTs will be prepared using pipetted blood within 6 hours of venipuncture.
- RDTs will be read after manufacturers specified waiting time and within 2 hours of preparation (the delay allows blinding procedures for readers of multiple tests).
- Readers will vary the RDT product being prepared and read daily. Reader ID will be noted for analysis.

Standard for comparison (further details to be refined by an expert group)

- Microscopy will be the main standard for comparison, and primary results stated on the basis of this.
- The Earle-Perez method for thick blood film preparation will be followed, and microscopy performed using a grid.
- Initial reading will involve 2 microscopists, with a third to read discordant slides. The organisation of microscopists will follow that used in the WRAIR MRDD trials in 2001, with pre-qualification of microscopists performed by the collaborating institution prior to the study.
- Microscopy reagents (stain etc.) will be standardised.
- Microscopists will be blinded to each other and to the RDT results and clinical findings.

Frozen blood and blood dried on filter paper will be retained and considered for use in clarifying discrepant results (e.g. high "false positive" rates).

Add-on study of "Ease of Use"

Once sufficient sample has been obtained, a small number of RDTs of each product will be prepared and read according to manufacturer instructions (i.e. using finger prick and blood transfer devices provided) by a panel of study and clinic personnel, and a qualitative assessment made of ease of use.

Results and Analysis

Analysis will be coordinated by WHO-WPRO and performed centrally. Results will remain closed to analysis until all 3 arms are completed. Analysis will include basic demographic information and recent treatment for malaria. Reporting will be on the basis of parasite density. An expert panel will peer-review prior to release.

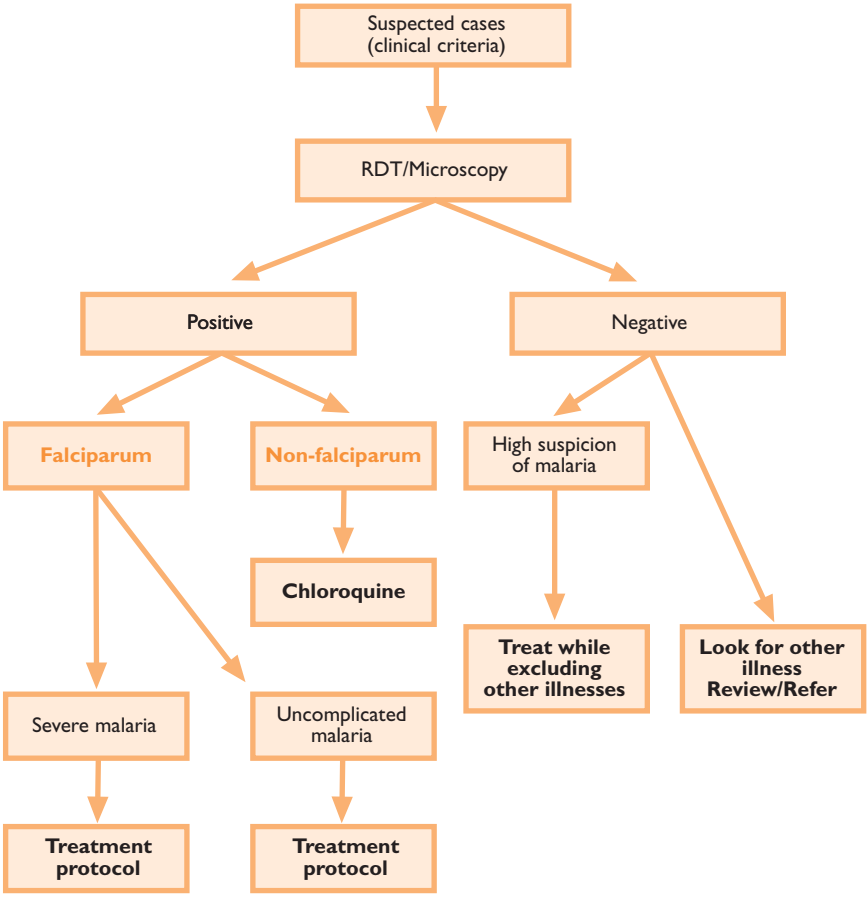
Basic results will be publicised through the WHO, and detailed publication sought in a peer-reviewed journal. WHO will not formally endorse an individual product, but publicise results as a guide to potential purchasers of RDTs.

Appendix 5

Diagnostic Algorithm

Figure A.1

Sample decision chart for treatment of malaria based on the results of a malaria RDT



Derived from model in National Treatment Guidelines for Malaria (2002), Ministry of Health, Kingdom of Cambodia.

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Bibliography

1. Nabarro, D.N. and E.M. Tayler, *The "roll back malaria" campaign.* Science, 1998. 280(5372): p. 2067-8.
2. WHO, *A global strategy for malaria control.* 1993, Geneva: World Health Organization.
3. WHO, *Roll Back Malaria. A global partnership. World Health Organization.* 2000, World Health Organization: Geneva.
4. Chandramohan, D., et al., *A clinical algorithm for the diagnosis of malaria: results of an evaluation in an area of low endemicity.* Trop Med Int Health, 2001. 6(7): p. 505-10.
5. Luxemburger, C., et al., *Clinical features cannot predict a diagnosis of malaria or differentiate the infecting species in children living in an area of low transmission.* Trans R Soc Trop Med Hyg, 1998. 92(1): p. 45-9.
6. Armstrong-Schellenberg, J.R.M. and e. al., *What is clinical malaria? Finding case definitions for field research in highly endemic areas.* Parasitology Today, 1994. 10: p. 439-442.
7. WHO, *New Perspectives: Malaria Diagnosis. Report of a joint WHO/USAID informal consultation 25-27 October 1999.* 2000, World Health Organization: Geneva.
8. Craig, M.H., et al., *Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests.* Trans R Soc Trop Med Hyg, 2002. 96(3): p. 258-65.
9. Cunha, M.L., F. Piovesan-Alves, and L.W. Pang, *Community-based program for malaria case management in the Brazilian Amazon.* Am J Trop Med Hyg, 2001. 65(6): p. 872-6.
10. Garcia, M., et al., *Immunochromatographic test for malaria diagnosis.* Lancet, 1996. 347(9014): p. 1549.
11. Beadle, C., et al., *Diagnosis of malaria by detection of Plasmodium falciparum HRP-2 antigen with a rapid dipstick antigen-capture assay.* Lancet, 1994. 343(8897): p. 564-8.
12. Palmer, C.J., et al., *Evaluation of the OptiMAL test for rapid diagnosis of Plasmodium vivax and Plasmodium falciparum malaria.* J Clin Microbiol, 1998. 36(1): p. 203-6.

13. Quintana, M., et al., *Malaria diagnosis by dipstick assay in a Honduran population with coendemic Plasmodium falciparum and Plasmodium vivax*. Am J Trop Med Hyg, 1998. 59(6): p. 868-71.
14. Rubio, J.M., et al., *Limited level of accuracy provided by available rapid diagnosis tests for malaria enhances the need for PCR-based reference laboratories*. J Clin Microbiol, 2001. 39(7): p. 2736-7.
15. Mason, D.P., et al., *A comparison of two rapid field immunochromatographic tests to expert microscopy in the diagnosis of malaria*. Acta Trop, 2002. 82(1): p. 51-9.
16. Gaye, O., et al., *Diagnosis of Plasmodium falciparum malaria using ParaSight F, ICT malaria PF and malaria IgG CELISA assays*. Parasite, 1998. 5(2): p. 189-92.
17. Huong, N.M., et al., *Comparison of three antigen detection methods for diagnosis and therapeutic monitoring of malaria: a field study from southern Vietnam*. Trop Med Int Health, 2002. 7(4): p. 304-8.
18. Iqbal, J., et al., *Diagnosis of imported malaria by Plasmodium lactate dehydrogenase (pLDH) and histidine-rich protein 2 (PfHRP-2)-based immunocapture assays*. Am J Trop Med Hyg, 2001. 64(1-2): p. 20-3.
19. Jelinek, T., et al., *Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in nonimmune travelers*. J Clin Microbiol, 1999. 37(3): p. 721-3.
20. Mankhambo, L., et al., *Evaluation of the OptiMAL rapid antigen test and species-specific PCR to detect placental Plasmodium falciparum infection at delivery*. J Clin Microbiol, 2002. 40(1): p. 155-8.
21. Leke, R.F., et al., *Detection of the Plasmodium falciparum antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria*. J Clin Microbiol, 1999. 37(9): p. 2992-6.
22. Ricci, L., et al., *Evaluation of OptiMAL Assay test to detect imported malaria in Italy*. New Microbiol, 2000. 23(4): p. 391-8.
23. Hernandez, E., et al., *[Evaluation of the OptiMal test in the diagnosis of imported malarial outbreak]*. Med Trop (Mars), 2001. 61(2): p. 153-7.
24. Stow, N.W., J.K. Torrens, and J. Walker, *An assessment of the accuracy of clinical diagnosis, local microscopy and a rapid immunochromatographic card test in comparison with expert microscopy in the diagnosis of malaria in rural Kenya*. Trans R Soc Trop Med Hyg, 1999. 93(5): p. 519-20.
25. Cho, D., et al., *Evaluation of rapid immunocapture assays for diagnosis of Plasmodium vivax in Korea*. Parasitol Res, 2001. 87(6): p. 445-8.
26. Tjitra, E., et al., *Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for detection of Plasmodium falciparum and Plasmodium vivax in patients with*

a presumptive clinical diagnosis of malaria in eastern Indonesia. J Clin Microbiol, 1999. 37(8): p. 2412-7.

27. Singh, N., A. Saxena, and N. Valecha, *Field evaluation of the ICT malaria P.f/P.v immunochromatographic test for diagnosis of Plasmodium falciparum and P.vivax infection in forest villages of Chhindwara, central India.* Trop Med Int Health, 2000. 5(11): p. 765-70.
28. Atkins, P.W., *Physical Chemistry*. 6 ed. 1998, Oxford: Oxford University Press.
29. Mayxay, M., et al., *Persistence of Plasmodium falciparum HRP-2 in successfully treated acute falciparum malaria.* Trans R Soc Trop Med Hyg, 2001. 95(2): p. 179-82.
30. Moody, A., et al., *Performance of the OptiMAL malaria antigen capture dipstick for malaria diagnosis and treatment monitoring at the Hospital for Tropical Diseases, London.* Br J Haematol, 2000. 109(4): p. 891-4.
31. Eisen, D.P. and A. Saul, *Disappearance of pan-malarial antigen reactivity using the ICT Malaria P.f/P.v kit parallels decline of patent parasitaemia as shown by microscopy.* Trans R Soc Trop Med Hyg, 2000. 94(2): p. 169-70.
32. Tavrow, P., E. Knebel, and L. Cogswell, *Using quality design to improve malaria rapid diagnostic tests in Malawi.* 2000, Quality Assurance Project (QAP) for the United States Agency for International Development: Bethesda, Maryland.
33. Funk, M., et al., *MalaQuick versus ParaSight F as a diagnostic aid in travellers' malaria.* Trans R Soc Trop Med Hyg, 1999. 93(3): p. 268-72.
34. Trachsler, M., P. Schlagenhauf, and R. Steffen, *Feasibility of a rapid dipstick antigen-capture assay for self-testing of travellers' malaria.* Trop Med Int Health, 1999. 4(6): p. 442-7.
35. Whitty, C.J.M., M. Armstrong, and R.H. Behrens, *Self-testing for falciparum malaria with antigen-capture cards by travelers with symptoms of malaria.* Am J Trop Med Hyg, 2000. 63(5-6): p. 295-7.
36. Fryauff, D.J., et al., *Performance of the OptiMAL assay for detection and identification of malaria infections in asymptomatic residents of Irian Jaya, Indonesia.* Am J Trop Med Hyg, 2000. 63(3-4): p. 139-45.
37. Jelinek, T., M.P. Grobusch, and H.D. Nothdurft, *Use of dipstick tests for the rapid diagnosis of malaria in nonimmune travelers.* J Travel Med, 2000. 7(4): p. 175-9.
38. Bell, D., et al., *Diagnosis of malaria in a remote area of the Philippines: comparison of techniques and their acceptance by health workers and the community.* Bull World Health Organ, 2001. 79(10): p. 933-41.
39. Kilian, A.H., et al., *Application of the ParaSight-F dipstick test for malaria diagnosis in a district control program.* Acta Trop, 1999. 72(3): p. 281-93.

40. Mharakurwa, S., B. Manyame, and C.J. Shiff, *Trial of the ParaSight-F test for malaria diagnosis in the primary health care system, Zimbabwe*. Trop Med Int Health, 1997. 2(6): p. 544-50.
41. Oduola, A.M., et al., *Plasmodium falciparum: evaluation of lactate dehydrogenase in monitoring therapeutic responses to standard antimalarial drugs in Nigeria.* Exp Parasitol, 1997. 87(3): p. 283-9.
42. Palmer, C.J., et al., *Field evaluation of the OptiMAL rapid malaria diagnostic test during anti-malarial therapy in Guyana.* Trans R Soc Trop Med Hyg, 1999. 93(5): p. 517-8.
43. Wongsrichanalai, C., et al., *Comparison of a rapid field immunochromatographic test to expert microscopy for the detection of Plasmodium falciparum asexual parasitemia in Thailand.* Acta Trop, 1999. 73(3): p. 263-73.
44. Xiao, J., et al., *[Cloning and sequence analysis of histidine-rich protein-II gene fragment of Plasmodium falciparum yunnan strain].* Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi, 1999. 17(3): p. 143-5.

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