

**WORLD HEALTH ORGANIZATION  
REGIONAL OFFICE FOR THE WESTERN PACIFIC**



**Informal Consultation on  
Laboratory Methods for Quality Assurance  
of Malaria Rapid Diagnostic Tests**

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## **MEETING REPORT**

### **WHO INFORMAL CONSULTATION ON LABORATORY METHODS FOR QUALITY ASSURANCE OF MALARIA RAPID DIAGNOSTIC TESTS**

Convened by:

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## **NOTE**

The views expressed in this report are those of the participants in the WHO Informal Consultation on Laboratory Methods for Quality Assurance of Malaria Rapid Diagnostic Tests and do not necessarily reflect the policies of the Organization.

This report has been prepared by the World Health Organization Regional Office for the Western Pacific for governments of Member States in the Region and for those who participated in the WHO Informal Consultation on Laboratory Methods for Quality Assurance of Malaria Rapid Diagnostic Tests, held in Manila, Philippines from 20 to 22 July 2004.

## CONTENTS

Page

ACKNOWLEDGMENT.....	
EXECUTIVE SUMMARY.....	
LIST OF ABBREVIATIONS.....	
1. INTRODUCTION .....	1
1.1 Background and rationale .....	1
1.2 Objectives of the consultation.....	1
1.3 WHO aims for laboratory testing methods for malaria RDTs.....	2
2. SUMMARY OF RECENT DEVELOPMENT ACTIVITY .....	3
2.1 RDT sensitivity and predictive values .....	3
2.2 Relationship between antigen concentration and parasite density .....	3
2.3 Antigen stability .....	4
2.4 The effect of genetic diversity on RDT sensitivity.....	4
2.5 The other issues relating to laboratory SOPs.....	5
2.6 Other RDT QA development.....	5
3. PRODUCTION OF GOOD PANELS .....	5
3.1 Principles for developing methods to produce panels .....	5
3.2 Principles for determining contents of panels .....	6
3.3 Detection of antigen.....	7
4. ASSESSMENT OF RDT STABILITY.....	7
4.1 Early outcomes of heat stability trial.....	7
5. GENERAL REQUIREMENTS FOR TESTING MALARIA RDTs.....	8
5.1 Setting standards for sensitivity .....	8
5.2 When should an RDT be tested?.....	9
5.3 Definition of a product and lot.....	10
5.4 RDT specifications to be assessed in an evaluation scheme .....	11
5.5 Management of an RDT testing scheme .....	11
5.6 Other specifications necessary for RDT procurement.....	12
6. DETAILED FUNCTION OF PROPOSED LABORATORY-BASED MALARIA RDT ASSESSMENT SCHEME.....	12
6.1 Product testing or prequalification scheme .....	12
6.2 Lot testing.....	13
6.3 Panels available to manufacturers .....	13
6.4 Composition of panels .....	13
7. FURTHER DEVELOPMENT OF TESTING METHODS AND PANELS.....	14

ANNEXES

ANNEX 1 - FURTHER DEVELOPMENT REQUIRED..... 17

ANNEX 2 - TIMELINE FOR DEVELOPMENT OF METHODS AND  
PRODUCT TESTING..... 19

ANNEX 3 - FUNDING AND SUSTAINABILITY..... 21

ANNEX 4 - LIST OF PARTICIPANTS..... 23

REFERENCES..... 27

## EXECUTIVE SUMMARY

The increasing use of artemisinin-based combination therapy (ACT) as first-line treatment for malaria has brought about an urgent need for affordable, accurate, parasite-based diagnosis. Rapid diagnostic tests (RDT), based on lateral flow technology, are the only practical way of providing such diagnosis on a wide scale in most endemic areas, as microscopy is difficult to sustain away from major centres. However, wide variations in RDT product quality have underlined the need for a product testing and quality assurance scheme that will ensure the suitability of RDTs for wide-scale use. An initiative to develop quality assurance (QA) methods for malaria RDTs, coordinated by the WHO Regional Office for the Western Pacific, WHO Roll Back Malaria Department (RBM) and the Special Programme for Research and Training in Tropical Diseases (TDR), has been in place since 2002. This report details the findings and recommendations of an informal consultation meeting, held in Manila in July 2004, that reviewed progress in laboratory-based testing methods and designed the laboratory component of a proposed global product-testing scheme.

Testing of RDT stability and sensitivity has been proceeding on a limited scale within a laboratory network coordinated by WHO, based on standard operating procedures developed as part of the malaria RDT QA initiative. Data on the extent of variation in target antigen structure and the influence of this variation on RDT sensitivity, on stability of RDTs and stored parasite panels, and procedures for panel preparation and characterization, were discussed during the consultation, and further research planned to inform the design and operation of RDT quality assessment. The meeting reviewed recommendations of previous WHO consultations on specifications for malaria RDTs, noting in particular a need to specify sensitivity recommendations in terms of antigen concentration and lower limits of detection, but further research on the antigen parasite relationship is required to facilitate this. An increased emphasis was recommended on laboratory-based, rather than field-based assessment of RDT accuracy and stability, with an urgent need for a global product-testing scheme (or pre-qualification), while maintaining and expanding post-purchase monitoring.

A strategy to develop a product-testing and quality assurance scheme was outlined, with clear roles for various institutions in the collaborative network, with development of a representative malaria parasite specimen bank to serve as a basis for:

- a product-testing scheme to guide procurement and set standards for manufacturers;
- methods for quality assurance of product lots after procurement; and
- availability of panels for use in product testing and improvement by developers.

While much of the development work is already underway, the rapidity with which the proposed scheme can be instituted will depend on the availability of further funding. It is recognized that the resultant increased quality of diagnosis will considerably increase the value of substantial funds already committed to malaria case management through improved targeting of antimalarial drugs.

## **List of Abbreviations**

Ab	Antibody
ACT	Artemisinin-based combination therapy
Ag	Antigen
AMI	Army Malaria Institute (Brisbane, Australia)
CDC	Centers for Disease Control and Prevention (Atlanta, United States of America)
CNM	National Center for Parasitology, Entomology and Malaria Control, Cambodia
DRD	Diagnostics Research and Development (TDR/PRD)
EHT	Essential Health Technologies (WHO)
ELISA	Enzyme-linked immunosorbent assay
IP	Institut Pasteur (Phnom Penh, Cambodia)
HIV	Human immunodeficiency virus
HRP2	Histidine-rich protein II
HTDL	Hospital for Tropical Disease (University College of London Hospitals, London, United Kingdom of Great Britain and Ireland)
LLD	Lower limit of detection
Mab	Monoclonal antibody
pLDH	Parasite lactate dehydrogenase
RBM	Roll Back Malaria (WHO)
RDT	Rapid diagnostic test
RITM	Research Institute for Tropical Medicine (Department of Health, the Philippines)
PRD	Product Research and Development (TDR)
QA	Quality assurance
QC	Quality control
PCR	Polymerase chain reaction
SDI	Sexually Transmitted Disease Diagnostics Initiative (TDR/PRD/DRD)
SOP	Standard operating procedure
TDR	UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases
UP	University of the Philippines
WHO	World Health Organization

## 1. INTRODUCTION

### 1.1 Background

Parasite-based diagnosis (etiological) of malaria is of increasing importance due to the increased use of artemisinin-based combination therapy (ACT) and other higher-cost drug combinations, which are necessitated by the development of resistance of malaria parasites to less expensive drugs. Malaria rapid diagnostic tests (RDTs), which detect parasite-specific antigen through lateral flow immunochromatography, have great potential to fill this need for testing in remote areas where good microscopy cannot be maintained. The success of RDTs in improving the targeting of drug therapy, and their acceptance in malaria management by remote health workers and patients, will depend on the reliability and accuracy of the tests and on the ability to demonstrate such accuracy through quality assurance (QA) programmes.

Quality assurance of malaria RDTs was addressed in WHO informal consultations in 1999 and 2003, and discussed in detail in the reports of those meetings [1, 2]. The consultation in 2003 outlined a plan for collection of wild-type parasites for laboratory-based QA, and an outline for multicentre field trials which could form the basis for a laboratory-based QA scheme and define the attributes of various products to guide procurement. The consultation also recommended further investigation of methods for quality control (QC) of RDTs in remote locations and making panels of parasites available to manufacturers to facilitate internal QA and development.

During 2003 and 2004, WHO, through the Regional Office for the Western Pacific, the Roll Back Malaria (RBM) Department of WHO Headquarters, and the [UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases \(TDR\)](#), and in collaboration with a number of institutions and developers, continued to develop a methods manual containing standard operating procedures (SOPs) and methods for QA of malaria RDTs. This work has raised a number of research questions and challenges to the development of a good RDT testing scheme. The continued rapid expansion and development of commercially available products (approximately 30 are now commercially available), the high cost of field trials, and emerging data on target antigens have resulted in a shift in focus towards laboratory-based testing rather than field studies as a means of product quality assessment. The increasing costs of malaria treatment and continued variation in product performance in published trials [3-11] has lent an increased urgency to the need for accurate, evidence-based information on the performance of commercially available RDTs. In view of this, a small informal consultation of experts associated with current development work on laboratory methods for malaria RDT quality assurance was convened in Manila, the Philippines, on 20-22 July 2004. This short report details the findings of the consultation, and plans for further development

### 1.2 Objectives of the consultation

The consultation aimed to bring together representatives of institutions currently collaborating or planning collaboration with WHO on RDT QA development, and some key product developers, to:

- review progress and problems in the development of RDT testing techniques;
- develop closer collaboration between the institutions present;
- plan laboratory-based development necessary to provide a functioning system for testing the accuracy and stability of malaria RDTs; and

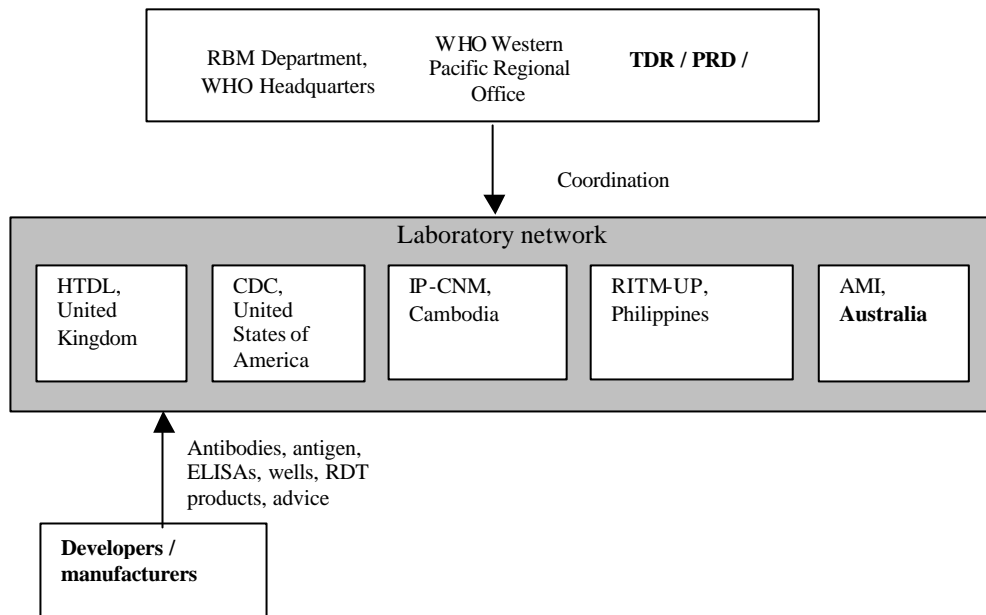


- outline an appropriate laboratory-based system suitable for WHO to adopt to test / pre-qualify malaria RDTs.

### 1.3 WHO aims for laboratory testing methods for malaria RDTs

Quality assurance for RDTs includes all processes for ensuring and maintaining a high quality of diagnostic performance from the manufacturing of the individual components of the devices to their use and interpretation by the end user [2]. These various aspects of QA are being addressed by the WHO Western Pacific Regional Office, RBM, and TDR (Figure 1). The development of laboratory methods for testing RDTs, the subject of this consultation, aims to test RDT accuracy and stability in a repeatable, transparent manner in order to:

- provide advice to countries on appropriate RDT procurement;
- maintain a public list of relative characteristics of commercially available products to guide purchase or recommendation by Malaria Medicines and Supplies Services, and other malaria control programmes and funding agencies;
- provide SOPs and other support to member countries to monitor RDT accuracy centrally and peripherally; and
- make panels available for internal QA and testing by manufacturers and developers.



**Figure 1: Current arrangements for the development of laboratory methods for RDT QA**

## 2. SUMMARY OF RECENT DEVELOPMENT ACTIVITY

Methods for collecting, diluting and preserving wild-type parasites, assembling evaluation panels, and using these panels for testing RDTs have been further developed since the 2003 consultation [2], and are detailed in SOPs developed for trial in laboratories in the Western Pacific Region [12]. For a global and repeatable testing system to be operational, further clarification of the effect of antigen variation, the relationship between antigen concentration and parasite density, and other issues concerning the use of parasites and recombinant antigen, is necessary. Considerable progress has been made in clarifying these issues, some results indicating a need to modify previous recommendations.

### 2.1 RDT sensitivity and predictive values

Previous WHO consultations concluded that 95% sensitivity at 100 parasites/ $\mu$ l of blood is appropriate [1, 2]. In this context, sensitivity is equivalent to the 'lower limit of detection' (LLD) of the RDT. The present consultation concluded that this target should be revised, and a more appropriate target would rely on parasite antigen concentration (see below). Tests that detect histidine-rich protein II (HRP2) were noted to have a lower LLD (i.e. they are more sensitive) than RDTs detecting parasite lactate dehydrogenase (pLDH), though this varied somewhat between commercial products. Aldolase LLD was variable between testing laboratories and products. Apart from product quality, the LLD may be affected by parasite stage, duration of infection, the amount of antigen released by the parasites (this varies between antigens and imposes an absolute threshold for LLD), variation in the structure of some antigens, and host immunity. A parasite density of 100 parasites/ $\mu$ l appears to be close to the absolute limit of detection for some antigens in the lateral flow assay formats presently in use, and may not always be attainable.

### 2.2 Relationship between antigen concentration and parasite density

At a given parasite density, the antigen concentration will depend on:

- the total parasite load (sequestered and circulating parasites: affects *Plasmodium falciparum* only);
- the developmental stage of parasites;
- the accumulation of persistent antigens (e.g. HRP2) with duration of infection;
- antigen expression by the parasite; and
- the persistence of antigen after elimination of parasites.

In parasite panels, the relationship may be further influenced by:

- technique (including microscopy accuracy, dilution accuracy and mixing);
- possible variation in donor blood; and
- possible effects of preservatives or anticoagulants.

Measurement of the relationship may be further influenced by the technique (ELISA) used for quantification of antigen, and the affinity of Mabs in the ELISA kit to the antigen of the particular parasite (isolate or strain).

Significant variation in antigen detection in parasite dilutions of 100 parasites/ $\mu$ l was noted at the consultation. Likely causes are natural variation in antigen concentration due to the reasons listed above, as investigation of possible flaws in technique indicated that such flaws had not occurred. The SOPs had recently been modified to use dilutions of 200 parasites/ $\mu$ l as an interim baseline for testing RDT sensitivity in view of this [12]. This issue needs further clarification.

Assessment of antigen concentration using quantitative ELISA on a limited number of parasite samples indicates a wider variation in the antigen-parasite relationship with HRP2 than pLDH.

### 2.3 Antigen stability

Antigen stability data presented at the consultation indicated greater stability of antigen in blood samples than the SOPs had allowed. Dilutions of 200 parasites/ $\mu$ l maintained at room temperature (20°C – 25°C) for 60 days remained positive to both HRP2 and pLDH RDTs at, by visual comparison, similar test-line intensity. Beyond this period, HRP2 remained detectable, but pLDH was reduced. It was noted that pLDH persisted at detectable levels in samples stored at 4°C for 12 months. Further clarification of these data using ELISA is necessary, but it appears that storage at 4°C for 2–3 days during preparation prior to freezing samples will not significantly impair panel quality.

During long-term storage, panels of *P. falciparum* collected 20 months earlier, diluted to 100 parasites/ $\mu$ l, and stored at -70°C, still produced stable positive reactions on quality-assured pLDH and HRP2 tests, indicating that parasite antigens will not degrade for months or years under such conditions.

### 2.4 The effect of genetic diversity on RDT sensitivity

Evidence presented to the consultation indicated an extensive variation in HRP2 structure from *P. falciparum* isolates within and between countries from which these parasites had been examined, and that this variation is likely to influence the sensitivity of HRP2-detecting RDTs at parasite densities below 500 parasites/ $\mu$ l. HRP2 variation, or at least the range of variation, may vary geographically, but more studies are needed to reach this conclusion. Some HRP2-detecting RDTs are likely to cross-react with other HRP2-like antigens, and this cross-reactivity may modulate the effect of HRP2 variation on RDT sensitivity.

The target epitopes of most existing anti-HRP2 monoclonal antibodies (Mabs) have yet to be defined. Establishing the target epitopes and mapping these epitopes on a range of falciparum malaria isolates from different regions will provide useful guidance concerning the potential for existing Mabs, or combinations of Mabs, to be used in RDTs. It is also necessary to determine the appropriateness of parasite samples and isolates, and use of recombinant HRP2, for assessment of RDT sensitivity.

The extent of variation in pLDH and parasite aldolase is less well defined. Evidence presented at the consultation suggests that pLDH may vary by less than HRP2, but that various isomers exist in some non-falciparum parasites that may potentially influence the sensitivity of pLDH-detecting RDTs. Further investigation is needed to determine whether variation in these antigens is likely to be of significance to RDT sensitivity.

As ELISAs for detection of target antigens generally rely on Mabs, variation in antigen structure and resultant variation in affinity of Mabs for antigen will affect the results of quantitative ELISAs, and will impact on characterization of the antigen concentration in quality control (QC) panels.

Further samples should be obtained from the major geographic regions of malaria endemicity to determine the extent of global antigen variation. Thus the samples should be relatively evenly

distributed and come from the major geographic divisions, including South-East Asia, Papua New Guinea / Oceania, South Asia, East Africa, West Africa, Southern Africa and Central/South America.

## 2.5 Other issues relating to laboratory SOPs

While further data on antigen concentration are being accumulated, interim measures to determine appropriate lower dilutions for RDT testing are needed. These include serial dilution and testing against a quality-assured RDT, to determine the dilution at which this RDT produces a weak, but clear positive result, if 200 parasites/ $\mu$ l dilutions do not produce a positive test line.

A consistently available supply of fresh, parasite-free Type O blood for dilution of blood containing parasites has been a problem. Assessment of the appropriateness of stored blood (e.g. blood-bank discards) and of the effect of various anticoagulants (e.g. citrate, heparin, EDTA) is needed.

Use of preservatives (antimicrobial agents) may be necessary if preparation times from blood extraction to freezing are extended.

## 2.6 Other RDT QA development

A summary of work on the development and use of positive control wells and temperature monitors for remote-area QA, and on assessment of blood transfer methods, was presented to the consultation.

Vaccine vial monitors may offer an inexpensive way of flagging exposure of RDTs to potentially damaging temperatures. One commercially available vaccine vial monitor is showing potential for operating within a useful range for commercially available RDTs, and other products will be investigated.

A study of field use of HRP2-positive control wells and stability of HRP2 and pLDH wells will begin in 2004 in Cambodia and the Philippines.

Densitometers suitable for reading malaria RDT test-line intensity have been assessed in the Philippines, and provide a method to increase consistency of results for laboratory assessment of RDTs by eliminating subjective interpretation and observer variability.

# 3. PRODUCTION OF GOOD PANELS

## 3.1 Principles for developing methods to produce panels

To develop testing panels which reliably predict the accuracy of RDTs in the field, it will be necessary to clearly characterize the range of antigen variation in wild parasites globally (particularly with HRP2 in falciparum malaria), determine the relationship of antigen concentration to parasite density, and then to clearly characterize the contents of samples in a panel, in terms of:

- antigen structure;
- antigen concentration; and

- characteristics of blood<sup>1</sup>:
  - anticoagulants,
  - preservatives,
  - age, and
  - mixing

It will be necessary to establish reliable antigen capture assays to achieve this, and to compare relative effects on RDTs of wild-type parasite specimens, parasites from culture, and recombinant antigens in blood. A drawback in using recombinant antigen is that, when it is added to parasite-free blood, this antigen will be present only in extracellular fluid in the sample, and antigen release through effective lysis of cells and release of parasites by the buffer on the RDT will not be tested.

### 3.2 Principles for determining contents of panels

- QC panels used for testing malaria RDTs must contain antigen that is representative of the range of structural variation (epitope expression) encountered in the field.
- In at least part of the panel, the antigen must be at a concentration representative of the lowest level of malaria infections that a good malaria management programme would expect to detect and treat.
- The panel should also represent the range of antigen concentrations that are likely to be encountered, or at least predict the sensitivity of the RDT to this range.
- All four major human malaria parasite species should be represented.
- The substrate in which the antigen is held must mimic the action of fresh blood on the RDT as closely as possible.
- The panel must be stable and reproducible.

With these characteristics in mind, it is necessary to determine the effects of antigen structure on RDT sensitivity, the relative antigen concentrations in different epidemiological situations and stages of infection, and stability of the panel.

All samples in a panel will need to be characterized by:

- microscopy (stage, species, parasite density);
- species, confirmed by PCR;
- determination of gene sequence of target antigen (species,  $\pm$ antigen structure);
- ELISA (antigen concentration); and
- geographical origin.

Under the SOPs currently in use, the integrity of parasite QC panels is checked using quality-assured RDTs that are stored at 4°C until used [12]. The SOPs need to be modified to specify

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<sup>1</sup> It is necessary to determine which characteristics of preserved blood affect RDT sensitivity, and then characterize the panel accordingly.

measurement of antigen content by ELISA, when adequate reliability of ELISA has been demonstrated.

### 3.3 Detection of antigen

It is necessary to accurately determine the concentration of antigen in each QC sample (at least for samples with low parasite density), and the range of concentrations expected to correlate with a certain parasite density in the field. If the antigen is characterized in terms of structure (HRP2, and other antigens if necessary), the likely field sensitivity of an RDT could be predicted from results of testing using the characterized sample.

Standard curves of antigen concentration in lysed samples and parasite density will therefore need to be developed using antigen capture by ELISA for a range of isolates characterized geographically and clinically. For HRP2, at least, the antigenic structure of each isolate will also need to be known.

Four ELISAs have been under trial for quantitative analysis of parasite antigen, two for HRP2 and two for pLDH. One pLDH and one HRP2 ELISA perform consistently with a linear range of about 200–5000 parasites/ $\mu$ l.

An aldolase ELISA still needs to be developed, and progress has been held up due to delays in obtaining raw materials.

## 4. ASSESSMENT OF RDT STABILITY

Assessment of RDT stability is a necessary component of any QA or RDT testing scheme, as degradation by heat and moisture is likely to be a significant factor in RDT failure in the field.

### 4.1 Early outcomes of heat stability trial

A heat stability trial of five commercial products had been underway in the Hospital for Tropical Diseases (HTDL), Centers for Disease Control and Prevention (CDC) and Research Institute for Tropical Medicine (RITM) prior to the consultation. An assessment of early results from two laboratories indicated:

- a marked difference in temperature stability of products between the two laboratories;
- significant test-to-test variation at borderline parasite density with at least two products;
- consistent difference in temperature stability between products detecting HRP2 compared with products detecting pLDH;
- significant difference in freeze-thaw stability between products detecting HRP2 compared with products detecting pLDH; and
- failure of one product from outset, which was overcome when the cassette was dismantled, the lateral flow strip removed, and blood and buffer placement modified.

Identical RDT lots and blood samples were used, and identical evaluation protocols followed in both laboratories. A difference in storage conditions for blood-parasite samples (liquid nitrogen prior to study and  $-80^{\circ}\text{C}$  during the study, vs.  $-20^{\circ}\text{C}$  at all times) may explain the difference in stability

between them, and this requires further investigation. Degradation during transport from manufacturers to testing sites is also possible.

The reasons for the difference in temperature stability between RDTs targeting different parasite antigens may relate to the:

- stability of other RDT components, and
- stability of the Mabs used in the RDTs.

Further investigation to determine this would be useful, though not necessary specifically for the design of an RDT testing scheme. If the Mabs are less stable, more stable Mabs may be available that target these antigens.

More detailed data on deterioration of RDTs at higher temperatures are required to allow long-term prediction from short-term data.

The heat stability testing protocol could be further improved by improving blinding, standardizing storage of blood, and assessing RDT test signal intensity using a densitometer rather than visual assessment.

## 5. GENERAL REQUIREMENTS FOR TESTING MALARIA RDTs

Requirements for testing RDTs are discussed in a previous publication [2], and issues concerning assessment of RDT sensitivity are discussed above. The 2003 consultation [2] recommended the use of wild-type parasites at high and low dilutions, and that recombinant antigen should be considered in future.

### 5.1 Setting standards for sensitivity

The LLD for RDTs recommended by the previous consultations was 100 parasites/ $\mu$ l [1, 2]. However, the evidence of variation in RDT sensitivity resulting from structural variation of the target antigen and the high variation in parasite density versus antigen concentration, discussed earlier, suggests that setting an LLD in terms of parasite density is not biologically credible. Evidence also suggests that an LLD of 100 parasites / $\mu$ l may not be achievable with some target antigens. This has three major implications:

- (1) The LLD may be better defined in terms of antigen concentration, based on good data on the parasite-antigen relationship.
- (2) Panel constituents should include well characterized wild-type parasites from a wide range of geographical and epidemiological backgrounds, and include culture and recombinant antigen specimens that can be better standardized and controlled.
- (3) Rating of RDTs in terms of LLD against well-characterized samples is likely to be more useful than recording the ability of the RDT to detect an arbitrary low parasite density.

## 5.2 When should an RDT be tested?

A testing scheme for RDTs should provide accurate information:

- to guide procurement of RDTs; and
- on the sensitivity, specificity and stability of RDTs that are in use.

A number of models for RDT product testing schemes exist, including those for HIV, hepatitis B and C, and Chagas disease [13-16], and those for syphilis rapid tests [17]. Disease diagnostics schemes for hepatitis, HIV and Chagas disease rely on product testing in a single reference laboratory according to SOPs developed by WHO. Manufacturers pay for testing, and retesting is at the behest of the manufacturer. The scheme for syphilis diagnostics has relied on testing in eight laboratories in various regions, coordinated by two reference laboratories. Testing has been laboratory-based, and is now moving to a field-based phase. A tuberculosis specimen bank (TDR / PRD / DRD; TDR Diagnostics Research and Development) is also under development for use in future testing schemes.

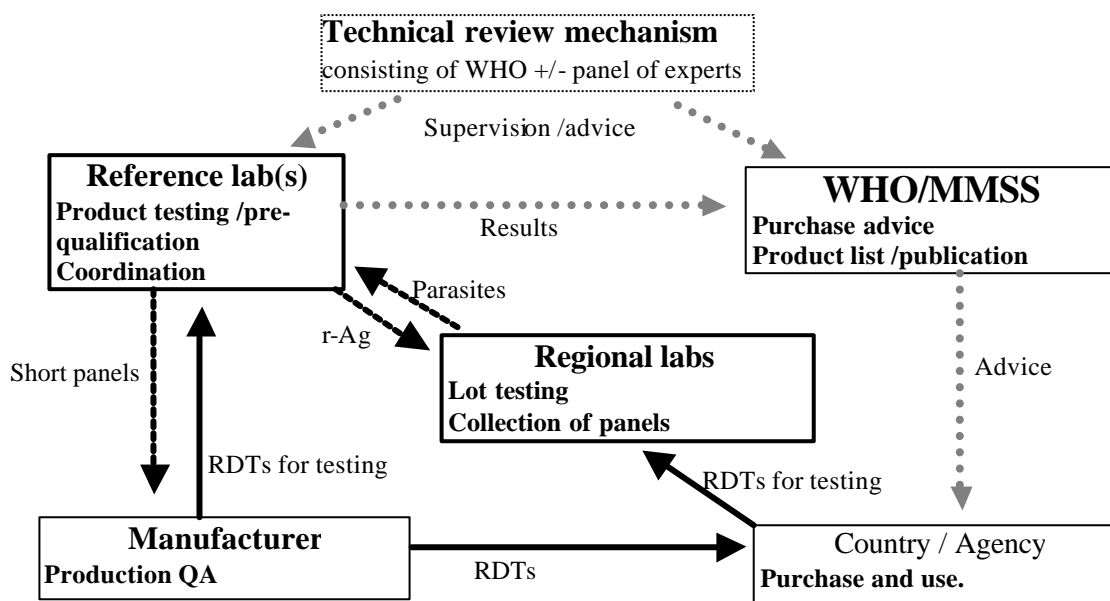
Although malaria RDTs are likely to be used primarily in resource-poor, remote field situations, the consultation agreed that field testing was not ideal for a long-term product testing scheme due to the high cost of recruiting sufficient numbers of malaria patients, the high rate of product modification in the commercial sector, and the difficulty in adequately characterizing the antigen content of blood samples. Evidence presented to the consultation demonstrating lot-lot variation in some products, and structural problems affecting RDT sensitivity, including cassette design and characteristics of nitrocellulose membranes, also indicate that one-time product testing is not sufficient alone to give confidence in product quality, although it is necessary for guiding procurement. Lot testing is therefore also necessary. Lot testing must be standardized and repeatable, simple and inexpensive, and based on parasites derived from the region where the product will be used. In view of this, a two-tier system for product testing was proposed (Figure 2):

- (1) **Product testing** would be used in (at least) two central reference laboratories, using standard panels and SOPs, to assess likely performance of a product in the field. A product need be tested only once, and should include samples from at least two lots.
- (2) **Lot testing** would be used after purchase at a regional level, using shorter panels of local parasite strains, to ensure performance is equivalent to the published product's test results. These panels should also be included in central reference laboratory panels used for product testing.

It was also considered important that manufacturers and developers have access to well-characterized panels to aid product development and for internal QA procedures, and to allow manufacturers to ensure products are suitable for submission to an external product-testing scheme.

The consensus of the consultation was that product testing should occur simultaneously in at least two laboratories, in view of the potential for testing results being affected by damage during transport and storage of RDTs, possible deterioration of panels, and variation in technique and interpretation. Models for RDTs for other diseases encourage manufacturers to visit testing laboratories and demonstrate testing techniques [13-16]. Lot testing should be organized on a regional basis, with enough laboratories to ensure maintenance of standards. These laboratories should have access to locally representative parasites.





**Figure 2: Potential way in which an RDT testing scheme, involving product testing, lot testing and provision of panels to developers and manufacturers, might be organized**

### 5.3 Definition of a product and lot

It is necessary to clearly define the terms 'product' and 'lot' to implement the proposed testing scheme, as product testing results should be applied only to a specifically defined and labelled product, and lot testing results to a clearly defined and labelled lot. The consultation made the following recommendations:

- (1) **Lot** – Defined as one manufacturing run of a product, using the same source and concentration of Mabs, same signal reagent, same buffer, same source of nitrocellulose and same consistency in other constituents of the RDT that may impact on accuracy.
- (2) **Product** – Defining a malaria RDT 'product' for the purposes of a product testing and pre-qualification scheme is more difficult. However, it be based on consistency in overall design and in major constituents of the RDT that are likely to have a significant impact on RDT stability or accuracy. Assuming that evidence of equivalent performance can be provided, the following is recommended:
  - (a) Monoclonal antibodies – A change in target epitope, or of the species from which target antigen for Mab development is derived, should constitute a new product. A change in source (manufacturer) or modifying the amount of Mab used in a test would not constitute a new product if the Mab cell line were originally from the same source.
  - (b) Dye conjugate (signal reagent) – A change in specification or type of label (e.g. colloidal gold, latex particle or liposome) should constitute a new product, but a change in manufacturer / source should not.
  - (c) Nitrocellulose – A change in specifications of the nitrocellulose membrane should constitute a new product. A change in manufacturer should not.

(d) **Format** – A change in assay presentation between, for example, a dipstick, cassette or card constitutes a new product.

(e) **Buffer** – A change in assay buffer constituents or pH does not constitute a new product.

(3) **Equivalence of performance** – Where changes are made that have the potential to significantly affect accuracy of the RDT, including changes in raw materials or components including Mabs, signal reagents, buffers, nitrocellulose membranes, or in cassette design, equivalence of performance data should be provided to the product testing coordinating body to demonstrate that the modified product has a performance equivalent to or better than that previously submitted to formal testing. As this is an activity that should be performed as part of routine internal QA by the manufacturer, demonstration and notification of equivalence should not result in additional costs or workload.

#### 5.4 RDT specifications to be assessed in an evaluation scheme

The aspects of malaria RDT that determine its usefulness vary depending on the situation in which it is intended to be used. Rather than defining certain standards that a test should achieve to be 'acceptable', it is recommended that results of testing and prequalification of various aspects of RDT performance should be listed, together with a guide to interpretation. Models exist for RDTs for other diseases [13-17].

**Sensitivity:** Test sensitivity should be based on serial dilutions of specimens with well characterized antigen down to below an equivalent of 100 parasites/µl. Sensitivity should also be tested against a panel of wild-type antigen variant parasites of approximately 200 parasites/µl. Using serial dilutions, it is useful to report sensitivity in terms of lower limit of detection (LLD; see section 5.1: Setting standards for sensitivity).

**Specificity:** Specificity should be measured with negative blood samples, including samples known to have induced false-positive reactions with malaria RDTs and samples from non-parasitaemic cases with malaria-like symptoms from malaria-endemic areas (see section 6.4: Composition of panels).

**Stability:** Accelerated temperature stability, and some form of assessment of the resistance of packaging to moisture, should be performed.

**Ease of use:** This rating should include the requirement for other equipment, number of steps involved, and clarity of test and control lines.

**Inclusion of necessary equipment:** Inclusion of lancets, swabs, etc. in the box.

**Clarity of instructions:** Instruction on all necessary steps and clarity of presentation.

Note on predictive values: Positive and negative predictive values are less useful in laboratory settings due to their dependence on prevalence, and are more relevant to field trials. If reported, it is useful to adjust predictive values to take the ratio of positive and negative samples in the panel into account.

#### 5.5 Management of an RDT testing scheme

Various issues pertaining to the function of the testing scheme (Figure 2) were identified by the consultation, and need to be addressed by WHO while development of methods for producing and characterizing panels is under way, including:

- supervision and certification of laboratories where testing takes place;

- mode of publishing results;
- legal issues, including potential for litigation by manufacturers or purchasers related to published test results;
- sustainability, and allocation of costs for product and lot testing;
- definition of a 'new product', and notification of product modifications;
- monitoring of transport and storage of RDTs prior to testing; and
- responsibility if RDTs fail the testing procedure.

Funding models include payment by manufacturers [13-16] and provision of RDTs free of charge, with external funding of evaluation [17]. In the long term, product testing at cost by manufacturers would appear more sustainable, while funding of lot testing may involve a combination of external funding and funding by purchasers as part of the QA budget of a diagnostic programme. Provision of panels to manufacturers may provide a means of supplementing programme costs.

#### 5.6 Other specifications necessary for RDT procurement

An important part of QA for RDTs, in addition to testing, is appropriate purchasing. The 2003 WHO consultation on RDTs emphasized issues to consider, including evidence of quality of the manufacturing process and evidence of stability [2]. Discussions with manufacturers since then indicate that many have not achieved certification under the ISO standards scheme, but that this should be achievable without significant cost implications. Requirements were discussed briefly at the 2004 consultation, with consensus that a move to recommend purchase only from manufacturers with such certification is appropriate. ISO13485:2003 is specific for manufacture of medical devices.

## 6. DETAILED FUNCTION OF PROPOSED LABORATORY-BASED MALARIA RDT ASSESSMENT SCHEME

The consultation concentrated on the design of laboratory methods for testing RDTs. Aspects of supervision, organization, funding and RDT assessment-other than sensitivity, specificity and stability-were discussed briefly, and should be the subject of further consultations by WHO and other appropriate bodies.

### 6.1 Product testing or prequalification scheme

WHO or a WHO-designated coordinating body, assisted by some form of expert technical working group, should oversee the overall scheme. A structured consultation with experts and manufacturers should occur when present development work is close to completion, and before the scheme commences. The WHO/coordinating body and technical working group will be responsible for overseeing maintenance of standards, coordinating collection of panels, and the operation of regional laboratories, in collaboration with the reference laboratories.

Product testing should evaluate malaria RDT products against well characterized panels, and against other criteria (section 5) according to SOPs provided by WHO. Testing should occur independently at two or more reference laboratories, and results consolidated for publication.

Products should be submitted for testing by manufacturers either free of charge, or at a charge to the manufacturer to cover costs of testing. This should be coordinated through WHO (RBM) or through a body designated by WHO. It was the consensus of the 2004 consultation that charging to cover the cost would be appropriate. Results should be published in hard copy and posted on the WHO website. This is similar to the model for evaluation of other RDTs [13-17]. Manufacturers will be encouraged to visit reference laboratories to demonstrate tests and discuss procedures.

Manufacturers should notify the WHO /coordinating body of equivalence of performance after changes in product format (section 5.3: Definition of a product and lot). This should then be noted on the website. Major product changes requiring re-testing should also be noted in the publication and on the website. WHO could then recommend that countries/agencies use the website and publication for guidance on purchasing.

## 6.2 Lot testing

Lot testing should use smaller panels and concentrate on ensuring adequate sensitivity, and that stability during shelf life is within the manufacturer's specifications. Testing should occur after procurement on batches submitted by countries and other agencies to QC laboratories, preferably in the same region, and tested according to standard SOPs. A sample from the purchased lot(s) would be sent to one testing laboratory. The WHO/coordinating body/reference laboratories and technical working group should oversee standards and procedures. It will be necessary for WHO regional offices to have a role in this process.

The laboratories would serve as sources of specimens containing wild-type parasites for the product-testing reference laboratories, and reference laboratories will be available to provide confirmatory testing of failed RDTs, using identical panels.

A model for the lot-testing scheme is operating in the Western Pacific Region, where two laboratories-RITM and Institut Pasteur (IP)-test RDTs from countries in the Region against a panel of specimens containing local parasites, store them at 28°C, and monitor sensitivity every three months through the product shelf life. Test reports are sent directly to WHO and the procuring country. There are no good models for sustainable funding for this activity; the Western Pacific Region pilot scheme is currently funded through WHO. An assessment of running costs is necessary, as is a feasibility assessment of possible mechanisms, including payment by users and external funding.

## 6.3 Panels available to manufacturers

Short panels should be available to manufacturers and developers, at a cost sufficient to cover their production and administration costs. Panels should consist of well characterized recombinant antigen and cultured parasites, and be identical to parts of the product-testing panel. Provision of wild-type parasites to manufacturers and developers is not considered an appropriate part of the WHO scheme, as allowing access of commercial bodies to patient blood samples may present potential legal complications which will limit access to parasites for the other arms of the QA scheme.

## 6.4 Composition of panels

Principle: Assess RDT sensitivity against common and unusual parasite isolates and predict sensitivity in different regions, and assess RDT specificity.

Proposed product-testing panel (P) will consist of 200 to 300 samples, lot-testing panel (L) 5 to 10 samples, and panel available to manufacturers (M) 20 to 25 samples (Table 1).

**Table 1. Proposed composition of RDT testing panels**

Sample type	Details	P	L	M
1. Cultured <i>P. falciparum</i>	>20 isolates high parasite density HRP2, pLDH, aldolase sequenced <sup>a</sup>	X		X
2. Recombinant antigen <sup>b</sup>	Serial dilutions to below 100 para/μl equivalent HRP2: ~3 alleles pLDH: x <sup>c</sup> alleles aldolase: x <sup>c</sup> alleles	X	X <sup>d</sup>	X
3. Wild-type <i>P. falciparum</i> <sup>g</sup>	Natural density and 200 para/μl dilution Sites: South Asia South East Asia Pacific (2) Africa (3) South and Central America (2) 10-15 isolates per site Characterized by ELISA, PCR (species), gene sequence ? Characterize by PCR for antigen sequence <sup>e</sup>	X	X <sup>d</sup>	
4. Wild-type <i>P. vivax</i>	5 isolates	X	X <sup>d</sup>	
5. ? chimp <i>P. vivax</i>	5 isolates	X		
6. Wild-type /chimp <i>P. ovale</i>	5 isolates	X		
7. Wild-type /chimp <i>P. malariae</i>	5 isolates	X		
8. Parasite-negative human blood	Anti-nuclear antibody (ANA) 5-10 RPR-TPHA positive 5-10 Rheumatoid factor positive 5-10 Heterophile antibody positive 5-10 Anti-mouse antibody positive 5-10 Clean negatives (none of above) 5-10 Other tropical diseases <sup>f</sup> , including: Chagas dengue typhoid leishmaniasis	X	X <sup>d</sup>	

a Use samples with wide range of epitope expression

b Common sequence

c 1 to 3 sequences, dependent on evidence of significant variation in epitope expression

d Lot testing would use local parasites (*vivax* only if combo RDT) for initial testing. Dilutions of single recombinant antigen used to test sensitivity. Reduced parasite-negative panel (clean negatives only)

e Need to characterize antigen sequence depends on evidence of significance of antigen variation.

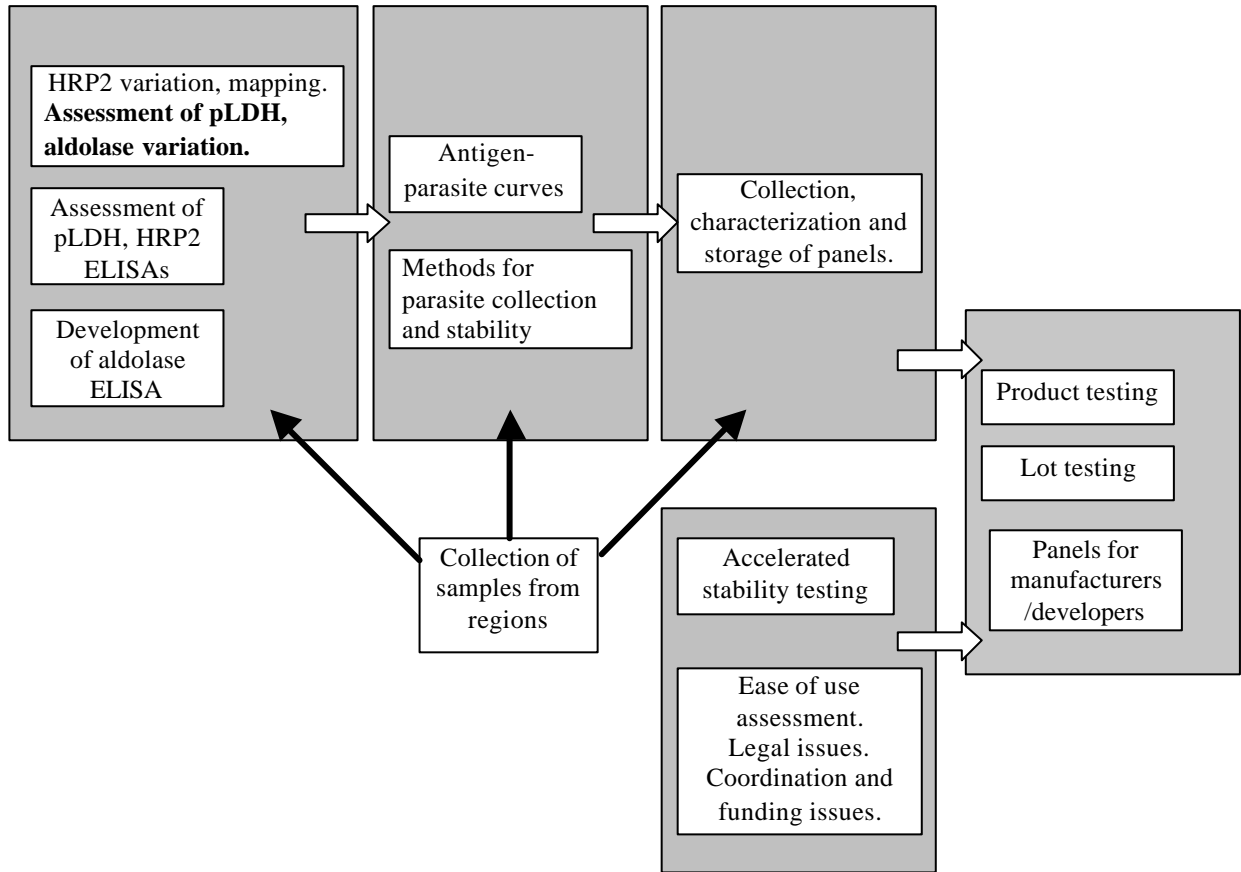
f Tropical fevers that are common differential diagnoses of malaria.

g Aliquots of 50μl provide sufficient volume to test = 4 of most RDT products.

## 7. FURTHER DEVELOPMENT OF TESTING METHODS AND PANELS

In order to develop well characterized panels of parasites for use in testing malaria RDTs, further research and development is necessary in a number of areas, most of which is currently under way or planned within the existing laboratory network (Figure 3). Plans for collaboration between WHO, the network of institutions currently involved with WHO in development work, and other institutions, were developed in more detail during the consultation and are the subject of a separate document for internal circulation. The activities involved are outlined in Annex 1. A potential

timeline for development is detailed in Annex 2. This will depend on adequacy and promptness of funding (Annex 3).



**Figure 3. Outline of development work necessary for RDT testing scheme .**



FURTHER DEVELOPMENT REQUIRED

<b>Wild-type Panel collection.</b>	
Microscopy.	Compare EP, WCC, RCC methods Intra-slide, inter-slide, inter-reader consistency Inter-lab consistency
Donor blood	Donor-donor variation Old vs. new blood Citrate: EDTA : Heparin
<b>Mixing time</b>	
Short-term Ag stability	Ag stability from collection to freezing of aliquots
<b>Panel stability.</b>	
Ag stability when frozen	pLDH, HRP2, aldolase Stability in liquid N <sub>2</sub> , -70°C, -20°C, 4°C,
Freeze-thaw effect on RDT	
<b>Other panel issues.</b>	
Obtain & characterize recombinant Ag	HRP2, pLDH, aldolase
Variation of Ag with parasite stage	
Half-life of HRP2, pLDH, aldolase in-vivo	This is considered useful for understanding the relationship between parasite density and antigen concentration, but this is not essential to QA /product testing development
Ag concentration vs. parasite density	HRP2, pLDH, Aldolase MULTIPLE SITES WITH MICROSCOPICALLY AND CLINICALLY-CHARACTERIZED SAMPLES.
Ag distribution in blood:	Ag activity in cells vs. plasma.
Ag. variation	HRP2 pLDH aldolase Multiple sites blood spot collection Characterization of culture lines
<b>RDT stability</b>	
Stability of component parts	Will determine importance of product changes
Temperature decay curves of whole RDTs, accelerated stability predictions	Test Expert opinion
Assessment of packaging	





TIMELINE FOR DEVELOPMENT OF METHODS AND PRODUCT TESTING

Activity	Approx. time:	2004						2005 <sup>a</sup>						2006+	
		J	A	S	O	N	D								
Technical consultations															
HRP2 variation, mapping.															
Assessment of pLDH, aldolase variation.															
Assessment of pLDH, HRP2 ELISAs															
Development of aldolase ELISA															
Antigen-parasite curves															
Methods for parasite collection and stability															
Collection, characterization and storage of panels						b								c	
Collection of samples from regions															
Ease of use assessment developed															
legal issues, coordination and funding issues.															
Product testing <sup>d</sup>															
Lot testing <sup>e</sup>															
Panels for manufacturers /developers															

a later activities dependent on arrival of funding.

b samples collected in field work and excess to research and on-going QA stored for use in panels

c further collection dependent on range already collected and stability in storage. Expected to need only sporadic replacement from field.

d product testing when sufficient range available in panels, then as needed.

e lot testing on-going in Western Pacific Region, and could commence elsewhere according to need and laboratory capacity.



## FUNDING AND SUSTAINABILITY

Funding to date for the RBM/TDR malaria rapid diagnostics initiative has been provided by AusAID, DFID and USAID through RBM and TDR. Scaling up development work, including procurement of materials and collection and transport of samples, will require a further infusion of funds over the coming months. This should be balanced against long-term savings to anti-malaria programmes in better procurement of RDTs, and resultant gains in more appropriate use of anti-malarial drugs. This is particularly relevant with the rapid increase in funding necessary to procure ACT, for Africa in particular. Capacity for lot-testing in various regions will require further funding. In addition to improvement in quality of diagnosis, this will offer benefits in laboratory capacity building and development of good laboratory practices contained in SOPs for RDT testing.

Long-term programme sustainability will depend on a degree of self-funding, institutionalization as a partially autonomous function, and wide acceptance of the need to maintain and demonstrate quality of malaria diagnosis.

### Initial funding needs:

- Continuing /expanding laboratory-based development work.
- Collecting /transporting samples
- Setting up regional laboratory capacity
- Accumulating and characterizing panels.

### Long-term funding needs:

- Storage of panels
- Product testing and publication
- Lot testing
- Replenishment of panels<sup>2</sup>

### Potential self-funding sources:

- Charge manufacturers fee to cover costs for product testing and publication.<sup>3</sup>
- Provide panels at cost to manufacturers and developers.
- Charge procurers for lot testing to cover (partial) costs.

### Short-term set-up costs:

External funding agencies (Aid agencies, foundations).

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<sup>2</sup> Replenishment of panels will depend on rate of use and stability in storage. Initial evidence indicates that panels may be stable for several years. Evidence will be accumulated through real-time monitoring of antigen activity.

<sup>3</sup> Models of financial sustainability for product testing for HIV and hepatitis rapid diagnostics are mentioned earlier (PART 4: Management of a testing scheme)



LIST OF PARTICIPANTS

1. TEMPORARY ADVISERS

Dr John Barnwell  
Senior Biomedical Research Service  
Chief, Research & Development Laboratories Unit  
Malaria Branch, Division of Parasitic Diseases  
Centers for Disease Control & Prevention  
MS F-36, Bldg.109, Room 1121  
4770 Buford Highway, NE  
Atlanta, GA 30341 U.S.A.  
Tel: +1-770-488-4528; Fax: +1-770-488-4253  
E-mail: wzb3@cdc.gov

Dr Martin Bubb  
Head, Biotechnology Division  
Natal Bioproducts Institute  
Private Bag X9043  
Pinetown 3610, Natal  
South Africa  
Tel: +27-31-7196514; Fax: +27-31-7085614  
E-mail: bubbm@nbi-kzn.org.za

Dr Qin Cheng  
Head, Drug Resistance and Diagnostics  
Australian Army Malaria Institute  
Weary Dunlop Drive, Gallipoli Barracks  
Enoggera QLD 4051  
Australia  
Tel: +61-7-3332 4834; Fax: +61-7-3332 4800  
E-mail: Qin.Cheng@defence.gov.au

Prof Peter Chiodini  
Hospital for Tropical Diseases  
London School of Hygiene & Tropical Medicine  
Department of Clinical Parasitology  
Mortimer Market, Capper Street  
London WC1E 6AU  
United Kingdom  
Tel: +44 20 7387 9300 ext 5418; Fax: + 44 20 7383 0041  
E-mail: peter.chiodini@uclh.org / peterlchiodini@doctors.org.uk

Ms Rebecca Guy  
Epidemiologist/Senior Research Officer  
Epidemiology and Social Research Unit  
The Burnet Institute for Medical Research and Public Health  
GPO Box 2284, Melbourne 3004  
Australia  
Tel: + 61 3 9282 2290; Fax: + 61 3 9282 2100  
E-mail: Rebecca.Guy@burnet.edu.au / becguy@hotmail.com

Ms Jennifer Luchavez  
Senior Science Research Specialist  
Research Institute of Tropical Medicine  
Filinvest Compound,  
Alabang, Muntinlupa City  
Philippines  
Tel: 8072628-32 loc 227; Fax: 842-2245  
E-mail: jluchavez@ritm.gov.ph

Dr Sandra Incardona Mazerand  
Research Scientist  
Laboratory of Molecular Epidemiology  
Pasteur Institute of Cambodia  
#5, Monivong Blvd, P.O. Box 983  
Phnom Penh, Cambodia  
Tel: (855) 12 812 003; Fax: (855) 23 725 606  
E-mail: sandra@pasteur-kh.org

Dr Anthony Moody  
Head Biomedical Scientist  
Department of Clinical Parasitology  
Hospital for Tropical Diseases  
Mortimer Market, London WC1E 3BG  
United Kingdom  
Tel: +44-207-387-9300 ext 5411; Fax: +44-207-383-0041  
E-mail: anthony.moody@uclh.org

Dr Milton Tam  
PATH  
1455 NW Leary Way  
Seattle, WA 98107-5136, U.S.A.  
Tel: +1-206-285-3500; Fax: +1-206-285-6619  
E-mail: mtam@path.org

## 2. OBSERVERS

RESEARCH INSTITUTE  
FOR TROPICAL  
MEDICINE

Dr Fe Esperanza Espino  
Medical Specialist III  
Research Institute for Tropical Medicine  
Filinvest Compound  
Alabang, Muntinlupa City 1781  
Philippines  
Tel: +632 842 2628 to 32 (loc. 801/804); Fax: +632 842 2245  
E-mail: fespino@ritm.gov.ph

NATIONAL INSTITUTES  
OF HEALTH  
UNIVERSITY OF THE  
PHILIPPINES

Dr Vicente Belizario, Jr.  
National Institutes of Health  
University of the Philippines Manila  
P. Gil St., Ermita, Manila  
Tel: +632 526 4266; Fax: +632 525 0395  
E-mail: vbelizar@yahoo.com

## 3. SECRETARIAT

WHO/WPRO

Dr Eva-Maria Christophel  
Medical Officer  
Malaria, Vectorborne and Other Parasitic Diseases  
World Health Organization  
Regional Office for the Western Pacific  
Tel.: +632 528 9723; Fax: +632 521 1036  
E-mail: christophele@wpro.who.int

Dr David Bell  
Scientist, Malaria Diagnostics  
Malaria, Vectorborne and Other Parasitic Diseases  
World Health Organization  
Regional Office for the Western Pacific  
Tel: +632 528 9756; Fax: +632 521 1036  
E-mail: belld@wpro.who.int

Ms Pernille Joergensen  
Associate Professional Officer  
Malaria, Vectorborne and Other Parasitic Diseases  
World Health Organization  
Regional Office for the Western Pacific  
Tel: +632 528 9760; Fax: +632 521 1036  
E-mail: joergensenp@wpro.who.int





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