WHO product testing round 1

WHO-FIND Malaria RDT Evaluation Programme

WHO, with the Foundation for Innovative New Diagnostics (FIND), is coordinating the development of a global specimen bank of cryo-preserved malaria parasite samples to support product testing (comparative performance evaluation of commercially-available antigen-detecting malaria rapid diagnostic tests – RDTs).

The first round of testing commenced in May 2008 at the Malaria Branch, Division of Parasitic Diseases, United States Centres for Disease Control and Prevention (CDC), involving 40 products from 21 manufacturers. Results of this testing will give a comprehensive guidance on the potential performance characteristics of malaria RDTs to national malaria control programmes and other major procuring agencies.

An outline of the testing programme (Product Testing Overview), and the place of product testing within the quality assurance recommendations for malaria rapid tests of the WHO-FIND malaria RDT evaluation programme (WHO Malaria QA Update), are outlined below, together with notices to manufacturers concerning the first testing round. A further round of product testing is expected to be announced later in 2008. Manufacturers who have not received previous notifications should contact WHO through the contact addresses below.

This document compiles all the steps followed during this process with all related letters, documents and supporting materials.

For more information concerning the other rounds, please see:

Round 2 | Round 3 | Round 4 | Round 5

For more information on the WHO-FIND Malaria RDT Evaluation Programme, please see:

http://www.who.int/malaria/areas/diagnosis/rapid-diagnostic-tests
28 August 2007 - Seeking expression of interest in participation in WHO Malaria Diagnostics Evaluation Programme

- Letter to manufacturers
- Annex 1: Information for manufacturers on WHO Malaria Diagnostics Evaluation Programme
- Annex 2: Sample WHO standard confidentiality agreement

31 October 2007 - Expression of interest in first round of product testing (closed)

Clarification of implications for WHO procurement and prequalification of malaria rapid diagnostic tests, and extension of deadline for submission of expressions of interest.

- WHO malaria diagnostics evaluation programme, WHO procurement through webbuy and prequalification
- Overview of malaria RDT product testing programme and testing panels against which evaluation will take place

28 January 2008 - Availability of manufacturer panels: further information on product testing

Information in attachments for manufacturers previously notified of acceptance into the product-testing programme:

- Limit on products to be submitted by each manufacturer (3 products per manufacturer).
- Availability of panels of recombinant antigen for preliminary testing of products by manufacturers.
- Details of payment to be made on submission of RDTs for testing.
- Summary of product testing procedure.
- Provisional timeline for product testing.
- Relationship of product testing to WHO RDT procurement, and to WHO prequalification of malaria RDTs.

Details of actual submission of product will be available at a later date. Note that panels are currently available only to manufacturers notified of acceptance to the first round of product testing, to assist manufacturers in decisions on product submission. Further panels will be available to all manufacturers later in 2008.
24 March 2008 - Evaluation of rapid diagnostic for malaria

Letter to manufacturers for round one of product testing:

- Annex 1: sequence of events and relevant deadlines, contact details and addresses
- Annex 2: WHO standard confidentiality and material transfer agreement
- Annex 3: products to be included for evaluation

15 May 2008 - Methods manual for product testing of malaria rapid diagnostic tests
(WHO-FIND Malaria RDT Evaluation Programme)

- Methods manual (version 1) pdf, 1.47Mb

2 June 2008 - Malaria rapid diagnostic tests accepted into round 1 of product testing

- Product summary list

15 June 2008 - Stability assessment at manufacturing site

Letter to manufacturers –Attachments:

- Information on manufacturing site stability test
- Stability test protocol
- Manufacturing site stability test results form 036a

25 April 2009 - Results of product testing round 1

- Results of WHO product testing of malaria RDTs: Round 1 (2008)
- Executive summary
- Résumé d'orientation
- Interactive performance guide
24 August 2007

Dear Sir/Madam,

RE: WHO Malaria Diagnostics Evaluation Programme

The World Health Organization (WHO) would like to explore the interest of your company in participating in a proposed WHO Malaria Diagnostics Evaluation Programme.

The proposed WHO programme, coordinated by the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR) and WHO Regional Office for the Western Pacific (WHO/WPRO), aims to evaluate commercially available, rapid simple point-of-care antigen-detecting diagnostics for malaria, that are appropriate for use at primary health care settings in developing countries.

Note that WHO is only seeking an Expression of Interest (EOI) at this time. This expression of interest, which should include an indication and description of the product(s) proposed for evaluation, should be submitted to WHO before 19 October 2007, at the latest. Although the submission of an expression of interest will not be binding on either party, such a submission is required for inclusion of products in the first round of testing scheduled to commence at the end of 2007.

/.../

... BNCL: As stated.

cc: MVP Chrono
Further details of the Evaluation Programme are provided in the enclosed information document (Annex 1), including the requirements for submission of a product and the conditions for participation in the Evaluation Programme (such as, for example, the full payment of a testing fee, the signature of a confidentiality agreement, as per the document attached as Annex 2 and the free supply of sufficient quantities of the product(s).

If your company is interested in participating in the WHO evaluation of malaria rapid diagnostics tests (RDTs), please read the enclosed documents carefully, and submit the required EOI within the permitted time-frame.

Please do not pay any amount as testing fee, sign the confidentiality agreement or supply any product(s), until invited by WHO to do so.

We look forward to receiving your company's EOI by 19 October 2007. Meanwhile, you can obtain additional information on the Evaluation Programme by visiting the website www.wpro.who.int/sites/rdt, and/or by sending an email to mal-rdt@wpro.who.int

Yours sincerely,

Dr David Bell
Scientist, Malaria Diagnostics
Malaria, other Vector-borne and Parasitic Diseases

Dr Rosanna Feuling
Diagnostics R&D, PDE
UNICEF/UNDP/World Bank/WHO Special Programme for Research & Training in Tropical Diseases (TDR)
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27, Switzerland
WHO MALARIA DIAGNOSTICS EVALUATION PROGRAMME
AUGUST 2007

ANNEX ONE

INFORMATION FOR MANUFACTURERS ON
WHO MALARIA DIAGNOSTICS EVALUATION PROGRAMME

Dissemination of this document: Email, hard copy and fax to all known malaria RDT manufacturers, Publication on WPRO RDT and TDR Websites.

This document includes details of the WHO Malaria Diagnostics Evaluation Programme and criteria for inclusion of products.

The only action necessary at present for a manufacturer interested in participating in the Evaluation Programme is to send the details of the products considered for submission to the first round of testing to WHO, in accordance with the instructions described below. Such an Expression of Interest will not be binding on either party, but should be received by WHO by 19 October 2007, at the latest, in order to allow for participation in the first round of testing (which is scheduled to commence at the end of 2007).

A. Introduction

WHO is proposing to undertake this Evaluation Programme with a view to assessing the performance of antigen-detecting malaria rapid diagnostic tests (RDT). All product testing will be conducted at the Malaria Branch, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, U.S.A. The programme will be coordinated jointly by the WHO-Regional Office for the Western Pacific (WHO/WPRO) and the UNICEF-UNDP-World Bank-WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR), under a joint work plan with the Foundation for Innovative New Diagnostics (FIND) and funded predominantly by AusAID and the Bill and Melinda Gates Foundation.

Testing will be conducted in two phases. 1100 tests from each of two lots will be required. Phase I of the testing process will be performed against a panel of cryo-preserved preparations of cultured parasites and recombinant antigens. Phase II will be performed against a panel containing diluted cryo-preserved preparations of wild parasites, and parasite-negative samples. The fee for this testing will be at or below cost, between $4000 and $8000. The exact figure will be provided to you before confirmation of submission of product, at the time of our future correspondence.

A short panel prepared from recombinant antigen and cultured *P. falciparum* malaria parasites will be made available to manufacturers for their own quality control testing prior to submission, and for a stability test at the manufacturing site required as part of the product-testing programme. This panel will be a subset of the phase I product testing panel. Manufacturers will be required to cover courier costs for delivery of the panel, to be shipped with dry ice.

Submission of a request for inclusion of products in the initial round of testing will close by 19 October 2007. After this date, further products may be submitted to WHO for assessment only after the initial round of testing is completed in 2008.
Data on product performance during testing will be published by WHO and may be used as a reference for future procurement of RDTs by WHO, other UN Agencies and national health authorities. Manufacturers will be informed of the performance results, in accordance with the terms of the attached sample confidentiality agreement. This confidentiality agreement should not be signed at this stage, but will be required at the time submitted products are accepted by WHO for evaluation.

WHO will list all products evaluated as part of the WHO Malaria Diagnostics Evaluation Programme together with their performance data on a dedicated page of the WHO website and in a hard copy publication. WHO may remove a product from the website list or require re-submission of a product for performance testing if changes in product specifications listed in Appendix A indicate that the RDT should be considered a new product, or performance data obtained from lot testing in the field are considered to be consistently outside those of the product testing programme published by WHO. The manufacturer of a listed product of which the product specifications have been changed, is required to inform WHO of such changes prior to the commercial release of the changed product.

Publication of data on product performance and/or inclusion in the above mentioned website list does not imply that the RDTs in question will actually be procured by WHO or any other party.

Participation in the Evaluation Programme, publication by WHO of the testing results and/or inclusion in the website list may not be used by the manufacturers and suppliers concerned for commercial or promotional purposes. Under no circumstances is a manufacturer or supplier authorized to refer to WHO, the manufacturers' or suppliers' participation in the Evaluation Programme, the publication of the testing results by WHO and/or inclusion in the website list, in any statement or material of an advertising or promotional nature, press release and/or similar public statement and/or other material aimed at promoting the manufacturer or supplier and/or its products.

Publication of the testing results and/or inclusion in the website list does not furthermore in any way imply an endorsement, certification, warranty of fitness or recommendation by WHO of any company or product for any purpose, and does not imply preference over products of a similar nature that are not mentioned. WHO will not accept any liability or responsibility whatsoever for any injury, death, loss, damage, or other prejudice of any kind that may arise as a result of, or in connection with the procurement, distribution and use of any product, as to which WHO has published the testing results and/or which is included on the list.

B. Criteria for entry

Conditions of entry are derived from the recommendations of WHO expert consultations at Geneva, Kismu and Atlanta in 2006.

**Conditions for testing of products as part of the WHO Malaria Diagnostics Evaluation Programme**

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1 It is planned to include a product specific audit as a criteria in the future, through a mechanism to be determined by WHO
1. Submission to WHO of an Expression of Interest by the deadline through completion of the Expression of Interest form attached as Appendix B.

2. Fulfillment of the requirements for listing of products in the WHO website list of commercially available malaria RDTs (http://www.wpro.who.int/sites/rdt) by WHO as detailed in the most recent version of the document Assurance of Good Manufacturing Practice for Malaria Rapid Diagnostic Tests (Appendix C), including:
   a. ISO 13485:2003 or US FDA 21 CFR 820^2
   b. Provision of an acceptable heat stability protocol of internal quality assurance.

   (Manufacturers who already fulfill these criteria and are included in the above-mentioned WHO website list of commercially available malaria RDTs, are not required to submit further evidence concerning this).

3. Signed confidentiality agreement and acceptance of conditions for product testing and publication of results, including the undertaking to perform a stability test of submitted product lots according to a protocol specified by WHO and to submit the results thereof to WHO for publication.

4. Advance payment of costs relating to the evaluation and testing process

5. Free supply and delivery of sufficient tests of single lot of product to testing site(s) designated by WHO (1100 tests per lot).

Note: Re-labelled products that are manufactured at the same site and under the same conditions as a tested product, and fulfill the criteria in Appendix E may be jointly listed with the tested product under the criteria and conditions listed in Appendices D and E.

C. Supply of product for testing

- Two lots of product are required. These should be provided separately, at an interval of at least 2 months (to be specified).
- All RDTs should be received at the testing centre by a given deadline (to be specified) in order to be accepted for product testing.
- All products will be stored at 4°C from time of receipt until actual testing occurs (the product testing site will determine the order in which testing will be conducted)
- Sufficient product of each lot should be provided to conduct both phases of testing against the challenge panel (Towards quality testing of malaria rapid diagnostic tests: Evidence and methods. Manila, World Health Organization, 2006). The first lot should be supplied in accordance with WHO's instructions at the time of the call for final submission of product and the second lot at a later date specified by WHO to the manufacturer. If a product does not display sufficient performance against the Phase 1 panel, the lot will not be tested against the Phase 2 panel.

Important note on RDT format

Manufacturers may submit products in any format. RDTs with the same product name but different format (e.g. cassette and dipstick) are considered separate products and will require

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^2 Acceptance of suitability of USFDA 21 CFR part 820 documentation shall be determined by WHO

separate submission and testing. On publication of the testing results by WHO, the introductory text accompanying the table of product performance characteristics will emphasize the current WHO recommendation that cassettes are preferred to dipsticks for field use in endemic countries. Manufacturers are therefore advised to consider submitting only tests in cassette format.

D. Oversight: Specimen Bank Review Committee

The malaria specimen bank is the repository of characterized samples against which product testing will occur, and includes recombinant antigen, culture-derived and wild-type malaria parasites, and negative samples. The wild-type parasites are collected from a geographically-diverse network of collection sites in Asia, Africa and South America, and prepared according to standard protocols.

A Specimen Bank Review Committee will oversee the technical and logistical aspects of the testing and evaluation process, including the development of Standard Operating Procedures (SOPs), oversight of ethical approval for the collection sites contributing to the Specimen Bank (including submission to the WHO Ethics Committee, and local ethical review board) and oversight of the product testing and reporting of results.

Terms of reference of the Specimen Bank Review Committee:

The Malaria RDT Specimen Bank Review Committee will provide recommendations to WHO on:

- Development and modifications of SOPs for specimen collection and use
- Accumulation and content of the Specimen Bank, and characterization and maintenance
- Policy on access to the Specimen Bank
- Protocols for laboratory-based testing of the accuracy and stability of malaria RDTs, including product testing and lot testing
- Interpretation of the results of product testing, prior to publication.

Composition

Core:

- WHO/TDR [4]
- WHO/WPRO [1]
- Foundation for Innovative New Diagnostics (FIND), [2]
- US Centers for Disease Control and Prevention (CDC), [2]
- Kenya Medical Research Institute (KEMRI) [1]
- Médecins sans Frontières, [1]

Collection sites:

- 1 African, [1]
- 1 non-African, [1]

Specimen characterization centres:

- Hospital for Tropical Disease, UK [1]
- Army Malaria Institute, AU, [1]

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Figure in brackets indicates the number of representatives.
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E. Check-list

Documents to be submitted to WHO by manufacturers before 19 October 2007, to ensure eligibility for product testing

1. Notification of intent to submit product, including product details (Appendix B) to WHO.

Malaria, other Vector-borne and Parasitic Diseases
World Health Organization - Regional Office for the Western Pacific
P.O. Box 2932
Manila, Philippines

Ph: +63 2 5289756
Fax: +63 2 5211036

Mal-rdt@wpro.who.int

For identical products, a letter requesting co-listing should be included when submitting the final requirements for entry into the Product testing programme, and need not be submitted at this stage.

Conditions for product testing, once products listed in the Expression of Interest have been accepted by WHO for evaluation.

WHO may accept products for evaluation, based on:

1. Receipt of a duly completed Expression of Interest by the required deadline; and
2. Fulfillment of the requirements for evidence of good manufacturing practice listed in Appendix C (and currently the criteria for listing of products in the WHO website list of commercially available malaria RDTs at www.wpro.who.int/sites/rdt), including:
   a. ISO 13485:2003 or US FDA 21 CFR 820
   b. Provision of an acceptable heat stability protocol of internal quality assurance.

Manufacturers who already fulfill these criteria and are included in the above-mentioned WHO RDT website list, are not required to submit further evidence concerning this.

If and when WHO accepts a product for evaluation and so notifies a manufacturer, the following actions should be undertaken:

1. Signature and submission of Confidentiality Agreement (as per the attached sample)
2. Payment of fee for product testing (as per WHO's instructions).
3. Supply and delivery of sufficient product to testing site (1100 tests per lot) as per WHO's instructions, at manufacturer expense.

The above actions should be undertaken if and when WHO so notifies the manufacturer. No product testing will take place unless the manufacturer has fulfilled the above conditions by the dates set by WHO and in accordance with WHO's instructions.

5 Acceptance of suitability of USFDA 21 CFR part 820 documentation shall be determined by WHO.
F. Further Information

This document and its Appendices and an outline of the methods for product testing can be found at www.wpro.who.int/sites/rdt. Further information on the Evaluation Programme can be found in:


at [www.wpro.who.int/sites/rdt](http://www.wpro.who.int/sites/rdt)
or can be obtained by sending an email to [mal-rdt@wpro.who.int](mailto:mal-rdt@wpro.who.int)
APPENDIX A

Definition of a 'Product' and 'Lot', (see Towards Quality Testing of Malaria RDT-Evidence and Methods 5.3 (WHO, 2006))

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 should be applied only to a clearly defined and labelled lot.

(1) **Lot.** The definition of a “lot” is the responsibility of the manufacturer. All manufacturers must have ISO 13485:2003 or US FDA 21 CFR 820 certification and an appropriate “lot” definition must be compatible with this.

(2) **Product.** Defining a malaria RDT “product” for the purposes of a product testing scheme is more difficult. However, this definition should be based on consistency in overall design and on the major constituents of the RDT that are likely to have a significant impact on RDT stability and accuracy. Assuming that evidence of equivalent performance can be provided, the following applies:

   (a) Similar but re-labelled products from various manufacturers should generally be considered different products (see joint listing of products below) but may be considered the same product if specifically indicated by the manufacturers concerned.

   (b) **Monoclonal antibodies (Mab)** – 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 originated from the same source.

   (c) **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.

   (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 made 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 WHO 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.
APPENDIX B: Expression of Interest - Notification of interest to submit an antigen-detecting malaria rapid diagnostic test to the WHO Malaria Diagnostics Evaluation Programme.

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1  F: falciparum, V: vivax, O: ovale, M: malariae, Pan: all.
2  'Dipstick' refers to simple nitrocellulose strip which requires placement in well. Cassette / card indicates strip enclosed in (plastic) cassette or card.

Signature_______________________ Name _________________________ Designation / position _________________________

Note: This Expression of Interest should be submitted to WHO [mal-rdt@wpro.who.int, or David Bell, WHO – Regional Office for the Western Pacific, UN Avenue, Ermita, Manila, Philippines] by **19 October 2007, at the latest**. Manufacturers should ensure that receipt is acknowledged. The provision of the required product details in this table, and the provision of evidence demonstrating compliance with the requirements set forth in Appendix C, is necessary in order for the products to be considered by WHO for inclusion in the initial round of product testing. The submission of an Expression of Interest and/or the aforesaid evidence will neither obligate WHO to accept, nor obligate the manufacturer to actually provide, the listed products for product testing. WHO will notify a manufacturer if and when products have been accepted for evaluation. Any product testing will furthermore be subject to the conditions outlined in the document, entitled for ”Information for Manufacturers on WHO Malaria Diagnostics Evaluation Programme”.

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APPENDIX C

ASSURANCE OF GOOD MANUFACTURING PRACTICE FOR MALARIA RAPID DIAGNOSTIC TESTS

July 2007 revision of
Interim criteria for WHO procurement of malaria rapid diagnostic tests (RDT) and listing of products on the WHO malaria RDT website
(First published in April 2006 [effective October 2006] and revised in July 2007)

Notes

1. This document only indicates the criteria which are applied by WHO for evidence of quality manufacturing for WHO's internal list for procurement of malaria rapid diagnostic tests by WHO (through WebBuy) and for the WHO Malaria RDT list (list of commercially available malaria RDTs) published on www.wpro.who.int/rdt, including ISO13485:2003 and US FDA 21 CFR 820. The requirement for a heat stability protocol is set forth in Note 2 below.
2. These revised criteria are effective from 31 October 2006 and have been revised in July 2007.

Purpose of these Criteria

The following criteria will be required by WHO for:
1. the possible procurement by WHO of malaria rapid diagnostic tests (RDT); and,
2. publication of product details on the WHO malaria RDT website (www.wpro.who.int/rdt) product list (list of commercially available RDTs).

Development, Scope and Limitations of these Criteria

The following criteria are derived from recommendations on product testing from WHO informal consultations on malaria rapid diagnostic tests,¹,² and the Informal Consultation on Development of Methods for Testing Malaria Rapid Diagnostic Tests, Geneva, Feb 28-March 2, 2006, the International Standards Organization (ISO)³ and European Union (EU) standards ⁴, WHO recommendations on good manufacturing practices⁵,⁶, and consultations with regulators and manufacturers.

Before including any products in the list of commercially available malaria rapid diagnostic tests as published on www.wpro.who.int/sites/rdt or in WHO's internal list of malaria RDTs for possible procurement by WHO, WHO requires manufacturers to provide information on the conditions under which their malaria RDT are manufactured and sold.

While the information to be provided is based on minimum criteria which are intended to demonstrate appropriate and consistent product quality and support, WHO does not verify the accuracy of the data provided. Acceptance of products for listing indicates that the manufacturer has provided this information. It does not constitute any acceptance by WHO of the accuracy of the information and/or that products in question are of acceptable, assured quality. WHO does not furthermore warrant or represent that the use of the products listed is in accordance with the national laws and regulations of any country, including patent laws. Bearing in mind that the data and information provided for a product depend largely on the manufacturer, these data and information are provided by WHO as is, and WHO makes no representation or warranty, either express or implied, as to their accuracy,
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completeness or fitness for a particular purpose. WHO accepts no responsibility or liability for the reliance on, or use of, such data and information.

Criteria for inclusion on list

In addition to meeting the criteria for inclusion in the WHO website list of commercially available malaria RDTs (http://www.wpro.who.int/sites/rdt/purchasing_rdt.htm), and for listing in internal WHO procurement lists, it will be necessary for manufacturers to provide the following information:

1. Evidence of good manufacturing practice, as documented by either:
   a. evidence of compliance with ISO 13485:2003 (or ISO13485:2001 while this remains current) ; or
   b. evidence of compliance with US FDA 21 CFR 820

   'Evidence' is intended to mean a certification or independent listing of compliance by an independent, accredited body.

2. Evidence of testing for product storage temperature and shelf life in the form of protocols for such testing, and confirmation that data from such testing is freely available to WHO and to the public (Note 2 below). In submitting protocols as evidence of testing, the manufacturer automatically confirms that WHO shall be entitled to disclose such evidence (including the results of the testing) to all interested parties on request. WHO will not be responsible for verifying the accuracy of data or analysis, but requires confirmation that testing took place. In addition, the protocol followed during the testing must be submitted. Products may be listed without this data, but a note provided by WHO on the product list may clarify whether the recommended shelf life is supported by accelerated or real-time data. New protocols should be submitted when significant modifications are implemented (Note 3 below).

Notes

Note 1.a Documentation of good manufacturing practice as per ISO13485:2003 or US FDA 21 CFR part 820 standard

Evidence for 1.a & b shall include a copy of the document clearly specifying compliance with the ISO13485:2003 or US FDA 21 CFR part 820 standard.

Note 2. Stability testing

As evidence of stability testing, WHO will accept protocols submitted by manufacturers that comply with the points listed below. By providing WHO with the required evidence of testing for product storage temperature and shelf life, and confirmation that products have been tested according to these protocols, the manufacturer automatically confirms that WHO shall be entitled to disclose such evidence.

The following standards for stability testing are modified from Hornback, L.A., originally published in IVD Technology, April 2004.


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6 http://www.iso-standards-international.com/iso-13485.htm
A stability study for in-vitro diagnostic device (IVD) reagents has the same elements as those dictated for stability testing of drugs including the following:

- A written stability testing program designed to assess the stability characteristics of IVDs.
- A stability protocol with predefined acceptance criteria that can be correlated to the label claims.
- Testing multiple unique product lots. A stability study is required to use three product lots that are manufactured when the manufacturing process has been well defined and can be consistently executed.
- Evaluation of each stability attribute via a statistically valid sample size and testing intervals. The sample size should be sufficient to overcome the precision of the test method used, considering the cumulative effect of all elements of the test system (i.e., individual reagents and instruments). The test intervals should be chosen so that trends may be discerned from variability of the data. At a minimum, stability testing should continue to one time interval past labeled expiration.
- Control of material storage. For real-time stability testing, the IVD reagents should be stored under the conditions stated on the label (e.g., temperature, humidity, light protection).
- Testing IVD in the same container-closure system as the marketed product.
- Use of reliable, meaningful and specific test methods.

The requirement set forth in the last bullet point implies the use of blood samples containing adequate parasite antigen to produce a clear test line on the RDT near the minimum equivalent parasite density that the RDT is expected to detect.

Use of Accelerated Study Data

Accelerated stability studies are useful for predicting the shelf life of IVD. Such accelerated studies subject IVD to extreme conditions—typically elevated temperatures—to the extent that the device endures significant and measurable deterioration during the testing period. Mathematical extrapolations, such as the Arrhenius equation, are then used to calculate the predicted shelf life of the IVD. However, not all IVD follow a predictable degradation rate. Some products will perform acceptably until they fail, in which case only real-time testing will suffice.

According to the United States Food and Drug Administration's Office of In Vitro Diagnostic Device Evaluation and Safety, accelerated stability studies are acceptable in the following situations:

- establishing preliminary claims in new products only if there is sufficient correlation to an existing product; and,
- supporting implementation of a change to an existing product.

The European standard EN 13640:2000 provides guidance on not only conducting real-time and accelerated stability studies but also making calculations using the Arrhenius equation. Only real-time stability data are acceptable for testing of either newly licensed IVD or major changes to existing IVD, but for purposes of malaria RDTs it is recognized by WHO that accelerated data, used with reference to accelerated and real-time data from pre-existing products, is acceptable as an interim measure.
Note 3. Definition of new and significantly changed products

The following criteria are derived from the recommendations of the Informal Consultation on Laboratory Methods for Quality Assurance of Malaria Rapid Diagnostic Tests, and modified at the Informal Consultation on Development of Methods for Testing Malaria Rapid Diagnostic Tests, Geneva, Feb 28-March 2, 2006.

Criteria for definition of a malaria RDT product requiring submission of new evidence of temperature stability testing at WHO’s request:

- Products from different manufacturers are considered different products unless they meet the criteria set forth in Appendix A (see note on joint-listing of products).
- Monoclonal antibodies – A change in target epitope, or of the species from which target antigen for monoclonal antibody (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.
- **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.
- Format – A change in assay presentation between, for example, a dipstick, cassette or card constitutes a new product.
- **Buffer** – A change in assay buffer constituents or pH does not constitute a new product.

References

APPENDIX D: Request for joint-submission and joint-listing of products as part of the WHO Malaria Diagnostics Evaluation Programme

The following products are manufactured under identical conditions at the same manufacturing site, and are considered re-labelled versions of the same product. It is requested that they be listed as such in the publication of the performance results of product testing by WHO.

It is understood that in the event WHO requires a product to be re-tested due to concerns regarding performance, jointly listed products will be considered as identical and will all be removed from the relevant WHO website lists.

Details of product submitted for testing and manufacturer of submitted product.

<table>
<thead>
<tr>
<th>Name of manufacturer of product submitted for testing</th>
<th>Product name</th>
<th>Plasmodium species targeted¹</th>
<th>Target antigen(s)</th>
<th>Format²</th>
<th>Cassette: Ca</th>
<th>Card: Cd</th>
<th>Contact person and designation</th>
<th>Address</th>
<th>Email URL</th>
<th>Contact numbers</th>
</tr>
</thead>
</table>

Details of product to be listed as identical (re-labelled)

<table>
<thead>
<tr>
<th>Name of owner / user of re-labelled product name</th>
<th>Product name</th>
<th>Contact person and designation</th>
<th>Address</th>
<th>Email URL</th>
<th>Contact numbers</th>
</tr>
</thead>
</table>

Signature_______________________ Name ______________________ Designation / position ______________________

Note: A separate signed letter must be provided by the manufacturer submitting product for testing, and the manufacturer/owner of the re-labelled product.
APPENDIX E

Joint-submission and joint-listing of products

In cases where products with different names are produced on the same production line, a single product may be submitted for testing and identical products may be jointly listed with the results. Such co-submission will require a written application from all the companies concerned and provision of evidence that the products are the same (in the form of a letter signed by the entity indicated as manufacturer (assembler), and a letter signed by the entity or entities indicated as owner(s) of the other products listed as identical). In this context, 'manufacture' or 'assembly' indicates production of the test to a state in which it is contained in packaging designed to protect it from environmental degradation (i.e. moisture-proof envelope), including conduct of the quality assurance process in place to ensure product quality. The name of the company which is the operator of the site of manufacture or assembly will be indicated in the list of jointly listed products, together with the name of the company or companies owning the identical products.

In the case of jointly listed products, if one product included in a list of jointly listed products is deemed by WHO to warrant de-listing due to poor performance on lot testing, all jointly listed products will be de-listed and require re-application for product testing. Where products are submitted individually for testing, they will be deemed to be independently manufactured and removal from the product list will involve only the product(s) named.\(^7\)

\(^7\) In cases where a product is de-listed due to poor performance, WHO may require specific lot testing of other products believed to be the same as the de-listed product.
ANNEX TWO
SAMPLE NOT FOR SIGNATURE
WHO STANDARD CONFIDENTIALITY AGREEMENT

Between

........................................................................................................ having its principal offices at ...........................................

........................................................................................................ (hereinafter referred to as “the Company”);

and

The World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland, (hereinafter referred to as “WHO”).

The Company has developed (a) rapid malaria diagnostic test(s), known under the trademark ……., which test(s) (is) (are) further described in Exhibit 1 attached hereto, (hereinafter referred to as “the Product(s)”, and information relating thereto (hereinafter referred as the “the Information”). WHO is interested in having the Product(s) evaluated and tested in the WHO malaria diagnostics evaluation programme, jointly coordinated by the WHO Regional Office for the Western Pacific (WHO/WPRO) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR), hereinafter referred to as "the WHO Malaria Diagnostics Evaluation Programme".

Therefore, the Parties have agreed as follows:

1. The Company shall disclose and furnish to WHO the Information and sufficient quantities of the Product(s) in order to enable WHO to assess the Information and arrange for such evaluations of the Product(s), as WHO may determine are reasonably necessary to assess the performance of the Product(s) and (its)(their) suitability for use at primary health care settings in developing countries. At the conclusion of the testing and evaluation process, WHO will report the results thereof to the Company and, at the Company’s request and cost, return or destroy the Information and any unused quantities of the Product(s). For the avoidance of doubt, "Information" as used herein does not include the data and information resulting from the testing and evaluation process (which comprises the stability test performed by the Company and submitted to WHO as part of the Evaluation Programme). Such
data and information shall belong to WHO (subject always, however, to the other provisions of this Agreement).

2. If and to the extent the Information has been marked by the Company as "Confidential", WHO shall treat such Information as confidential and proprietary for a period of 5 years after disclosure to it. In this connection, WHO shall take all reasonable measures to ensure that the Information in question is not used for any purpose other than the aforementioned evaluation and testing activities and is not disclosed or provided to any person who is not bound by similar obligations of confidentiality and restrictions on use as contained in this Agreement.

3. WHO shall not be bound by any obligation of confidentiality or restriction on use to the extent it is clearly able to demonstrate that any part of the Information:
   a) was known to WHO prior to any disclosure by the Company to WHO; or
   b) was in the public domain at the time of disclosure by the Company to WHO; or
   c) becomes part of the public domain through no fault of WHO; or
   d) becomes available to WHO from a third party not in breach of any legal obligations of confidentiality to the Company.

4. Except as provided in paragraph 6 below, each Party furthermore undertakes to abide by similar obligations of confidentiality and restrictions on use as contained in paragraphs 2 and 3 above with regard to the testing results and reports generated as a result of this Agreement (regardless of whether or not such results and reports have been marked as "confidential").

5. The provision of Product(s), Information, testing results and reports hereunder shall not in itself be construed as conveying rights under any patents or other intellectual property which either Party may have or may hereafter obtain.

6. Subject to the protection of each Party’s confidential information and the provisions of this paragraph 6, testing results generated under this Agreement may be published by either Party. In order to avoid prejudicing confidential information of the other Party, the submitting Party will transmit to the other Party for its review, the material intended to be published at least 60 (sixty) working days before a proposed publication is submitted to any editor, publisher, referee or meeting organizer. In the absence of an objection by the other Party within that 60-day period concerning
prejudice to its confidential information, and provided that all other conditions of this paragraph 6 have been met, the publication may proceed.

In connection with the foregoing, it is understood and agreed that notwithstanding any other provisions in this Agreement, WHO shall be entitled to evaluate and publish the trial results, and to exclusively control this evaluation and the content of the aforesaid publication, provided that in order to avoid prejudice to the Company’s confidential Information disclosed to WHO pursuant to paragraphs 1 and 2 above, WHO shall submit any proposed publication to the Company for review in accordance with the provisions of this paragraph 6. For the avoidance of any doubt, the Company shall only be entitled to object to a proposed publication if and to the extent it contains any confidential Information of the Company, and not on the grounds that the Company is not satisfied with the trial results and/or does not agree with WHO's evaluation thereof. Similarly, the Company shall not proceed to publication of the testing results without having first submitted its proposed publication to WHO for review in accordance with the provisions of this paragraph 6, it being agreed furthermore that the Company’s publication (or other public disclosure) shall be placed under embargo until WHO has been able to publish the testing results.

All publications of the results of any evaluation and testing activities carried out under this Agreement shall include the following statement:

“This investigation was carried out as part of the WHO Malaria Diagnostics Evaluation Programme”.

Other than as provided herein before, neither Party shall, in any statement or material of an advertising or promotional nature, refer to the relationship of the Parties under this Agreement or to the relationship of the other Party to the Product(s).

7. The Company shall provide the Information and sufficient quantities of the Product(s) to WHO, or WHO’s designee(s), free of charge. Upon receipt of a written request to that effect, the Company shall furthermore pay any and all costs relating to the evaluation and testing process hereunder to WHO, or WHO’s designee(s), in advance, in accordance with WHO’s instructions. In the event that WHO, or its designee(s), do not receive the Information, sufficient quantities of the Product(s) and/or the aforesaid advance payment of costs, WHO shall be under no obligation to arrange for the performance of any evaluation or testing activities in relation to the
Product(s). Any balance of funds provided by the Company, and remaining unspent upon the conclusion of the testing and evaluation process shall be returned to the Company, unless otherwise agreed by the Parties.

8. The Company hereby furthermore confirms that it has taken good note of, agrees with and to the extent applicable, shall abide by, the provisions contained in the document, entitled "Information for Manufacturers on WHO Malaria Diagnostics Evaluation Programme."

9. Any dispute relating to the interpretation or application of this Agreement shall, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the Parties or, in the absence of agreement, with the rules of arbitration of the International Chamber of Commerce. The Parties shall accept the arbitral award as final.

On behalf of WHO:       On behalf of the Company:
Signature:              Signature:
Name:                   Name:
Title:                  Title:
Date:                   Date:
8 October 2007

Dear Sir/Madame:

RE: WHO Malaria Diagnostics Evaluation Programme,
WHO Procurement through Webbuy and Prequalification

This communication provides important information regarding the recent call for Expressions of Interest (EOI) to manufacturers for submission of rapid diagnostic tests to the WHO malaria diagnostics evaluation programme. This call for EOI was issued on 27 August 2007 and asked for responses by 19 October 2007.

In deviation of what is stated in the call for EOI, it has now been decided that, starting 2008, procurement through the WHO WebBuy system will (i.e. as opposed to "may") be restricted to those products that have been accepted for inclusion in the WHO malaria diagnostics evaluation programme. In addition, once the performance data resulting from the first round of testing by the WHO malaria diagnostics evaluation programme has been published, procurement through WHO WebBuy will be further restricted to those products that have been evaluated as part of the aforementioned programme and whose performance has been deemed by WHO to be acceptable (based on criteria developed by WHO as a result of the first round of testing).

It is moreover expected that in 2008, the WHO prequalification programme will be expanded to malaria antigen-detecting rapid diagnostic tests. Priority will be given to rapid diagnostic tests included in the WHO WebBuy system. For further information on the prequalification process, please contact WHO / Essential Health Technologies / Diagnostics and Laboratory Technology (DLT) section.

cc: MVP Chrono
In view of the above changes, the deadline for the submission of expressions of interest in the first round of product testing of antigen-detecting malaria rapid tests will be extended to 31st October 2007. Products submitted after this date will not be eligible for testing by the WHO malaria diagnostics evaluation programme and manufacturers will not be invited to include them in the tender for WHO procurement until the first round of testing and publication of the performance data resulting from this round has been completed (which is expected to be in late 2008).

Information regarding the submission of Expressions of Interest and inclusion of products in the evaluation programme can be obtained at http://www.wpro.who.int/sites/rdt/call_for_testing.htm, or by email to:

mal-rdt@wpro.who.int
belid@wpro.who.int
peelingr@who.int

This communication will be sent by email and facsimile.

Yours sincerely,

[Signature]

Dr David Bell
Scientist, Malaria Diagnostics
Malaria, other Vector-borne and Parasitic Diseases
Aims and Scope

This document provides an overview of the product testing scheme for antigen-detecting malaria rapid diagnostic tests under the WHO – FIND Malaria RDT Evaluation Programme. It describes the composition, characterization and management of the specimen bank, the protocols for product testing and use of product testing results, as well as the evidence and methods on which the specimen bank and protocols are based. The evaluation described provides performance data against a panel designed to mimic natural infections as closely as possible while fulfilling the necessity for standardization of testing. It is not, therefore, intended to fully replace field testing of RDTs.

This paper aims to provide manufacturers and procurers of malaria RDTs with information to guide submission of products to the programme, and to guide interpretation of results.

Acknowledgements

The malaria RDT evaluation programme is a partnership of the World Health Organization - Regional Office for the Western Pacific (WHO/WPRO), the Foundation for Innovative New Diagnostics (FIND) and the UNICEF / UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Disease (TDR).

The specimen bank collection and characterization is performed by RITM, Philippines; CNM, Cambodia; Inst. Pasteur, Cambodia; DMR, Myanmar; University of Lagos, Nigeria; Inst. Pasteur, Central African Republic; IHRDC, Tanzania; KEMRI Kisumu, Kenya; Inst. Pasteur, Madagascar; CIDEIM, Colombia; IMT, Peru; US CDC, Atlanta, USA; Hosp. Trop. Disease, UK; AMI/QIMR, Australia, NBI, South Africa;.


Funding has been provided predominantly by The Bill and Melinda Gates Foundation (through FIND), AusAID (through WHO/WPRO), and WHO/TDR. This document was compiled by Sandra Incardona-Mazerand, under contract to FIND.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of the frontiers or boundaries. The mention of specific companies or of certain manufacturers’ products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this publication is complete and correct and shall not be liable for any damages incurred as a result of its use.
# INTRODUCTION

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-based combination therapy</td>
</tr>
<tr>
<td>AMI</td>
<td>Army Malaria Institute (Queensland, Australia)</td>
</tr>
<tr>
<td>ANA</td>
<td>Anti-nuclear antibody</td>
</tr>
<tr>
<td>BET</td>
<td>Ethidium Bromide</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention (Atlanta, United States of America)</td>
</tr>
<tr>
<td>CIDEIM</td>
<td>Centro Internacional de Entrenamiento y Investigaciones Médicas (Cali, Colombia)</td>
</tr>
<tr>
<td>CNM</td>
<td>National Center for Parasitology, Entomology and Malaria Control (Phnom Penh, Cambodia)</td>
</tr>
<tr>
<td>DMR</td>
<td>Experimental Medicine Research Division (Department of Medical Research, Yangon, Myanmar)</td>
</tr>
<tr>
<td>DNA</td>
<td>Desoxypentose 6-phosphate</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetraacetic Acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
</tr>
<tr>
<td>FIND</td>
<td>Foundation for Innovative New Diagnostics</td>
</tr>
<tr>
<td>GMP</td>
<td>Good Manufacturing Practices</td>
</tr>
<tr>
<td>HAMA</td>
<td>Human Anti-mouse Antibody</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HRP2</td>
<td>Histidine-rich Protein 2</td>
</tr>
<tr>
<td>HTD</td>
<td>Hospital for Tropical Diseases (London, United Kingdom of Great Britain and Ireland)</td>
</tr>
<tr>
<td>ID</td>
<td>Identification number</td>
</tr>
<tr>
<td>IHRDC</td>
<td>Ifakara Health Research and Development Center (Bagamoyo, Tanzania)</td>
</tr>
<tr>
<td>IMT</td>
<td>Instituto de Medicina Tropical (Universidad Peruana Cayetano Heredia, Lima, Peru)</td>
</tr>
<tr>
<td>IPB</td>
<td>Institut Pasteur de Bangui (Bangui, Central African Republic)</td>
</tr>
<tr>
<td>IPC</td>
<td>Institut Pasteur du Cambodge (Phnom Penh, Cambodia)</td>
</tr>
<tr>
<td>IPM</td>
<td>Institut Pasteur de Madagascar (Antananarivo, Madagascar)</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute (Nairobi, Kenya)</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactic Dehydrogenase</td>
</tr>
<tr>
<td>MABs</td>
<td>Monoclonal Antibodies</td>
</tr>
<tr>
<td>MB</td>
<td>Molecular Biology</td>
</tr>
<tr>
<td>NBI</td>
<td>National Bioproducts Institute (xxx, South Africa)</td>
</tr>
<tr>
<td>Non-Pf</td>
<td>'Non Plasmodium falciparum species' (P. vivax, P. malariae, P. ovale)</td>
</tr>
<tr>
<td>Pan</td>
<td>Plasmodium</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Pf</td>
<td>Plasmodium falciparum</td>
</tr>
<tr>
<td>Pm</td>
<td>Plasmodium malariae</td>
</tr>
<tr>
<td>Po</td>
<td>Plasmodium ovale</td>
</tr>
<tr>
<td>Pv</td>
<td>Plasmodium vivax</td>
</tr>
<tr>
<td>QA</td>
<td>Quality Assurance</td>
</tr>
<tr>
<td>QC</td>
<td>Quality Control</td>
</tr>
<tr>
<td>RDT</td>
<td>Rapid Diagnostic Test</td>
</tr>
<tr>
<td>RITM</td>
<td>Research Institute of Tropical Medicine (Manila, Philippines)</td>
</tr>
<tr>
<td>RPR</td>
<td>Rapid Plasma Reagin</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Diagnostics (Seoul, South Korea)</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>TDR</td>
<td>UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases</td>
</tr>
<tr>
<td>UL</td>
<td>University of Lagos (Lagos, Nigeria)</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WPRO</td>
<td>Western Pacific Regional Office of the World Health Organization</td>
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</table>
I. Introduction

Malaria rapid diagnostic tests (RDTs) are filling a valuable and growing role in providing parasite-based diagnosis of malaria in areas where good quality microscopy cannot be sustained. In this role, they can bring great improvements in the early recognition and management of both malarial and non-malarial fever, through demonstration (or exclusion) of antigens specific for malaria parasites in host (patient) blood. Parasite-based diagnosis of malaria is becoming increasingly important as rising resistance of malaria parasites to less expensive drugs has led to rising use of artemisinin-based combination therapy (ACT) and other higher-cost drug combinations. The term 'RDT' in this document refers specifically to antigen-detecting lateral flow (immunochromatographic) tests.

While RDT-based diagnosis is applicable in a range of management conditions, the predominant role is in malaria diagnosis away from major health centres in tropical and subtropical (malaria-endemic) areas. In these areas, transport, storage, re-supply, and supervision are difficult and often limited. In such conditions, health workers must consistently provide an accurate diagnosis to guide the management of malarial and non-malarial fevers that may be rapidly fatal. Therefore, it is essential that malaria RDTs are sensitive and specific, simple to use, and stable in ambient conditions of storage and transport, retaining their accuracy for long periods.[1]

Manufacturers of malaria RDTs are faced with a number of challenges in producing high quality products. Among these has been a lack of established standards for sensitivity, specificity, and stability, and a lack of access to good quality reference material for use in assessing compliance with such standards (an unpublished World Health Organization (WHO) review of 26 manufacturer stability test protocols shows a high variability in both protocols and reference standards). National regulatory authorities in endemic countries face similar problems in designing and implementing appropriate regulatory standards to ensure the tests used in national programmes are of appropriate quality. Procuring agencies require high quality comparative performance data to determine appropriate products for the intended area of use.

The published literature contains evidence of highly variable sensitivity of RDTs in field use (reviewed in [2-6]). While this may partly reflect study design, it is clear that significant problems arise with the accuracy of malaria RDTs in the hands of their intended users, and this is supported by unpublished data from national programmes. Deficiencies in manufacture are likely to account for some shortfalls in quality, but variability in thermal stability,[7] variability of the target antigen (Pf HRP2),[8, 9] and technical requirements for RDT preparation and interpretation also play a role in reducing diagnostic accuracy.

The WHO, in cooperation with a number of research institutions (Annex 1), has been developing standardized methods to test RDT performance, guided by a number of technical consultations since 2002. This programme is currently overseen by the WHO-Regional Office for the Western Pacific (WHO/WPRO) and the UNICEF / UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Disease (TDR) in partnership with the Foundation for Innovative New Diagnostics (FIND), and aims to ensure and demonstrate RDT quality relevant to their predominant area of use.[2, 10] This programme is developing three tiers of quality control (Figure I-1):
• Product testing (comparative performance data of available products) and provision of reference standards for developers and manufacturers
• Lot-testing (testing of product conformity to expected standards at time of purchase)
• Testing at the level of the end-user (to demonstrate accuracy to both health workers and patients).

Figure I-1: Schematic representation of the Malaria RDT quality assurance programme

While there are other essential elements to RDT use, including appropriate training and instructions (job-aids),[11, 12] and the development and implementation of appropriate algorithms for the use of positive and negative results,[13] providing demonstrably good quality RDTs is a precondition to addressing these other aspects of use.

RDT-lot testing for control programmes and manufacturers has been conducted through this programme since 2003 at the Research Institute for Tropical Medicine (RITM) in the Philippines and the Institute Pasteur Cambodge (IPC) and National Malaria Control Centre (CNM) in Cambodia, with support from the Hospital for Tropical Disease (HTD), London, UK, the Centres for Disease Control and prevention (CDC), Atlanta, USA, and the Army Malaria Institute (AMI), Brisbane, Australia.[14, 15] This network is now expanding to include other institutions in Asia, Africa and South America. Development of improved positive control wells suitable for use in remote clinics has been proceeding through a partnership of these institutions and the National Bioproducts Institute in South Africa.[16]

This document describes the first tier of the RDT evaluation programme, Product Testing, and the specimen bank that will support this process. It provides an overview of the protocols used for product testing, composition and management of the specimen bank and of the product testing results, and the evidence and methods on which the specimen bank and protocols are based. The performance data derived from product testing will guide WHO procurement as

Global Specimen Bank
Product Testing

Manufacturer
Good Manufacturing Practice and QA

Regional / country laboratory
Lot Testing

Appropriate storage and timely distribution

District / remote area
Quality Control
Training and Community Education

Good procurement

Appropriate storage

Manufacturers

Good Manufacturing Practice and QA

Regional / country laboratory
Lot Testing

Appropriate storage and timely distribution

District / remote area
Quality Control
Training and Community Education

Global Specimen Bank
Product Testing

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well as procurement recommendations to member states, and form the performance data set for future WHO prequalification of malaria RDTs. This will complement the current WHO requirement for certification of conformity with ISO13485:2003, and for provision of a heat stability protocol and specific product information.
II. Product testing of Rapid Diagnostic Tests

The RDT product testing consists in a laboratory-based assessment of RDTs in terms of sensitivity, specificity, stability and ease of use, against a large specimen panel of well-characterized antigen and parasite samples, using standardized protocols, controlled manipulations and a unique testing location.

The composition of the product testing panel and the testing procedures are designed to ensure that parameters critical to RDT performance remain essentially constant while individual samples in the bank are replaced as it becomes depleted. The results of product testing must be interpreted in light of this, and the assessment is not intended to completely replace field trials in real conditions of use.[3]

A. Testing Site

The rapid diagnostic tests will be evaluated at the Malaria Branch, Division of Parasitic Diseases at the Centers of Disease Control and Prevention (CDC, Atlanta, USA), one of the major operating components of the Department of Health and Human Services (HHS) of the United States of America. This laboratory houses the global specimen bank against which performance is assessed. Part of the specimen bank samples are prepared at the CDC itself, others are shipped from distant production facilities and/or collection laboratories to the CDC for characterization and inclusion in the specimen bank.

B. Contents of the specimen bank

1. Sub-panels of the specimen bank

The specimen bank is intended to be a repository for a comprehensive global reference standard against which the performance of malaria rapid diagnostic tests can be assessed. To supply a reference for tests that detect the targeted Plasmodium antigens, the bank must:

• Provide a range of clinically-relevant antigen concentrations (detection sensitivity)
• Provide a representative variety of antigens, both in terms of polymorphism of the antigen itself (known antigen variants), and in terms of geographic origin of the parasites from which these antigens are derived
• Provide a clinically-relevant standard for stability testing
• Provide a panel of parasite-negative samples that include probable causes of false-positive results and likely differential diagnoses of malarial fever

To achieve these aims, and to allow comparisons between large numbers of RDTs and between RDTs over time with well-characterized and stable testing parameters, the specimen bank has been designed to include:

• Serial dilutions of recombinant antigens, at precise concentrations, for which the equivalence to parasite densities has previously been studied (sub-panel 1)
• Culture-derived parasite samples, diluted to precise parasite densities (sub-panel 2)
• Wild parasite samples derived from infected hosts and diluted to the same precise parasite densities (sub-panel 3)
• Negative control samples, partly with blood factors possibly causing false-positive results, partly corresponding to so-called “clean negative samples” (sub-panel 4)

The contents of each sub-panel and the sample preparation protocols are described in detail in Chapter III and Annex 2.

2. Technical issues and choices

Variations from malaria RDT testing in field trials

While RDTs are designed to test fresh human blood, the requirement for a large and stable panel necessitates the use of cryo-preserved samples. These are prepared in conditions designed to minimize loss of antigen content, and to provide a sample that mimics fresh blood infected with naturally-occurring parasites as much as possible, while fulfilling the requirement for standardized and repeatable testing.[14] However, some differences may occur, both through the effects of preparation and storage on the target antigens, and on the effect of freeze-thaw induced lysis of cells on flow on the RDT. As RDTs lyse cells in fresh blood as an initial step, lysis of the frozen sample is of limited significance, and is essential for standardized internal quality assurance in the manufacturing process.[7]

A further variation from field equivalence is the use of a micro-pipette to supply blood to the RDT device rather than the blood transfer device provided by the manufacturer. This is necessary as blood is obtained from a cryo-tube rather than a finger-prick. It also ensures consistency of testing by reducing the likelihood of operator error (page 13).

Predominance of *P. falciparum* in the parasite sample panels

The four major human malaria species are represented in the specimen bank, with a predominance of *P. falciparum* reflecting the higher importance of this species in terms of disease severity and case numbers. Furthermore, *P. falciparum* is expected to present higher variations between parasite samples in antigen content, due to parasite sequestration and structural variation of the *P. falciparum* specific antigen HRP2 (Page 33).

Antigen levels and parasite densities used for malaria RDT testing

For RDT product testing with recombinant antigens, relevant antigen concentration ranges have been selected on the basis of previous studies of the relationship between the parasite density and the antigen content in the blood. The purpose is to allow comparison of the RDTs lower detection limits, usually situated around 100 parasites per microlitre of blood [17, 18]

Culture-derived and wild parasite samples are diluted to well-calibrated parasite densities, chosen according to the following rationales:

(i) High parasite density = 2000 or 5000 parasite/µL = clinically relevant parasite density which is significantly above the density at which RDT sensitivity variations have been reported. Specimen bank samples at this dilution level are expected to produce strongly positive RDT results, and intend to reveal major dysfunctions of malaria RDTs.
(ii) Low parasite density = 200 parasite/µL = a parasite density close to the lower limit of clinical-relevance, and close to the limit of sensitivity of RDTs reported in field evaluations and of standard field microscopy. Specimen bank samples at this dilution level are expected to be detected, possibly with a faint RDT result, and are likely to detect clinically-significant product and lot-to-lot variations.

(iii) Medium parasite density = 500 parasite/µL = intermediate parasite density prepared for non-\(P. falciparum\) species only (\(P. vivax\), \(P. malariae\) and \(P. ovale\)), for which detection of very low parasite densities is not clinically so critical. Furthermore, various malaria RDTs have been reported to be less sensitive for detection of non-\(P. falciparum\) species, compared with \(P. falciparum\).[4, 19, 20] Specimen bank samples at this dilution level should be detected even when the 200 parasite/µL samples produce negative RDT results.

C. Outline of the product testing protocol

The general principle of the testing algorithm is shown in Figure II-1. The RDT manufacturer must:

• Provide evidence of good manufacturing practice in the form of accreditation of full conformity with ISO13485:2003 (quality manufacturing of medical devices). The close US FDA equivalent US FDA 21 CFR part 820 can be used if a clear indication of full compliance is provided by the US FDA,

• Undertake an in-house real-time stability test and provide results for publication,

• Agree to publication of all performance data once the product is submitted to the programme.

The manufacturer is provided with a short panel of calibrated parasite samples from five \(P. falciparum\) culture lines at high and low parasite densities (high: 2000 or 5000 parasites/µL, low: 200 parasites/µL). This ‘manufacturer panel’ is a subset of the sub-panel 2 of the Malaria Specimen Bank, described into more detail in Chapter III and Annex 2. It allows quality control testing at the manufacturing site prior to product submission, and one of the culture lines is later used for stability testing by the manufacturer. Manufacturers will be required to cover courier costs for delivery of these panel samples.

The testing procedure is performed on two different lots of RDTs provided by the manufacturer. The testing algorithm includes four main steps:

• Phase 1 challenge against recombinant antigens and culture-derived parasites,

• Phase 2 challenge against samples derived from wild parasites and the parasite-negative panel,

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1 NOTE ON USE OF 200 parasites/µL AS LOWER LIMIT FOR RDT EVALUATION: The WHO informal consultations in 1999 and 2003 recommended that RDTs should detect 100 parasites/µL with 95% sensitivity.[2] This is considered a parasite density likely to be indicative of current or impending significant malarial illness in a host with low immunity, and consistent with the lower limit of good quality field microscopy. The relevance of detection of parasites below this density is controversial. The RDT evaluation described here does not indicate a departure from that standard. The variation in the relationship between parasite density and antigen concentration (Page 33), the inherent inaccuracy of microscopy due to chance variation, human error and parasite loss from thick blood films, and the potential for antigen loss during harvesting and cryo-preservation, necessitate the use of a higher parasite density for the product testing.
• Heat thermal stability test (at CDC, and at the site of manufacture),
• A descriptive Ease-of-use assessment.

A product must perform satisfactorily against the Phase 1 panel to proceed to further evaluation. This performance will be determined by the specimen bank steering group and communicated to manufacturers before product submission.

1. 'Phase 1' Panel Test

A first screening step allows the selection of RDT products meeting certain minimal quality requirements. This phase 1 panel test uses samples from sub-panels 2 and 3, for which critical characteristics and production conditions remain unchanged over the years (page 18). RDTs are tested against serial dilutions of recombinant antigens and well-characterized culture-derived *P. falciparum* samples at high and low parasite densities. Only those RDTs with adequate performance will be included in further testing. The minimal requirements for further testing are less stringent, to prevent exclusion of products that may perform adequately on wild parasite samples but not in culture-derived parasite samples: positive RDT results must be obtained with at least one recombinant antigens at the concentration equivalent to 200 parasites/μL, or with a minimal proportion of samples at high parasite density (90% detected samples at 2000 or 5000 parasites/μL) and at low parasite density (50% detected samples at 200 parasites/μL).

2. 'Phase 2' Panel Test

Performance of both RDT lots is further assessed against a panel with a larger number of wild parasite samples from the blood of infected hosts, and with negative control samples (*Figure II-2*). This phase 2 panel test is based on the sub-panel 3, intended to better reflect the variety of parasites originating from different endemic areas of the world, and sub-panel 4 for evaluation of the RDTs specificity using a carefully chosen series of parasite-negative samples.

Sub-panel 3 comprises 100 *P. falciparum*, 20 *P. vivax*, 5 *P. malariae* and 5 *P. ovale* isolates. Due to the difficulty in obtaining mono-species infections of non-*P. falciparum* parasites, *P. vivax*, *P. ovale* and *P. malariae* from non-human primates may be substituted. *P. falciparum* samples are included at low (200 parasites/μL) and high (2000 or 5000 parasites/μL) parasite densities, and additional 500 parasites/μL dilutions are included for non-*P. falciparum* samples. The rationales for deciding the sample numbers and dilution levels for the four species have been previously described (page 9). For blinded reading by technicians, low parasite density samples and *Plasmodium*-negative samples are re-labeled and batches combined before testing. A summary of such working rules ensuring the quality of the whole testing process is given later (page 13).

The sub-panel 4 consists of “clean negative” samples from the blood of healthy individuals, and samples containing blood factors or components known to be associated with RDT cross-reactions, and likely differential diagnoses of clinical malaria.

The results of the phase 2 panel test are expressed in terms of percentage of detected samples at high and low parasite densities (sensitivity), and of true negative results obtained with the
different sets of *Plasmodium*-negative control samples (specificity). Detailed performance results against individual components of the panel will also be available.

3. **Stability testing**

RDTs heat stability is assessed on 2 different RDT lots against a reference *P. falciparum* culture line (Nigeria XII strain), at two parasite densities (including 200 parasites/µL). The choice of a reference culture line allows more standardization of stability tests over the years. The reference parasite strain originates from Africa, and expresses the Pf HRP2 structural group B with a typical antigen concentration (Page 36).

The CDCs stability test algorithm is schematically shown in *Figure II-3*. After baseline testing, the RDTs are incubated for two months at 4°C, 35°C and 45°C (at 75% humidity), and re-tested. If the results indicate a good stability of the product (more than 80% positive results with RDTs stored two months at 45°C), testing may be repeated with RDTs stored 6 months at 4°C and 45°C.

Manufacturers will be required to perform real-time testing on the same 2 RDT lots at the upper limit of their recommended storage temperature and until their specified expiry date, using Nigeria XII strain provided by the specimen bank.

4. **Ease of use assessment**

The ease of use assessment consists in a descriptive evaluation of the RDT product (*Figure II-4*). Assessment focuses on the following critical aspects:

- RDT format
- Total preparation time
- The necessity of time control
- Blood safety
- The quality of the instructions and the languages in which the instructions are provided
- The blood transfer device
- The items included in the RDT package

The description will be accompanied by recommendations on the appropriateness of various parameters for different conditions of use.

5. **Review and publication of results**

All results are reviewed by the Malaria Diagnostics Specimen Bank and Evaluation Steering Group (page 30), which may recommend repeating certain evaluation steps or performance of complementary tests if contradictory results were obtained. A performance report will be sent to the RDT manufacturer, and all results published. Results of the real-time stability assessment by the manufacturer will be published as they become available.

Data on product performance obtained through the WHO Malaria Diagnostics Evaluation Programme may be used as a reference for future procurement of RDTs by WHO, other UN agencies and national health authorities. Manufacturers will be informed of all performance
results, but these results can not be used as evidence of WHO endorsement or 'certification' of a product.

D. Quality assurance of the RDT product testing

Product testing will be conducted according to Standard Operating Procedures (SOPs) developed through prior testing experience and based on recommendations of expert consultations on this subject. A dry run using a limited number of tests and abbreviated panel has been conducted to refine these procedures. The quality of critical steps is controlled, as follows:

- Quality of the malaria RDTs and their use:
  All RDTs are stored in a controlled environment at \( \leq 25^\circ\text{C} \); the pouch is opened and desiccant checked immediately before use; manufacturer instructions are followed with the exception of use of the blood transfer device provided by the manufacturer (a micropipette is used to ensure correct blood volume). A temperature-monitoring device will be included with the RDTs for shipment to the testing site.

- Quality and objectivity of the RDT reading results:
  Three different readings are performed: two independent readings by two technicians, and a third reading with a densitometer to give a quantitative determination of intensity for weak positive reaction. All wild parasite samples at low parasite density are first randomized with a similar number of negative control samples and re-labeled for blinded reading of the RDT results.

- Quality of the specimen bank samples:
  SOPs have been established for the preparation of all specimen bank samples [21]. Recombinant antigens, culture lines of parasites, and wild-type samples are selected taking into account previous evidence and data from specifically conducted studies. All diluted parasite samples are stored and transported at -70°C, and are used only once within 8 hours of thawing (REF Ag Stability Studies if available).

The detailed SOPs will be published prior to commencement of product testing.
Figure II-1: Overview of RDT product testing algorithm

Evidence of quality manufacturing
Signed agreement between manufacturer and WHO

Manufacturer panel
For QC testing by manufacturer

Receive RDTs, store below 25°C

For each of 2 different RDT lots

**PHASE 1 panel test**

**Recombinant Antigens (sub-panel 1): 7 antigens**
- HRP2: 3 variants x 6 dilutions → test with 2 RDTs
- LDH: 2 species (Pf, Pv) x 6 dilutions → test with 2 RDTs
- Aldolase: 2 species (Pf, Pv) x 6 dilutions → test with 2 RDTs

**P. falciparum culture lines (sub-panel 2): 20 lines**
- High parasite density: → test with 1 RDT
- Low parasite density: → test with 2 RDTs

Further testing of RDT products, if:
Reach pass criteria on sub-panel 2 (cultured parasites)
RDT are expected to detect at least 50% of high density samples

**PHASE 2 panel test**

**Sensitivity / Specificity**
Sub-panel 3: wild parasites,
Sub-panel 4: negative controls

**Stability**
Sub-panel 2:
Reference Pf culture line

**Ease of use**
Description of test

Review of results (Malaria Specimen Bank Steering Group)

Product testing report sent to manufacturer and published

Stability
Manufacturer’s agreed protocol

Updated Report (including manufacturer-published stability results) published

Reference:
Pf: culture line

Figure II-2, III-2

Figure II-3

Figure II-4

Figure II-2: Sensitivity and specificity testing of malaria RDTs

For each of 2 different RDT lots

**RDTs detecting P. falciparum**

<table>
<thead>
<tr>
<th>Parasite Type</th>
<th>Density</th>
<th>Samples</th>
<th>Test with</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> wild parasites and negative controls (sub-panels 3 and 4)</td>
<td>High/medium parasite density:</td>
<td>100</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Low parasite density:</td>
<td>100</td>
<td>2 RDTs</td>
</tr>
<tr>
<td></td>
<td>Negative controls:</td>
<td>100</td>
<td>2 RDTs</td>
</tr>
</tbody>
</table>

**RDTs detecting non-P. falciparum species**

<table>
<thead>
<tr>
<th>Parasite Type</th>
<th>Density</th>
<th>Samples</th>
<th>Test with</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vivax</em> wild parasites and negative controls (sub-panels 3 and 4):</td>
<td>High parasite density:</td>
<td>20</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Medium parasite density:</td>
<td>20</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Low parasite density:</td>
<td>20</td>
<td>2 RDTs</td>
</tr>
<tr>
<td></td>
<td>Negative controls:</td>
<td>20</td>
<td>2 RDTs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasite Type</th>
<th>Density</th>
<th>Samples</th>
<th>Test with</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. malariae</em> wild parasites and negative controls (sub-panels 3 and 4):</td>
<td>High parasite density:</td>
<td>5</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Medium parasite density:</td>
<td>5</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Low parasite density:</td>
<td>5</td>
<td>2 RDTs</td>
</tr>
<tr>
<td></td>
<td>Negative controls:</td>
<td>5</td>
<td>2 RDTs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parasite Type</th>
<th>Density</th>
<th>Samples</th>
<th>Test with</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. ovale</em> wild parasites and negative controls (sub-panels 3 and 4):</td>
<td>High parasite density:</td>
<td>5</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Medium parasite density:</td>
<td>5</td>
<td>1 RDT</td>
</tr>
<tr>
<td></td>
<td>Low parasite density:</td>
<td>5</td>
<td>2 RDTs</td>
</tr>
<tr>
<td></td>
<td>Negative controls:</td>
<td>5</td>
<td>2 RDTs</td>
</tr>
</tbody>
</table>

**Report on sensitivity and specificity, Review of results (Malaria Specimen Bank Steering Group)**
An additional stability test is performed by the manufacturer using samples of the same *P. falciparum* culture line (product stability at the maximum recommended storage temperature for the recommended shelf-life).

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Figure II-3: Stability testing of malaria RDTs

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2 An additional stability test is performed by the manufacturer using samples of the same *P. falciparum* culture line (product stability at the maximum recommended storage temperature for the recommended shelf-life).
Figure II-4: 'Ease of use' testing of malaria RDTs
III. Specimen bank contents and samples preparation

The malaria specimen bank, hosted at the Malaria Branch, Division of Parasitic Diseases, (CDC, Atlanta, USA), comprises four sub-panels (Annex 2). Sub-panel 1 consists in dilution series of recombinant proteins, which are the three major antigens used for malaria diagnosis by RDTs: *P. falciparum* histidine-rich protein 2 (Pf HRP2), *Plasmodium* lactate dehydrogenase (pLDH) and *Plasmodium* aldolase. Sub-panel 2 contains a series of cultured *P. falciparum* strains, while sub-panel 3 consists in wild parasite samples prepared with the blood of naturally infected individuals or, in some cases, of experimentally infected primates. Sub-panel 4 provides a range of negative control samples. All samples are prepared according to Standard Operating Procedures (SOPs) which are regularly reviewed, based on the experience of the collection laboratories and on recommendations of the Malaria Diagnostics Specimen Bank and Evaluation Steering Group (page 30).

A. *Sub-panel 1: Recombinant antigens*

This set allows performance assessment of malaria RDTs against precisely known concentrations of recombinant target antigens of known amino-acid sequence and manufacturing quality.

1. Contents

In the case of pLDH and *Plasmodium* aldolase, species-specific detection sensitivity is assessed with recombinant proteins of both *P. falciparum* and *P. vivax*. Both antigens are structurally well conserved within these species (page 35).[22, 23] One recombinant protein will be chosen for each of pLDH and aldolase antigens for *P. falciparum* and *P. vivax*. Selection is based on comparative data of relative detection of available recombinant antigens against multiple available RDTs, and ELISA, to minimize the potential of structural differences between recombinant and natural antigens introducing bias into RDT performance data. Available recombinant antigens have been compared against RDT and ELISA and further recombinant antigens are currently being cloned and expressed for assessment of suitability.

In the case of the *P. falciparum* specific antigen HRP2, high structural variability occurs and this affects the lower limit of detection of HRP2-detecting RDTs (page 36) [8, 9] HRP2 has been classified into three structural variant groups for the purpose of the testing programme (Type A – high repeat frequency, B – intermediate repeat frequency and C/Borderline – low repeat frequency), on the basis of the frequency of repeats of major target epitopes. A recombinant antigen from each structural group is included.

Six dilutions of each recombinant antigen are used, designed to cover the lower range of antigen detection of commonly-used RDTs, providing a comparison of detection at very low antigen concentration.

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3 HRP2 has been divided by the Programme into three structural groups based on the frequency of repeats of common target epitopes (discussed in more detail later).
2. **Samples preparation**

Currently, some recombinant proteins are obtained from commercial suppliers who produce well-characterized, recombinant proteins using standard good manufacturing practices (GMPs), such as CTK Biotech (California, USA), the National Bioproducts Institute (South Africa) and Standard Diagnostics (South Korea).

An independent production of recombinant proteins in laboratories of the Malaria RDT Evaluation Programme network is also being developed, so that the entire process can be controlled and remain unchanged over years. Proteins are derived from *P. falciparum* laboratory reference strains and from recently collected, culture-adapted parasites at the Army Malaria Institute (Australia). The gene coding for the antigen is cloned into JM109 cells, then protein expression and purification is performed at the National Bioproducts Institute using their GMP-labelled facilities and manufacturing process. Determination of the gene sequence at different cloning stages and characterization of the protein end-product ensure that no sequence change has occurred during the entire process.

The recombinant proteins are diluted to the six target concentrations in venous donor blood, which has previously been tested by microscopy and malaria RDTs to ensure absence of malaria infection. The dilutions are then aliquoted in 50 µL volumes and stored at -70°C. The quality of the dilutions is ensured by strict working rules (manipulation of the samples at 4°C, reverse pipetting of blood, etc., page 23) and by subsequent characterization of the samples by ELISA quantification of the antigen contents.

**B. Sub-panel 2: *P. falciparum* culture lines**

Twenty culture-adapted strains of *P. falciparum* of widely varied geographic origin have been selected to constitute a stable source of well-characterized parasites, continuously available over years. Parasite culture-derived samples allow better control of the effects of parasite stage and accumulation of stable antigens on RDT performance.

1. **Contents**

Twenty (20) *P. falciparum* culture lines have been selected among strains that had been adapted to culture and cryostabilized after only a few culture cycles in order to minimize culture-induced changes of the parasites. The selection of the parasite strains would be based on three main criteria:

- The concentration of the three antigens HRP2, pLDH and aldolase, measured by ELISA in parasite sample dilutions at 200 parasites per microliter (parasite/µL).
  - Previous studies have determined the natural variation of antigen concentration at this parasite density in geographically widely distributed wild parasite samples. For inclusion into the sub-panel 2, the parasite strain must display an antigen content in the middle 90% of the observed range of antigen concentrations at 200 parasite/µL.
- The structural group of the Pf HRP2 antigen, to ensure the natural variation of this antigen in parasites of reflected in the panel.
  - Previous studies have described the natural variation of the Pf HRP2 antigen, the impact of this variation on RDT results (classification into the three
structural groups A, B and C/borderline), and the relative proportions of HRP2 variants in different endemic areas. Similar proportions have then been applied to the sub-panel 2 composition (inclusion of 3, 14 and 3 culture lines possessing an HRP2 antigen of type A, B and C/borderline, respectively). Diversity of geographic origin is maintained, with inclusion of parasites originating from Africa, Asia and the Pacific, and the Americas.

For each culture line, dilutions at high (2000 or 5000 parasite/µL) and low (200 parasite/µL) parasite densities are prepared for product testing (choice of parasite densities, page 9). A summary of the culture lines, their geographic origins and their HRP2 variant groups is shown in Annex 2.

A subset of these 20 culture lines has been selected for the so-called ‘manufacturer panel’ (page 10). This consists in dilutions of 5 P. falciparum culture lines that are provided to the manufacturer for in-house quality control testing before actually starting the product testing. Selection of these 5 culture lines follows the same rationale as described above: the strains originate from 3 different continents (Africa, the Americas, and the Western Pacific), and representatives from each Pf HRP2 structural groups are chosen with the same proportions as above (1, 3 and 1 culture lines with Pf HRP2 type A, B and C, respectively).

A continuous culture of the parasite strains is based on a previously published standard protocol.[24] Before dilution to the target parasitaemias, the cultures are synchronized at the young trophozoïte stage with a standard protocol based on sorbitol treatment.[25] After resuspension in a 40% hematocrit mixture of O+ blood cells and AB+ plasma, the parasite density is determined by two independent microscopists, based on a red cell count. Dilutions at 5000 parasite/µL, 2000 parasite/µL and 200 parasite/µL are then prepared, using venous donor blood uninfected by Plasmodium parasites (screened by microscopy and malaria RDTs). The dilutions precision and stability of antigen during the dilution process are ensured by the same working rules as for dilutions of wild parasite samples (page 23).

All samples are additionally characterized by PCR to ensure single-strain cultures, and antigen is quantitated by ELISA to ensure antigen concentration is within the mid-90th percentile of the expected range.

C. Sub-panel 3: Wild parasite samples

Wild parasite samples of the four human Plasmodium species are prepared from venous blood of naturally infected patients in the Western Pacific Region, South-East Asia, Africa and South-America. In the case of P. vivax, P. malariae and P. ovale, single-species infections are relatively uncommon in most endemic areas. Some samples are therefore derived from primates experimentally infected with parasite strains which have been initially isolated from human patients. This sub-panel approaches most closely the real conditions of use of a malaria RDT, since the samples contain naturally infecting parasites.

1. Contents
Sub-panel 4 comprises 100 *P. falciparum*, 20 *P. vivax*, 5 *P. malariae* and 5 *P. ovale* samples, with each one being derived from a single infected source case. Samples are prepared from the venous blood of the infected host by dilution to precisely calibrated parasite densities: high (2000 or 5000 parasites/µL) and low (200 parasites/µL), with additional dilutions at a medium parasite density (500 parasites/µL) for non-*P. falciparum* species only. The selection criteria of the parasite densities have been described above (page 9). A summary of the samples, their geographic origin and their Pf HRP2 structural group is shown in Annex 2. The selected collection sites and preparation protocols are detailed in the following paragraphs. The final dilutions are characterized by microscopy, molecular species typing, Pf HRP2 sequencing, antigen quantitation and screening of viral infections (page 24). Recurrent collections will ensure availability of wild parasite samples over coming years. Replacement is planned to ensure that the composition of the wild parasite sample set remains stable in terms of antigen variant group and geographic origin (page 28).

### 2. Collection sites and laboratories

Laboratories are contracted by WHO and FIND in different endemic areas of the Western Pacific Region, of the African continent and of South-America in order to ensure a broadly scattered geographic origin of the wild parasite samples (Figure III-1). Selection of these laboratories is based on a series of criteria:

- Geographic area
- Quality of infrastructures and staffing
- Quality of previous / ongoing work in the field of malaria, particularly on malaria diagnostics
- Ease of access to malaria endemic recruitment sites
- Experience in field work logistics

Malaria patient recruitment is conducted in health facilities with skilled local health staff, good working conditions, availability of HIV counselling services, and within easy reach of the laboratory where the samples are processed.

All collection laboratories have obtained authorization from the respective National and WHO Ethics Committees before recruiting patients and preparing specimen bank samples. All initial collections have been attended by a consultant experienced in the use of the collection and preparation protocols, and staff have been specifically trained. The collection laboratories will have an annual external quality assessment of infrastructure, working conditions, equipment, staff supervision and management, quality assurance documentation, internal and external quality control, and safety. The preparation of wild parasite samples will be regularly reviewed.
3. Sample preparation

The preparation of wild parasite samples can be divided into three main steps: i) recruitment of *Plasmodium*-infected patients in the field and collection of venous blood ('parasitized blood'), ii) procurement of blood from non-infected donors ('parasite-free blood'), and iii) dilution of the 'parasitized blood' with the 'parasite-free blood' to high, medium and low level parasite densities (high level: 5000 or 2000 parasites per microliter of blood, medium level: 500 parasite/µL, low level: 200 parasite/µL), aliquoting and freezing. Inclusion of samples in the specimen bank is based on final characterization results. The whole process is schematically presented in Figure III-2 and Figure III-3.

Patient recruitment and blood collection (Figure III-2)

Febrile patients aged 5 years or more (age limits are higher in some sites, according to country-specific ethics requirements) and not having taken any known anti-malaria treatment in the past month are screened for malaria by RDT and/or thick blood film. If a strongly positive RDT result and/or a sufficient parasite density are detected, the patient is informed and his consent for venous blood collection and screening for viral infections (hepatitis B and C, HIV I and II) is requested. If positive, patients are informed of viral screening results, through the locally-appropriate health service mechanism.

A unique identification number (ID) is assigned to the patient, venous blood is collected (10 mL in EDTA tubes, 5 mL in a plain tube), and two slides with thick and thin blood films and two blood spots on filter paper are prepared. All samples are immediately transported to the laboratory in appropriate storage conditions.
Preparation of parasite-free blood (Figure III-3)

For dilution of the *Plasmodium*-infected patient blood, “parasite-free” blood is prepared by centrifugation of O- or O+ whole blood and replacement of the O- or O+ plasma by AB+ plasma (ensures compatibility with all patient blood groups). The whole blood and the plasma are obtained from informed and consented volunteer donors or from accredited blood banks (mostly National Blood Transfusion Centres). Blood donors are tested for malaria (microscopy, RDT) and viral infections (hepatitis B and C, HIV I and II, by ELISA). Screening of these infections by the blood banks is verified and completed if necessary. Blood is only used if all tests are negative. The quality of the “parasite-free” blood mixture is ensured by rapid preparation at 4°C, good homogenization and use within 36 hours.

Dilution of the parasitized blood (Figure III-3)

The patient’s infection is characterized for species and parasite density by thin/thick film analysis by two experienced, prequalified microscopists, using a discrepancy limit of 20% and a white cell count of the patient’s blood for calculations (see below). The mean parasite density is used for calculating the dilution steps down to a high-level (2000 or 5000 parasite/µL), a medium-level (500 parasite/µL, for non-*P. falciparum* species only) and a low-level parasite density (200 parasite/µL). The dilutions are first prepared in a small test volume of 1 mL, to check for fulfillment of two criteria: i) no agglutination of red blood cells, ii) at least a faint positive RDT result at the low-level (for *P. falciparum* infections) or at the medium-level (for non-*P. falciparum* infections) parasite densities. Larger volumes of dilutions are then prepared and checked again for the same criteria. If satisfactory, these dilutions are aliquoted in 50 µL volumes in pre-labeled cryotubes and immediately frozen at -70°C.

4. Quality assurance of the sample preparation

Quality of the malaria microscopy

The species and parasite density of the patient’s infection are determined by blinded thin/thick film analysis by two prequalified microscopists. If at least one microscopist identifies a mixed species infection, the sample is not processed further. Parasite densities are calculated against an accurate white cell count of the patient blood, or the Earl-Perez method is used for parasite quantitation. The two calculated parasite densities must have a maximal discrepancy of ≤20%. If not, the two counts are repeated and, if discrepancy remains higher than 20%, the sample is discarded.

Quality of the malaria RDTs used for screening

Malaria RDTs can be used for the initial patient screening prior to microscopy. Two different RDT brands are used, usually to detect Pf HRP2 and Pf LDH, with pan-specific LDH and/or pan-specific aldolase. RDTs are stored at a controlled temperature. If locally prepared specimen bank samples are available, the quality of the RDTs is assessed prior to use.

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4 Previous studies have shown that thick film parasite quantitation using the Earl-Perez method and against an accurate white cell count are equivalent.
Quality of blood dilution

All dilutions are performed with successive dilution steps and factors between 2 and 10. Calibrated micropipettes and the reverse pipetting method are used (large volumes of blood are slowly dispensed with sterile plastic pipettes), and the blood is homogenized on slowly rotating hematology wheels and a minimal mixing time depending on the volume (≤ 1 mL: at least 15 min, > 1 mL: at least 1 hour). To avoid degradation of the parasite antigens, the maximal delay between blood collection and freezing of the final dilution aliquots is 24 hours. During this time, all blood samples and dilutions are maintained at 2°C to 8°C.

5. Characterization of the samples

Summary of characterization of wild parasite samples from venipuncture to inclusion in the specimen bank:

- Parasite densities of the patient’s infection are determined by expert microscopy analysis of a thick film (see above).
- Parasite species are initially identified by microscopy, but the definitive species determination is performed with molecular biology tools (nested PCR, page 35). Samples with mixed species infections are excluded from the specimen bank.
- The parasite antigen activity of dilutions is tested with malaria RDTs. If the low-level (for Pf infections) or medium-level (for non-Pf infections) dilutions produce negative results with all test RDTs, the samples may be excluded from the specimen bank. (In all cases, samples are tested by quantitative ELISA before being include in a testing panel).
- Antigen contents of dilutions are quantified with ELISA, against established standard curves. Only samples having an antigen content within the middle 90% of previously observed variations are included in the specimen bank (page 34).
- HRP2 variation is determined by sequencing of the Pf HRP2 gene and classification in type A, type B and type C/borderline structural groups. Multi-clone infections producing indeterminate sequence results are considered to be in the HRP2 structural group B. Precise proportions of type A, B, C/borderline samples are included in the specimen bank, consistent with previously observed proportions in geographically widely distributed parasite samples (page 37).
- Infections by hepatitis B, hepatitis C, and HIV I and II viruses are eventually screened with rapid diagnostic tests upon patient recruitment, but a definitive and systematic screening is done with ELISA (commercial reference kits, according to the national guidelines of the respective countries). Positive samples are excluded from the specimen bank.
- Some aliquots of the initial patient blood (whole blood, serum and cell pellet) are retained at -70°C for additional tests in the future if warranted (e.g. additional molecular analyses).
- The diagram in Figure III-4 shows a summary of all tests.

Conditions for patient screening:
1) Febrile patient
2) 5 years or older (higher age limits are used in some sites)
3) No recent intake of anti-malarials (time limit 14–30 days)
4) Not anaemic (in some sites only)
Figure III-2: Patient recruitment and blood collection in the field
Figure III-3: Preparation of wild parasite samples in the laboratory

Parasitized blood

10 mL blood (in EDTA) → 2 thin / thick films → 2 blood spots → 5 mL blood (plain)

- white cell count per μL of blood
- 2 independent counts by 2 microscopists: parasite density (p/µL)

If ≤ 20% discrepancy: calculate mean density and blood dilutions

>20% discrepancy: repeat

Mixed infections: to be excluded

If positive: to be excluded

Parasite-free blood

- 'Test' dilutions (1 mL volume): Dilute down to 200 p/µL (Pf) or to 500 p/µL (non-Pf).

Procure O-/+ blood and AB+ plasma (donors / blood bank)
Screen for:
- Malaria (microscopy, RDT),
- Hepatitis B/C, HIV (ELISA).

If yes
- Replace O+-/ plasma by AB+ plasma, mix.

If no
- QC dilutions (large volumes):
  - High-level: 2000 or 5000 p/µL (Pf),
  - Medium-level: 500 p/µL (non-Pf),
  - Low-level: 200 p/µL

Agglutination of red blood cells? Malaria RDT negative?

If yes
- STOP processing this sample

If no
- If yes
  - Agglutination of red blood cells? Malaria RDT negative?
  - If yes
    - Aliquot dilutions in 50 µL volumes and freeze at -70°C

Characterization and in/exclusion in bank

Wild parasite samples
(Sub-panel 4 of specimen bank)
D. **Sub-panel 4: Negative control samples**

The parasite-negative challenge panel is designed to include:
- Samples from cases with characteristics previously recorded to cause, or suspected to cause, false-positive RDT results
- Samples from cases with possible differential diagnoses of malarial fever
- Samples with no known disease or cause of false-positive results.

1. **Contents**

It has been reported that blood factors unrelated to malaria, mostly antibodies, can produce a non-specific positive result with some malaria RDTs. Published studies have demonstrated or strongly suggested that the rheumatoid factor and heterophile antibodies (HAMA) have been responsible for the occurrence of false-positive RDT results.[26-28] In the case of other blood factors, like anti-nuclear antibodies (ANA), anti-mouse antibodies and the RPR positivity, their eventual cross-reaction with the monoclonal antibodies in malaria RDTs is rather suspected.

It is similarly important to evaluate the risk of false-positive malaria RDT results in patients suffering from a disease with malaria-like symptoms that is commonly occurring in malaria endemic areas. Selected diseases for the RDT testing programme are dengue fever, typhoid
fever, schistosomiasis, leishmaniasis and Chagas disease. Samples from HIV-infected individuals are also included in this sub-panel, because of commonly occurring co-infection of malaria and HIV, especially in some African countries.

For each of these selected characteristics, blood, serum or plasma samples from at least five different individuals are prepared with a low and a high titer of the relevant antibodies.

Finally, sub-panel 4 also comprises a set of “clean negative control samples”, consisting in blood samples from 50 healthy volunteers in which none of the previously listed blood factors or infections have been detected.

2. Samples preparation

Whole blood samples are obtained from accredited blood banks or collected from volunteer donors. The protocols for recruitment and sample preparation are based on those described for the wild parasite sample preparation (page 20), with the following three main differences:

• Malaria infection is excluded by microscopy and RDT during recruitment, and subsequent confirmatory diagnosis by nested PCR (page 35),
• Specific tests for detecting relevant blood factors or non-malaria infections are performed during the patient recruitment, according to relevant national or international guidelines or protocols.
• Collected venous blood is distributed in 50 µL aliquots, without previous dilutions, within a timeframe of 24 h during which the blood is kept at 4°C.

Serum or plasma samples are derived from archived samples at the Malaria Branch, Division of Parasitic Diseases (CDC, Atlanta, USA), or from commercial sources that have performed any relevant tests of blood factors or non-malaria infections. All samples are subjected to screening of malaria by nested PCR, then aliquoted in 50 µL volumes.

E. Maintenance of the specimen bank

Consistency over the years will be ensured for each sub-panel by the following mechanisms:

• It is verified that the recombinant antigens (sub-panel 1) manufacturing process does not undergo major changes, and batch-to-batch consistency is tested against comparative ELISA standard curves. In production laboratories of the QA-RDT network, expression clones are cryopreserved, and regular sequencing of the HRP2, pLDH and aldolase genes ensures consistency of all sequences.
• The *P. falciparum* culture lines (sub-panel 2) are cryopreserved between each sample preparation period. Each batch of diluted samples is tested for parasite density, consistent antigen amounts and unchanged Pf HRP2 sequence.
• For wild parasite samples (sub-panel 3), a steady replacement cycle ensures that there is no large panel change from year to year. Newly prepared dilutions undergo the usual characterization process (page 24). Antigen contents must be in the middle 90% of previously studied antigen levels (page 34) at least for two of the three antigens Pf HRP2, LDH and aldolase. Inclusion of replacement samples in the specimen bank is then decided according the structural group of Pf HRP2 (if applicable), by maintaining the relative proportions of groups A, B and C in the panel (page 37), and according to the geographical origin, by ensuring that each structural group has representatives from Africa, Asia / Western Pacific, and the Americas (in this order of emphasis).
• For replacement of negative control samples (sub-panel 4), the collection sites or commercial sources are maintained as much as possible, and the samples are tested for consistent levels of antibodies / blood factors (if applicable).
IV. Management of the specimen bank and RDT product testing

A. The Malaria Diagnostics Specimen Bank and Evaluation Steering Group

The technical and logistical aspects of the malaria specimen bank and the malaria RDT evaluation programme is mainly driven by the recommendations of the Malaria Diagnostics Specimen Bank and Evaluation Steering Group (subsequently referred to as the Steering Group). This group is constituted by representatives of the following Organizations or Institutions (number of representatives in brackets), and meets at least one time per year:

- WHO/TDR (Geneva, Switzerland) (2)
- WHO/WPRO (Manila, Philippines) (1)
- FIND (Geneva, Switzerland) (2)
- CDC (Atlanta, USA) (1)
- Collection laboratories (annual rotation)
- 1 laboratory in the African Region (1)
- 1 non-African laboratory (Asia, Western Pacific, South-America) (1)
- Médicins Sans Frontières (Holland) (1)
- Hospital for Tropical Diseases (London, UK) (1)
- Army Malaria Institute (Queensland, Australia) (1)
- External expertise from consultants may temporarily be included

Between full meetings of the Steering Group, decisions may be made by a subgroup consisting of TDR (1), WPRO (1), FIND (1), CDC (1) and other members as expertise requires.

The Terms of Reference of the Steering Group consist in providing recommendations on:

- The management of the malaria specimen bank, including:
  - Content, characterization and maintenance of the specimen bank
  - Policy on access to bank samples
- Development and modification of protocols for specimen collection and use
- Including Product Testing and Lot Testing of malaria lateral flow RDTs
- Development and modification of protocols for laboratory-based testing of other antigen-detecting diagnostics
- Review product testing results prior to publication

B. Outline of management policies

1. Specimen bank management and policy of access

The decisions concerning the inclusion, exclusion and characterization of samples for the specimen bank are based on criteria of published and unpublished evidence, discussed and developed by the Steering Group. These criteria are described in detail in specific paragraphs

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5 Figures in brackets indicate the number of representatives.
concerning the different specimen bank sub-panels (page 18), their characterization (page 24), as well as contents and variation of the antigens (page 33 and 35).

The protocols for collection and/or preparation of specimen bank samples (recombinant proteins, parasite culture lines, wild parasite samples and parasite-negative samples) have been developed in collaboration with the laboratories included in the QA-RDT network, and are agreed by the Steering Group. SOP modifications require the approval of the responsible WHO Officer, with eventual consultation of the Steering Group.

Inclusion of the collection laboratories of the QA-RDT network is based on a series of criteria detailed elsewhere (page 21) and is decided within the WHO – TDR – FIND partnership, with consultation of the Steering Group.

Access to the specimen bank samples and associated information is at the final discretion of WHO, in collaboration with FIND, and on the advice of the Steering Group. In general, wild parasite samples are only available for testing of RDTs within the network supported and coordinated by WHO and FIND. Culture-derived parasite samples may be accessed by diagnostics manufacturers and developers, through the approval mechanism described above, and at a cost decided by WHO (aimed at covering costs incurred in providing the sample).

2. Management, rules and definitions of the product testing

Main rules

The criteria for inclusion of RDT submitted products in the malaria RDT Evaluation Programme have been outlined previously (page 10), and access to a more detailed list of criteria is available online.[29] The manufacturer can withdraw products from the Evaluation Programme up to 2 months after having received the manufacturer panel samples for in-house testing, but not after commencement of the product testing. Submission of the physical product indicates agreement to test the product. RDT products first undergo a phase 1 evaluation, and are then subjected to the complete phase 2 evaluation if the phase 1 criteria for adequate test performance are fulfilled (page 11). The Steering Group will give recommendations on equivocal results.

The phase 1 and phase 2 testing results are systematically reviewed by the Steering Group. All product testing results will be published and made available to the manufacturer, but cannot be used for promotional purposes or as evidence for product certification (page 12).

WHO will list all evaluated products and performance data on a dedicated page of the WHO website and in a hard copy publication. WHO may remove a product from the website list (de-listing) or require its re-submission if changes of the RDT product justify its re-definition as a new product, or if data obtained from field testing are considered to be consistently outside those of the product testing programme.

Joint-submission and joint-listing of identical RDT products with different names is possible if they are produced on the same production line, and if the concerned companies provide all required documentations demonstrating that these RDT products are the same. In this case, only one product can be submitted for testing, and the identical RDT products will be jointly listed with the performance results.
Main definitions

A RDT product is considered to be different or to be a new product if one of the following specifications have changed:

(i) Monoclonal antibodies (MABs): change of the target epitope, or of the species from which the target antigen for MAB development is derived
(ii) Dye conjugate: change of specifications or type of label
(iii) RDT format: change in the assay presentation (dipstick, cassette, card, etc.)
(iv) RDT manufacturer: production of similar RDTs by different manufacturers, except if joint listing of RDTs has been justified and accepted (see below)

Other changes, which could have the potential to significantly affect RDT performance, such as changes in raw materials or components (MABs, buffers, nitrocellulose membranes, etc.), do not require the re-definition of a RDT as a new product, but the manufacturer should provide WHO with data demonstrating the equivalence of performance of the changed RDT product.

The definition of a RDT lot is the responsibility of the RDT manufacturer, and must be compatible with the ISO13485:2003 or US FDA 21 CFR part 820 certification.

A more detailed description of the Malaria Diagnostics Specimen Bank and Evaluation Steering Group, policies, rules and definitions is available on the WHO-WPRO Malaria Rapid Diagnostics website.[29]
V. Evidence and methods for RDT product testing

A. Antigen contents

The detection sensitivity of malaria RDTs is generally mentioned in terms of lowest detectable parasite density (lower limit of detection). However, a positive or negative RDT result rather depends on the amount of antigen contained in the blood sample. The antigen content in infected venous blood can be quantified by ELISA, by establishing a reference standard curve with known amounts of recombinant antigens. ELISA protocols and standard curves have been set up for the three antigens HRP2 (*P. falciparum*), pLDH and aldolase, the relationship between parasite density and antigen content in blood samples is being assessed. The results of this study will provide the basis for deciding about the recombinant proteins and their concentrations in dilution series to be used for RDT product testing.

1. Quantification of antigens by ELISA

After analysis of standard curves and range of antigen detection, the CELISA kit (Cellabs, Australia) and SD Malaria Antigen ELISA kit (Standard Diagnostics, South Korea) were selected for quantitation of HRP2 and pLDH respectively.

Protocols follow the manufacturers’ recommendations, but are optimized to improve quantitation. Samples are diluted to operate within the linear range of the standard curves of the respective ELISAs.

A quantitative ELISA has been developed for the programme by US CDC, using recombinant aldolase and anti-aldolase monoclonal antibodies provided through WHO, to assess aldolase content.

2. Relationship between antigen content and parasite density

Using the above described protocols and standard curves, antigen concentrations have been determined in culture line and wild parasite samples at calibrated parasite densities. The preparation of blood samples at 200, 500, 2000 and/or 5000 parasites per microliter of blood was previously described, for parasite culture lines in this document.

The results showed a correlation between antigen concentration and parasite density. Relatively weak correlation factors were observed, especially in the case of Pf HRP2, because of varying antigen contents in different samples despite identical parasite densities, and probably due to variation in binding of antigen by monoclonal antibodies:

Various factors can cause antigen content variation at a given parasite density. Some are of a technical nature, e.g. eventual effects of blood sample components (donor blood used for dilution, anticoagulants) or inaccuracies during sample preparation (microscopy, pipetting, mixing). Antigen content is also influenced by the parasite stage (particularly in the case of pLDH), the duration of the infection, the inherent antigen expression level of the parasite and antigen variant type (in the case of Pf HRP2, page 36), variations in the parasite load (parasite
sequestration in the case of *P. falciparum*), and persistence of antigen after parasite elimination (particularly of Pf HRP2).

The SOPs of the sample preparation process have been designed to minimize the effect of these factors (page 20).

A published study has shown that the amount of secreted Pf HRP2 protein per parasite indeed varies from one parasite stage to another.[30] It is similarly possible that the level of HRP2 transcription and of Pf HRP2 protein production varies from one parasite strain to another. However, the significance of this to clinical sensitivity is not clear, as HRP2 persists and therefore accumulates in the circulation over subsequent cycles of parasite development. The relationship between antigen concentration and parasite density would be further clarified by future studies.

3. **Study-based choices for the specimen bank**

The results of the above described study have driven decisions concerning the different sub-panels of the product testing specimen bank.

**Sub-panel 1**

This sub-panel consists of dilution series of recombinant Pf HRP2 (three variant types, according to page 35), Pf LDH, Pv LDH, Pf Aldolase and Pv Aldolase, with the purpose to compare the lower limits of RDT detection. The range of recombinant antigen concentrations is therefore situated around the equivalent of a parasite density of 200 parasite/µL, which has been previously determined for each recombinant protein. In the case of Pf HRP2, where different protein variants produce different ELISA results, the equivalent of 200 parasite/µL has been specifically assessed for each variant of sub-panel 1.

**Sub-panels 2 and 3**

Parasite culture lines for sub-panel 2 and wild parasite samples for sub-panel 3 were chosen and prepared according to the criteria described in the corresponding paragraphs. Subsequently, the contents of HRP2 (*P. falciparum* only), LDH and aldolase in all 200 parasite/µL dilutions were determined by ELISA. Based on the range of antigen content observed at 200 parasite/µL in all collected wild parasite samples, those with atypical antigen concentrations, (i.e. being situated within the 5% upper and 5% lower range of antigen), were excluded.

In the case of Pf HRP2, quantitation by ELISA is affected by the variation of this antigen. ELISA results can therefore not be regarded as an accurate assessment of antigen content, but they ensure that the specimen bank samples have antigen contents within a wide acceptable range. By excluding only the “far outliers” (i.e. samples with antigen content in the 5% upper and lower ranges), and by including parasites with different HRP2 variants in given proportions (page 12), the introduction of any bias is limited and the specimen bank mimics as much as possible the wide diversity occurring in wild parasites from different malaria endemic areas.
B. DNA extraction and molecular diagnosis of parasite species

The infecting species present in the wild parasite samples of the specimen bank has to be precisely identified, and more particularly mixed infections have to be excluded. The sample preparation procedure includes a high quality microscopy analysis, with species diagnosis on both a thick and a thin film by two experienced microscopists. Nevertheless, it has amply been reported that more sensitive molecular diagnosis methods detect higher rates of mixed infections, because minor species at very low parasite density are frequently overlooked by microscopy.[31-34] Whole blood in EDTA or blood-spots on filter paper are therefore collected from the patient, subjected to DNA extraction, and the infecting species is diagnosed by nested PCR analysis.

Commercial kits are used for extraction of DNA from venous blood samples (QIAamp DNA Blood BioRobot MDx Kit) and from filter paper blood spots (QIAamp Media MDx, QIAamp DNA Mini, or QIAamp DNA Blood Mini Kits). A robot is used for automated high-throughput extraction, minimizing the risk of contamination and manipulation errors (BioRobot MDx). The two kits procedures are based on the same DNA extraction principle: after lysis and protease digestion, the sample is mixed with ethanol and filtered through silica columns fixing the genomic DNA. After several wash steps, the DNA is eluted and stored at -20°C until PCR analysis.

The infecting species are identified by species-specific nested PCR amplification, using a published protocol which is currently regarded as the reference and has been used in numerous clinical and field studies since its publication.[32-37] This protocol is based on amplification of the 18S ribosomal RNA gene of the parasite. Two Plasmodium-specific primers are used for the primary amplification step, then four different species-specific primer pairs are used for four separate and specific amplification reactions of P. falciparum, P. vivax, P. malariae and P. ovale. Additionally, the primate parasite P. knowlesi is detected by using a previously published protocol, since this parasite has been shown to infect humans in various Asian countries.[38, 39] The presence or absence of each of these species is assessed by standard agarose gel electrophoresis and BET staining of the PCR products.

C. Antigen variation

The robustness of malaria RDTs is based, among others, on their capacity to recognize epitopes in the target antigen, whatever the geographical origin and the genetic background of the malaria parasite. It is therefore essential to assess possible sequence variations which may alter the number and binding affinity of the epitopes in the parasite antigen targeted by the monoclonal antibodies (MABs) in the RDTs, as well as the eventual impact of such variations on the detection performance of the RDTs. The targeted epitopes of the HRP2, pLDH, and aldolase antigens of commercial RDTs are not publicly known. The genetic diversity of the antigens has therefore been assessed by sequencing the entire coding region of the pLDH, aldolase and HRP2 genes of geographically widely distributed parasites by collaborating institutions.

1. Plasmodium LDH and aldolase variation

The genetic diversity of LDH has recently been studied in wild isolates of the four Plasmodium parasite species infecting human (P. falciparum: 49 isolates from Africa, the
Americas, Asia, the Western Pacific, and the Middle East; *P. vivax* and *P. malariae*: 10 and 17 isolates, respectively, from Africa, Asia and the Americas; *P. ovale*: 13 isolates from Africa and South-East Asia.[23] The resulting amino acid sequences of *P. falciparum*, *P. vivax* and *P. malariae* LDH were 100% identical within each species, with only a few cases of synonymous nucleotide variations. Only one non-synonymous mutation was noted in a published *P. falciparum* laboratory strain sequence. In the case of *P. ovale*, three different LDH protein sequences were observed because of non-synonymous mutations in three positions.

Even though larger confirmatory studies would be useful, these findings suggest that LDH variation is very limited. It appears unlikely that LDH variation is the reason for the varying RDT detection sensitivities of *P. falciparum*, *P. vivax* and *P. malariae*. It could possibly account for the rather poor detection of *P. ovale* by LDH-based RDTs, but this hypothesis would need to be confirmed by further investigations.[4, 20]

Another recently published study of the entire aldolase gene sequence in *P. falciparum* and *P. vivax* isolates has similarly shown very little sequence variation within each species.[21] The study was based on 36 *P. falciparum* isolates originating from Africa, the Western Pacific Region and South-East Asia, and on 18 *P. vivax* isolates from the Western Pacific and South-East Asia. A small number of synonymous mutations were found in the wild parasite isolates, and only one non-synonymous mutation was found in a laboratory strain of each species.

As for LDH, it seems highly unlikely that antigen diversity could be responsible for different performances of aldolase-based malaria RDTs, at least for the detection of *P. falciparum* and *P. vivax*.

2. *P. falciparum* HRP2 variation and its impact on RDT results

**Published studies**

A recently published study based on 75 *P. falciparum* culture lines and isolates from 19 different malaria endemic countries has shown an extensive diversity of Pf HRP2 nucleotide sequences.[8] The translated Pf HRP2 protein sequences consisted of various amino acid repeats which vary in composition, number and order. Detection of a subset of these parasite isolates with two different RDTs revealed that the detection of relatively low parasitaemias (≤ 250 parasites per microliter of blood, parasite/µL) was highly dependent on the Pf HRP2 sequences, while all isolates were well detected at parasitaemias higher than 1000 parasite/µL. More particularly, the RDT detection sensitivity could be predicted with a regression model, establishing a threshold for highly probable sensitive test results (*p* > 0.5) based on the multiplied numbers of type 2 and type 7 repeats (number of type 2 x type 7 repeats > 43).

The observation that HRP2 sequence variation affects RDT performances was further supported by a study demonstrating variable reactivity of HRP2-specific monoclonal antibodies (MABs) with geographically distinct *P. falciparum* isolates and different MABs recognizing different epitopes.[9]

**Further studies in the context of the RDT product testing scheme (unpublished data)**
The two published reports justified the need of a more extensive study of the worldwide Pf HRP2 sequence variation in *P. falciparum* parasites in the context of this RDT product testing scheme. Pf HRP2 sequences have been determined in a total number of 373 parasite samples (as at 2 November 2007) originating from Africa, Asia, South-East Asia, the Western Pacific, South America and the Caribbean. The resulting Pf HRP2 sequences have been classified according to their predicted detection sensitivity by Pf HRP2-based RDTs (Figure V-1), based on the previously described regression model,[8] as well as further statistical analysis with a larger number of samples: types A and B are predicted to be well detected at parasitaemias below 250 parasite//µL, while the probability of detection of types C and borderline below this parasitaemia is predicted to be low.

In all investigated geographical areas, the major HRP2 variant was of type B. type C and borderline HRP2 variants represented at least 30% of the parasite samples in Asian countries and the Western Pacific, while they were much less prevalent in Africa (15%) and were not detected in South and Central America in this sample.

<table>
<thead>
<tr>
<th>Area</th>
<th>n</th>
<th>A &gt; 100</th>
<th>B 50 - 100</th>
<th>C &lt; 43</th>
<th>Borderline 44 - 49</th>
</tr>
</thead>
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<td>Africa</td>
<td>135</td>
<td>14 (10%)</td>
<td>101 (75%)</td>
<td>12 (9%)</td>
<td>8 (6%)</td>
</tr>
<tr>
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<td>2 (20%)</td>
<td>5 (50%)</td>
<td>3 (30%)</td>
<td>0</td>
</tr>
<tr>
<td>Pacific</td>
<td>84</td>
<td>9 (11%)</td>
<td>50 (77%)</td>
<td>9 (11%)</td>
<td>16 (19%)</td>
</tr>
<tr>
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<td>54</td>
<td>9 (16%)</td>
<td>45 (83%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S.E. Asia</td>
<td>90</td>
<td>8 (9%)</td>
<td>47 (52%)</td>
<td>31 (34%)</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>373</td>
<td>42 (11%)</td>
<td>248 (66%)</td>
<td>55 (15%)</td>
<td>28 (8%)</td>
</tr>
</tbody>
</table>

**Figure V-1: Distribution of HRP2 variant types in malaria endemic areas**

*P. falciparum* parasite samples were obtained from Africa (Benin, Burkina Faso, Cameroon, Gambia, Ghana, Guinea, Kenya, Liberia, Malawi, Nigeria, Niger, Sierra Leone, Sudan, Tanzania, Uganda, Zambia), China, the Western Pacific Region (East Timor, Papua New Guinea, Solomon Islands, Vanuatu), South America and the Caribbean (Brazil, Columbia, Ecuador, Haiti, Honduras, Peru, Santa Lucia, Suriname, Columbia), as well as South-East Asia (Cambodia, Indonesia, Malaysila, Philippines, Thailand, Vietnam).

HRP2 variants were classified according to the multiplied number of type 2 and type 7 amino acid repeats, with 50 being the threshold for sensitive detection by HRP2-based malaria RDTs (below 50: type C and borderline, 50 and above: type A and B).[8]

3. **Study-based choices for the specimen bank**

The results of this study were used to decide the composition of the product testing specimen bank, in terms of HRP2 variants. The rationale was to test malaria RDT products against all three categories of HRP2 variants (type A with high repeat number, type B with intermediate repeat number, and type C and borderline, with low repeat numbers), and to apply the relative

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[6] AMI working classification
proportions of the investigated sample set (11% ‘Type A’, 66% ‘Type B’, 23% ‘Type C and borderline’) to all sub-panels made of *P. falciparum* parasite samples.

- Sub-panel 1 (recombinant antigen panel) contains three recombinant HRP2 preparations, with one for each HRP2 variant category,
- Sub-panel 2 (parasite culture lines) is made of 20 *P. falciparum* culture lines, with 3, 14 and 3 lines harboring a type A, type B a type C / borderline HRP2 sequence, respectively,
- Sub-panel 3 (wild parasite samples) contains 100 *P. falciparum* parasite samples prepared from infected individuals, with HRP2 sequences belonging to ‘Type A’ in 11 samples, to type B in 66 samples and to type C / borderline in 23 samples,
- The ‘manufacturer panel’ consists of 5 *P. falciparum* culture lines, with 1, 3 and 1 lines expressing the Pf HRP2 structural groups type A, B and C / borderline, respectively,
- For stability testing, one *P. falciparum* culture line with the major type B Pf HRP2 structural group has been selected.
Annex 1: Laboratories of the Malaria RDT Evaluation Programme Network

Global Specimen Bank / Samples Characterization

Division of Parasitic Diseases
National Centers for Disease Control and Prevention (CDC)
4770 Buford Highway
MS F36 Bldg 109, Room 1121
NE Atlanta GA 30341
USA

Samples Characterization

Australian Army Malaria Institute (AMI)
Weary Dunlop Drive
Gallipoli Barracks
Enoggera QLD 4051
AUSTRALIA

Department of Clinical Parasitology
Hospital for Tropical Diseases (HTD)
Mortimer Market, Capper Street
London WC1E 6AU
UK

Production of Recombinant Proteins

National Bioproducts Institute (NBI)
Private Bag X9043
Pinetown 3610, Natal
SOUTH AFRICA

Collection laboratories

Research Institute of Tropical Medicine (RITM)
Filinvest Compound
Alabang, Muntinlupa City
PHILIPPINES

Laboratory of Molecular Epidemiology
Institut Pasteur du Cambodge (IPC)
#5, Monivong Blvd, P.O. Box 983
Phnom Penh
CAMBODIA
Experimental Medicine Research Division,
Department of Medical Research (DMR)
No. 5, Ziwaka Road, Dagon P.O., Yangon 11191
MYANMAR

Department of Medical Microbiology and Parasitology
College of Medicine (RM 308) of the University of Lagos (UL)
University of Lagos
Idiaraba, Lagos
NIGERIA

Institut Pasteur de Bangui (IPB)
BP 923 Bangui
CENTRAL AFRICAN REPUBLIC

Centre for Clinical Research
Kenya Medical Research Institute (KEMRI)
Po Box 54
Kisumu
KENYA

Ifakara Health Research and Development Centre (IHRDC)
360 Kiko Avenue
Mikocheni, Dar-es-Salaam
UNITED REPUBLIC OF TANZANIA

Institut Pasteur de Madagascar (IPM)
Unité du Paludisme - Malaria Unit
Institut Pasteur de Madagascar
BP 1274 - Antananarivo 101
MADAGASCAR

Centro de Entrenamiento y Investigaciones Médicas (CIDEIM)
Avenida 1-N 3-03
Cali
COLOMBIA

Instituto de Medicina Tropical Alexander von Humboldt
Universidad Peruana Cayetano Heredia
Av. Honorio Delgado 430
Urb. Ingenieria, San Martin de Porres
AP 4314 Lima
PERU
### Annex 2: Summary of the Malaria Specimen Bank

<table>
<thead>
<tr>
<th>Sample type</th>
<th>n</th>
<th>Antigen</th>
<th>Origin</th>
<th>Testing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHASE ONE PANEL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recombinant antigen (sub-panel 1)</td>
<td>7</td>
<td>Pf HRP2 type A</td>
<td>1</td>
<td>6 serial dilutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type B</td>
<td>1</td>
<td>range of antigen concentrations:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type C</td>
<td>1</td>
<td>equivalent to 500 p/µL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf LDH</td>
<td>1</td>
<td>To be finalized</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pv LDH</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf aldolase</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pv aldolase</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>P. falciparum culture lines (sub-panel 2)</td>
<td>20</td>
<td>Pf HRP2 type A</td>
<td>3</td>
<td>SE Asia / WP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type B</td>
<td>14</td>
<td>Africa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type C</td>
<td>3</td>
<td>S America</td>
</tr>
<tr>
<td><strong>PHASE TWO PANEL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild parasites, derived from human (sub-panel 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. falciparum</td>
<td>100</td>
<td>Pf HRP2 type A</td>
<td>10</td>
<td>SE Asia / WP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type B</td>
<td>60</td>
<td>Africa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pf HRP2 type C</td>
<td>30</td>
<td>S America</td>
</tr>
<tr>
<td>Wild parasites, derived from human or primates (sub-panel 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. vivax</td>
<td>20</td>
<td>dilutions at 200 p/µL (low).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. malariae</td>
<td>5</td>
<td>500 p/µL (medium).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. ovale</td>
<td>5</td>
<td>2000 or 5000 p/µL (high)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmodium-negative samples (sub-panel 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-nuclear Ab positive</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPR positive</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor positive</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterophile Ab positive</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-mouse Ab positive</td>
<td>5-10</td>
<td>dilutions at high and low titres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chagas</td>
<td>5-10</td>
<td>of relevant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>5-10</td>
<td>antibodies / blood factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhoid</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>5-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean negative (none of above)</td>
<td>5-10</td>
<td>no dilution (whole blood)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: all Plasmodium-positive samples are characterized by microscopy, molecular species diagnosis, Pf HRP2 sequencing (if applicable), and antigen quantitation by ELISA (paragraph xxx).*

Where relevant, the parasite antigen is indicated (Pf HRP2 types A, B, C : see paragraph xxx).

*Recombinant proteins are either commercially available or are produced in laboratories of the QA-RDT network.*

The origins of the parasite strains are grouped into: South-East Asia / Western Pacific, Africa, South America.

n = number of samples, p/µL = parasites per microlitre of blood.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>n</th>
<th>Antigen</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MANUFACTURERS PANEL (subset of the sub-panel 2 of the specimen bank)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. falciparum culture lines</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name of the Pf strains:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin I</td>
<td>Pf HRP2 type A</td>
<td>Benin, Africa</td>
<td></td>
</tr>
<tr>
<td>Santa Lucia</td>
<td>Pf HRP2 type B (low)</td>
<td>El Salvador, C America</td>
<td></td>
</tr>
<tr>
<td>Nigeria XII</td>
<td>Pf HRP2 type B (med.)</td>
<td>Nigeria, Africa</td>
<td></td>
</tr>
<tr>
<td>FC27/A3</td>
<td>Pf HRP2 type B (high)</td>
<td>Papua New Guinea, WP</td>
<td></td>
</tr>
<tr>
<td>PH1</td>
<td>Pf HRP2 type C</td>
<td>Philippines, WP</td>
<td></td>
</tr>
</tbody>
</table>

*Note: samples characterized by microscopy, molecular species diagnosis, Pf HRP2 sequencing, and antigen ELISA.*

Indication of the parasite antigen (Pf HRP2 types A / B / C and antigen content high / medium / low).

*Geographical origins of parasite strains: Africa, Central America, Western Pacific.*

*Pf culture line used as the reference standard for RDT stability testing at the CDC and by the manufacturer.*

n = number of samples, p/µL = parasites per microlitre of blood.
References


Letter to Manufacturers

RE: World Health Organization Malaria Diagnostics Evaluation Programme

The attached document contains important information for manufacturers who have had Expressions of Interest (EOI) accepted for the first round of product testing at US CDC in Atlanta, under the WHO-FIND malaria rapid diagnostic test (RDT) product testing programme.

Contents:
(1) Limit on products to be submitted by each manufacturer.
(2) Availability of panels of recombinant antigen for preliminary testing of products by manufacturers.
(3) Details of payment to be made on submission of RDTs for testing.
(4) Summary of product testing procedure.
(5) Provisional timeline for product testing.
(6) Relationship of product testing to WHO RDT procurement, and to WHO prequalification of malaria RDTs.

Note: This letter is being sent by email, facsimile and mail.

PLEASE ACKNOWLEDGE RECEIPT OF THIS LETTER TO mal-rdt@wpro.who.int

Enquiries should be addressed to:
David Bell WHO belld@wpro.who.int and mal-rdt@wpro.who.int

Yours sincerely,

Dr David Bell
Scientist
Malaria Diagnostics
World Health Organization Malaria Diagnostics Evaluation Programme

WHO-FIND Product Testing of Malaria RDTs

1. Limit on number of products to be submitted by each manufacturer.

The EOI received a very high response rate from RDT manufacturers. It has therefore been decided to limit to a maximum of three the number of products to be submitted by each manufacturer in the first round of product testing. Further products may be submitted subsequently, once the results of the initial testing round has been completed. The figure of three is considered to cover the range of products needed to cover most of the needs of a malaria programme (e.g. HRP2-detecting test, pLDH-detecting tests for various target species, and tests targeting aldolase).

2. Availability of panels of recombinant antigen for preliminary testing of products by manufacturers

A panel of samples derived from cultured *P. falciparum*, diluted and preserved at -80°C, are now available. This panel will be a subset of the Phase One panel used for product testing (see Annex One), and is intended to allow manufacturers to assess product quality prior to submitting lots to the Product Testing programme. The panel is detailed in Table 1. Accessing this panel is optional, but recommended.

This 'manufacturer's panel' has been prepared at US CDC and consists of aliquots of 5 cultured *P. falciparum* parasite lines from geographically different endemic areas. Each of these samples is provided at different parasite dilutions: 200, and 5,000 parasites per µl. The panel has been tested against a number of commercially-available HRP2-detecting and pLDH-detecting RDTs. The results indicate that all 5000 parasite/microL concentrations are expected to be detected by good quality RDTs. The 200 parasite/microL samples can be detected by some HRP2-detecting pLDH and HRP2 RDTs but these samples are close to the limit of currently-available tests. Should your products fail to detect samples at 5000 parasite/microL, it is therefore likely that these products will not perform well in terms of antigen detection against the complete product testing panel, and your company should seriously consider whether or not you submit these products for further evaluation. As stated previously, all results of RDTs submitted to WHO for evaluation will be published.
Table 1. Sample panel available to manufacturers for screening prior to product submission.

<table>
<thead>
<tr>
<th>Sample</th>
<th>HRP2 sequence type</th>
<th>Derivation of sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benin I</td>
<td>A</td>
<td>Benin, Africa</td>
</tr>
<tr>
<td>Santa Lucia</td>
<td>B (high)</td>
<td>El Salvador, Central America</td>
</tr>
<tr>
<td>Nigeria XII</td>
<td>B (medium)</td>
<td>Nigeria, Africa</td>
</tr>
<tr>
<td>FC27/A3</td>
<td>B (low)</td>
<td>Papua New Guinea, Australasia</td>
</tr>
<tr>
<td>PH1</td>
<td>C</td>
<td>Philippines, Southeast Asia</td>
</tr>
</tbody>
</table>

Each manufacturer may receive a labelled panel consisting of frozen samples of each of these culture lines, as 15 aliquots of 50 microL diluted to 200 parasites/microL, and 5000 parasites/microL (Total 15 x 2 x 5 = 450 aliquots). Panels are provided free of charge, but the manufacturer is responsible for organizing and paying the costs of a courier, and organizing all passage through ports and customs once the sample has left the production site at the United States Centers for Disease Control and Prevention (US CDC) in Atlanta, USA. Note the samples should be shipped on Dry Ice (-78°C). Loss or spoilage of shipments is the responsibility of consignee, and the shipper engaged by the consignee. Due to the limited number of available samples, WHO can not guarantee that spoilt or lost shipments will be replaced.

**Contact details for ordering panels:**

<table>
<thead>
<tr>
<th>Order by email:</th>
<th>Lucian Marts: <a href="mailto:gko1@cdc.gov">gko1@cdc.gov</a></th>
<th>cc: <a href="mailto:mal-rdt@wpro.who.int">mal-rdt@wpro.who.int</a></th>
</tr>
</thead>
</table>
| Consigner address for courier | Lucian Marts  
Centers for Disease Control and Prevention  
Malaria Branch, Division for Parasitic Diseases  
Bldg 109, Room 1118.6, MS F-36  
4770 Buford Hwy NE  
Chamblee, GA 30341  
ph: 770-488-3771  
fax 770-488-4454  
Email: gko1@cdc.gov | |

All orders must be in the format detailed in Annex 2.
3. **Details of payment to be made on submission of RDTs for testing**

Previous correspondence indicated a fee to be paid for product testing. Manufacturers will be required to cover transport costs of the reference standard sample for stability testing that each manufacturer is required to undertake as part of the product testing programme (in addition to the transport of the optional manufacturers panel for preliminary testing). Therefore, the direct payment will be waived for this round of testing.

4. **Summary of product testing procedure**

A more detailed description of the product testing programme and its development will be sent to participating manufacturers and posted on the WHO RDT Website by early February 2008, and posted on [www.wpro.who.int/site/rdt](http://www.wpro.who.int/site/rdt). The full Standard Operating Procedures for product testing will be available at the time of product submission.
Outline of RDT assessment against Product Testing Performance Panel.
Each lot must detect a majority of the high density culture samples in Phase 1 before being tested against the Phase Two panel.
5. **Provisional timetable for product testing (times may change)**

<table>
<thead>
<tr>
<th>Jan 08</th>
<th>Feb 08</th>
<th>Mar 08</th>
<th>Apr 08</th>
<th>May 08</th>
<th>Jun 08</th>
<th>Jul 08</th>
<th>Aug 08</th>
<th>Sep 08</th>
<th>Oct 08</th>
<th>Nov 08</th>
<th>Dec 08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel available to manufacturers</td>
<td>Payment of fee for testing</td>
<td>Product arrival at US CDC</td>
<td>Commencement of product testing</td>
<td>Finish this round of product testing</td>
<td>Publish results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. **Relationship of product testing to WHO RDT procurement, and to WHO prequalification of malaria RDTs**

From 2008, eligibility for procurement of RDTs through the WHO procurement system (WebBuy) will be restricted to products submitted to the product testing programme. When the initial round of product testing is completed, WebBuy may remove poorly-performing products from the procurement programme. Future procurement will be restricted by this performance data until such time as a full WHO prequalification process for malaria RDTs is in place.

The WHO is currently developing a prequalification programme for malaria rapid diagnostic tests. This programme, coordinated by the WHO Diagnostics and Laboratory Technology unit (WHO/DLR) will include inspections of manufacturing sites, and evaluation of manufacturer dossiers. The product testing programme detailed here will provide the data through which performance of RDTs will be assessed in the prequalification process. WHO/DLR will begin contacting manufacturers in early 2008 regarding eligibility for future inspections and dossier submission. Participation in the prequalification programme will entail a fee per production facility to cover examination of the prepared dossier at the site inspection.
Annex One: Challenge Panel for malaria RDT Product Testing

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Details</th>
<th>P</th>
<th>L</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recombinant antigen</strong></td>
<td>HRP2-A  6 serial dilutions from 500 parasites/µL equivalent</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-B                                            to below 100 parasites/ µL equivalent</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-C                                            HRP-2 types refer to frequency of common HRP2 epitope repeats</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Pf pLDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pf aldolase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pv pLDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pv aldolase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cultured P. falciparum</strong></td>
<td>HRP2-A (2) 200, 5000 parasite/µL</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-B (11)</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Culture subset (included in main culture panel)</td>
<td>HRP2-A (1) African</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-B (3)  African (1)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-C (1)  200, 500, 5000 p/µL stability test standard</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>African (1)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asian (1)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRP2-C (1)  Asian (1)</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>PHASE TWO</strong></td>
<td>Wild-type malaria parasites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P. falciparum</strong> (Common HRP2)</td>
<td>100 200, 5000 (2000) parasites/µL dilutions</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sites: South East Asia (3)</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Africa (5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>South and Central America (2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10-15 isolates per site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type/chimp P. vivax</td>
<td>20 Chimp only if lack wild-type</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wild-type/chimp P. ovale</td>
<td>5 200, 500, 5000 (2000) p/µL</td>
<td>X</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Wild-type/chimp P. malariae</td>
<td>5 200, 500, 5000 (2000) p/µL</td>
<td>X</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Parasite-negative human blood</td>
<td>Anti-nuclear antibody (ANA) 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>RPR positive  5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Rheumatoid factor positive 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Heterophile antibody positive (EBV) 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Anti-mouse antibody positive 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Chagas 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Leishmaniaasis 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Schistosomiasis 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Typhoid 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>HIV 5-10</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Clean negatives (none of above) 50 Whole blood</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Note: The final panel may vary slightly according to the availability of samples acceptable for testing after characterization is completed.
Annex Two: Table for ordering culture-derived sub-panel by manufacturers

<table>
<thead>
<tr>
<th>Manufacturer Name</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>(consignee) address</td>
<td></td>
</tr>
<tr>
<td>Courier company</td>
<td></td>
</tr>
<tr>
<td>contracted for</td>
<td></td>
</tr>
<tr>
<td>transport (including</td>
<td></td>
</tr>
<tr>
<td>full contact</td>
<td></td>
</tr>
<tr>
<td>details).</td>
<td></td>
</tr>
<tr>
<td>Date requested for</td>
<td></td>
</tr>
<tr>
<td>collection of</td>
<td></td>
</tr>
<tr>
<td>samples from CDC</td>
<td></td>
</tr>
<tr>
<td>(dd/mm/yy):</td>
<td></td>
</tr>
</tbody>
</table>

Note: It is entirely the manufacturers responsibility to contract the shipping agent (courier) and undertake all necessary payments for transport, including supply of packaging materials and dry ice.

The manufacturer should be experienced at transporting goods on dry ice (-78°C). Note that the consignment contains dead malaria parasites in human blood:

- **Category B Biological Substance - UN 3373**
- Human Blood Samples (Frozen) containing dead malaria parasites
- Quantity: 150 vials containing 50 μL
- For Laboratory Testing Only
- Human material containing no animal material and not of tissue culture origin.

The shipping agent (courier) **MUST** contact CDC beforehand to arrange pick-up of samples. US CDC has the discretion to vary the preferred pick-up date.

**Contact details for ordering panels:**

<table>
<thead>
<tr>
<th>Order by email:</th>
<th>Lucian Marts: <a href="mailto:gko1@cdc.gov">gko1@cdc.gov</a></th>
<th>cc: <a href="mailto:mal-rdt@wpro.who.int">mal-rdt@wpro.who.int</a></th>
</tr>
</thead>
</table>
| Consigner address for courier | Lucian Marts
Centers for Disease Control and Prevention
Malaria Branch, Division for Parasitic Diseases
Bldg 109, Room 1118.6, MS F-36
4770 Buford Hwy NE
Chamblee, GA 30341
ph: 770-488-3771
fax 770-488-4454
Email: gko1@cdc.gov |
ANNEX ONE

SEQUENCE OF EVENTS AND RELEVANT DEADLINES,
CONTACT DETAILS AND ADDRESSES

(1) Applicant submits a request for one electronic temperature monitor (optional) to WHO

Recommended by: 5 April 2008 to allow time for shipment with product to CDC

By email to: belld@wpro.who.int, mal-rdt@wpro.who.int

(2) Applicant submits completed and signed confidentiality and materials transfer agreement in two signed copies (ANNEX TWO), together with the final list of products submitted for evaluation and product inserts (ANNEX THREE), to WHO

Not later than: 11 April 2008

By courier to:

Dr David Bell
Malaria Diagnostics
Malaria, other Vector-borne and Parasitic Diseases
World Health Organization - Regional Office for the Western Pacific
P.O. Box 2932
Manila, Philippines

(3) For each product, applicant submits 1100 tests from each of two separate lots to US CDC. Transportation and insurance during transportation shall be at applicant's cost.

For delivery at US CDC not later than: 30/April/2008

By courier to:

Lucian Marts
Centers for Disease Control and Prevention
Malaria Branch, Division for Parasitic Diseases
Bldg 109, Room 1118.6, MS F-36
4770 Buford Hwy NE
Chamblee, GA 30341
USA

ph: 770-488-3771
fax 770-488-4454
email: gkol@cdc.gov
(4) Applicant arranges for transfer by courier of panel (cryo-preserved parasite samples) from US CDC to the manufacturer site for stability test for each product (transferred on dry ice). Transportation and insurance during transportation shall be at Applicant's cost. The courier company engaged must be capable of transporting blood specimens packaged in dry ice.

On a date to be specified later by WHO, from:

Lucian Marts  
Centers for Disease Control and Prevention  
Malaria Branch, Division for Parasitic Diseases  
Bldg 109, Room 1118.6, MS F-36  
4770 Buford Hwy NE  
Chamblee, GA 30341  
USA

ph: 770-488-3771  
fax 770-488-4454  
email: gko1@cdc.gov

(5) Applicant submits results of stability test performed at the site of manufacture, in a format to be provided by WHO, 12 months after commencement of testing and at the end of the specified shelf life, to WHO (address to be provided).

Note: Receipt of the signed confidentiality and material transfer agreement, final list of products, product inserts, required number of tests for evaluation, and/or results of the stability test for each submitted product after the deadline or otherwise not conforming to the conditions set forth in this letter and/or its Annexes 1 to 4 may result in termination of the evaluation of one or more of the products submitted by the applicant. Please also see Annex 4 ("Information for Manufacturers on WHO Malaria Diagnostics Evaluation Programme"). Applicant will be deemed to have accepted the terms of this document.
ANNEX TWO

WHO STANDARD CONFIDENTIALITY AND MATERIAL TRANSFER AGREEMENT

Between

..............................................................having its principal offices at ...................................................

..............................................................(hereinafter referred to as “the Company”);

and

The World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland, (hereinafter referred to as “WHO”).

The Company has developed (a) rapid malaria diagnostic test(s), known under the trademark ……, which test(s) is/are further described in Exhibit 1 attached hereto, (hereinafter referred to as “the Product(s)”, and information relating thereto (hereinafter referred to as the “the Information”). WHO is interested in having the Product(s) evaluated and tested in the WHO malaria diagnostics evaluation programme, jointly coordinated by the WHO Regional Office for the Western Pacific (WHO/WPRO) and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (WHO/TDR), hereinafter referred to as "the WHO Malaria Diagnostics Evaluation Programme".

Therefore, the Parties have agreed as follows:

(1) The Company shall disclose and furnish to WHO the Information and sufficient quantities of the Product(s) in order to enable WHO to assess the Information and arrange for such evaluations of the Product(s), as WHO may determine, are reasonably necessary to assess the performance of the Product(s) and its/their suitability for use at the primary health care settings in developing countries. At the conclusion of the testing and evaluation process, WHO will report the results thereof to the Company and, at the Company’s request and cost, return or destroy the Information and any unused quantities of the Product(s). For the avoidance of doubt, "Information" as used herein does not include the data and information resulting from the testing and evaluation process (including the stability test(s) performed by the Company and submitted to WHO as part of the Evaluation Programme), any other testing results and
the reports generated as a result of this Agreement (all the foregoing hereinafter jointly referred to as "the Testing Results"). Such Testing Results shall belong to WHO (subject always, however, to the other provisions of this Agreement).

(2) If and to the extent that the Information has been marked by the Company as "Confidential", WHO shall treat such Information as confidential and proprietary for a period of five years after disclosure to it. In this connection, WHO shall take all reasonable measures to ensure that the Information in question is not used for any purpose other than the aforementioned evaluation and testing activities and is not disclosed or provided to any person who is not bound by similar obligations of confidentiality and restrictions on use as contained in this Agreement.

(3) WHO shall not be bound by any obligation of confidentiality or restriction on use to the extent it is clearly able to demonstrate that any part of the Information:

a) was known to WHO prior to any disclosure by the Company to WHO; or
b) was in the public domain at the time of disclosure by the Company to WHO; or
c) becomes part of the public domain through no fault of WHO; or
d) becomes available to WHO from a third party not in breach of any legal obligations of confidentiality to the Company.

(4) The Company undertakes to abide by similar obligations of confidentiality and restrictions on use as contained in paragraphs 2 and 3 above with regard to the Testing Results (regardless of whether or not such Testing Results have been marked as "confidential").

(5) The provision of Product(s), Information, and Testing Results shall not in itself be construed as conveying rights under any patents or other intellectual property which either Party may have or may hereafter obtain.

(6) Subject to the protection of each Party’s confidential information and the provisions of this paragraph 6, Testing Results may be published by either Party. In order to avoid prejudicing confidential information of the other Party, the submitting Party will transmit to the other Party for its review, the material intended to be published at least 60 (sixty) working days before a proposed publication is submitted to any editor, publisher, referee or meeting organizer. In the absence of an objection by the other Party within that 60-day period concerning prejudice to its confidential information,
and provided that all other conditions of this paragraph 6 have been met, the publication may proceed.

In connection with the foregoing, it is understood and agreed that notwithstanding any other provisions in this Agreement, WHO shall be entitled to evaluate and publish the Testing Results, and to exclusively control this evaluation and the content of the aforesaid publication, provided that in order to avoid prejudice to the Company's confidential Information disclosed to WHO pursuant to paragraphs 1 and 2 above, WHO shall submit any proposed publication to the Company for review in accordance with the provisions of this paragraph 6. For the avoidance of any doubt, the Company shall only be entitled to object to a proposed publication if and to the extent it contains any confidential Information of the Company, and not on the grounds that the Company is not satisfied with the Testing Results and/or does not agree with WHO's evaluation thereof.

The Company shall not proceed to the publication (or any other public disclosure) of any of the Testing Results until such Results have been published by WHO and until the proposed publication has been submitted to WHO for review in accordance with the provisions of this paragraph 6.

All publications of the results of any evaluation and testing activities carried out under this Agreement shall include the following statement:

"This investigation was carried out as part of the WHO Malaria Diagnostics Evaluation Programme".

Other than as provided herein before, neither Party shall, in any statement or material of an advertising or promotional nature, refer to the relationship of the Parties under this Agreement or to the relationship of the other Party to the Product(s). The Company shall not, at any time, use, nor allow any other parties to use, the participation in the Evaluation Programme and/or publication by WHO of the Testing Results for commercial or promotional purposes. Under no circumstances shall the Company or any other party be authorized to refer to WHO, the Company's participation in the Evaluation Programme, and/or the publication of the Testing Results by WHO, in any statement or material of an advertising or promotional nature, press release and/or similar public statement and/or other material aimed at promoting the Company, any other party and/or the Product(s).
The Company shall provide the Information and sufficient quantities of the Product(s) to WHO, or WHO's designee(s), free of charge. Upon receipt of a written request to that effect, the Company shall furthermore pay any and all costs relating to the evaluation and testing process hereunder to WHO, or WHO's designee(s), in advance, in accordance with WHO's instructions. In the event that WHO, or its designee(s), do not receive the Information, and sufficient quantities of the Product(s) by the required deadlines, WHO shall be under no obligation to arrange for the performance of any evaluation or testing activities in relation to the Product(s). Any balance of funds provided by the Company, and remaining unspent upon the conclusion of the testing and evaluation process shall be returned to the Company, unless otherwise agreed by the Parties.

The Company hereby furthermore confirms that it has taken good note of, agrees with, accepts and to the extent applicable, shall abide by, the provisions contained in the document, entitled "Information for Manufacturers on WHO Malaria Diagnostics Evaluation Programme."

Any dispute relating to the interpretation or application of this Agreement shall, unless amicably settled, be subject to conciliation. In the event of failure of the latter, the dispute shall be settled by arbitration. The arbitration shall be conducted in accordance with the modalities to be agreed upon by the Parties or, in the absence of agreement, with the rules of arbitration of the International Chamber of Commerce. The Parties shall accept the arbitral award as final.

On behalf of WHO:

Signature:

Name:

Title:

Date:

On behalf of the Company:

Signature:

Name:

Title:

Date:
ANNEX THREE

PRODUCTS TO BE INCLUDED FOR EVALUATION

Up to three products, from the list already submitted to WHO through the Expression of Interest (note that details of products have previously been submitted to WHO, and that for this round of testing no new products can be submitted).

<table>
<thead>
<tr>
<th>Manufacturer's Name</th>
<th>Product 1</th>
<th>Product 2</th>
<th>Product 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Name: ____________________________ Signature: __________________________

Designation / position: __________________________ Date: __________________________

Submit this form by email to:

belld@wpro.who.int or mal-rdt@wpro.who.int

Not later than: 11 April 2008
# WHO-FIND malaria RDT evaluation programme

Malaria rapid diagnostic tests submitted to current round of product testing at US Centres for Disease Control and Prevention

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Product accepted into WHO-FIND Product Testing Programme at US CDC, 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access Bio, Inc.</td>
<td>CareStart Malaria HRP2 (Pf)</td>
</tr>
<tr>
<td></td>
<td>CareStart Malaria HRP2/pLDH (Pf/PAN) COMBO</td>
</tr>
<tr>
<td></td>
<td>CareStart Malaria pLDH (PAN)</td>
</tr>
<tr>
<td>ACON Laboratories, Inc.</td>
<td>Malaria Plasmodium falciparum Rapid test Device (Whole blood)</td>
</tr>
<tr>
<td>Amgenix International, Inc.</td>
<td>OnSight – ParaQuick (Pan, Pf) Test</td>
</tr>
<tr>
<td>AZOG, Inc.</td>
<td>AZOG Malaria pf (HRP-II) /pv (pLDH) Antigen Detection Test Device</td>
</tr>
<tr>
<td>Biosynex</td>
<td>Immunoquick Malaria Falciparum</td>
</tr>
<tr>
<td></td>
<td>Immunoquick Malaria +4</td>
</tr>
<tr>
<td>Diagnostics Automation Inc.</td>
<td>Malaria P.F/Vivax</td>
</tr>
<tr>
<td>DiaMed</td>
<td>OptiMAL-IT</td>
</tr>
<tr>
<td>Human GmbH</td>
<td>Hexagon Malaria</td>
</tr>
<tr>
<td></td>
<td>Hexagon Malaria Combi</td>
</tr>
<tr>
<td>IND Diagnostic Inc.</td>
<td>One Step Malaria Antigen Strip</td>
</tr>
<tr>
<td>Innovatek Medical Inc.</td>
<td>Quickstick Malaria Antigen Test (Co- listing with IND Diagnostics Inc. One Step Malaria Antigen Strip)*</td>
</tr>
<tr>
<td>Intec Products Inc.</td>
<td>ADVANCED QUALITY™ One Step Malaria (p.f.) Test (Whole blood)</td>
</tr>
<tr>
<td></td>
<td>ADVANCED QUALITY™ MALARIA (p.f) POCT</td>
</tr>
<tr>
<td>Inverness Medical Innovations</td>
<td>Binax Now Malaria</td>
</tr>
<tr>
<td>J. Mitra Company Pvt Ltd</td>
<td>Advantage Pan Malaria Card</td>
</tr>
<tr>
<td></td>
<td>Advantage P.f. Malaria Card</td>
</tr>
<tr>
<td></td>
<td>Advantage Mal Card</td>
</tr>
<tr>
<td>Orchid Biomedical Systems</td>
<td>Paracheck Pf Rapid test for P. falciparum Malaria ( Device)</td>
</tr>
<tr>
<td></td>
<td>Paracheck Pf Rapid test for P.falciparum Malaria ( Dipsick)</td>
</tr>
<tr>
<td>Premier Medical Corporation Ltd.</td>
<td>First Response Malaria Ag HRP2</td>
</tr>
<tr>
<td></td>
<td>First Response Malaria Ag Combo (pLDH/HRP2)</td>
</tr>
<tr>
<td>ICT Diagnostics</td>
<td>ICT Malaria Pf Cassette Test (ML01)</td>
</tr>
<tr>
<td></td>
<td>ICT Malaria Combo Cassette Test (ML02)</td>
</tr>
<tr>
<td>Span Diagnostics</td>
<td>Parahit-f DIPSTICK FOR FALCIPARUM MALARIA</td>
</tr>
<tr>
<td></td>
<td>Parahit-f TEST DEVICE FOR FALCIPARUM MALARIA</td>
</tr>
<tr>
<td></td>
<td>Parahit-Total Device Rapid test for P. falciparum and Pan malarial species.</td>
</tr>
<tr>
<td>Standard Diagnostics, Inc.</td>
<td>SD BIOLINE Malaria Ag</td>
</tr>
<tr>
<td></td>
<td>SD BIOLINE Malaria Ag Pf</td>
</tr>
<tr>
<td></td>
<td>SD BIOLINE Malaria Ag Pf/Pan</td>
</tr>
<tr>
<td>Unimed International, Inc.</td>
<td>FirstSign – Malaria Pf Card Test</td>
</tr>
<tr>
<td></td>
<td>FirstSign – ParaView-2 (Pv + Pf) Card Test</td>
</tr>
<tr>
<td>Vision Biotech</td>
<td>Malaria Rapid Pf</td>
</tr>
<tr>
<td></td>
<td>Malaria Rapid Combo</td>
</tr>
<tr>
<td></td>
<td>Malaria Rapid Dual</td>
</tr>
<tr>
<td>Guangzhou Wondfo Biotech Co., Ltd</td>
<td>Wondfo One Step Malaria Pf/Pan Whole Blood Test</td>
</tr>
<tr>
<td>Zephyr Biomedicals</td>
<td>Parascreen Rapid Test for Malaria Pan/Pf (Device)</td>
</tr>
<tr>
<td></td>
<td>Malascan Rapid Test for Malaria Pf/Pan (Device)</td>
</tr>
<tr>
<td></td>
<td>Parabank Rapid Test for Malaria Pan (Device)</td>
</tr>
</tbody>
</table>

*Co-listing: One product is submitted for assessment. Manufacturers confirm that second product is identical with different label.
WHO-FIND Malaria RDT Evaluation Programme
Product Testing of Malaria Rapid Diagnostic Tests (RDTs)
Manufacturing Site Stability Test

This note outlines requirements for the manufacturing site stability test of malaria RDT products submitted for product testing. The testing process is detailed in the accompanying standard operating procedures (SOP 2.2.d), and a standard report form is provided in electronic format (by email) and hard copy. Please keep this note with SOP 2.2.d for future reference.

This stability test was specified in previous correspondence as a requirement for participation in the product testing programme, which has now commenced. The test is intended to provide real-time data on thermal stability at the maximum specified temperature recommended for each product, for the duration of the recommended shelf-life, against standardized reference parasite-positive blood samples. The products of your company accepted into the programme are listed in a letter document accompanying this document.

**Production lots to be tested**

The manufacturing site stability test should be performed using the same production lots as those submitted for product testing to the US Centers for Disease Control and Prevention (CDC), Atlanta, USA. If this is not possible, two other lots of the same product should be used.

**Duration of Testing**

The initial test should be conducted by 30 June 2008, and both RDT lots should be under incubation by this date. If this is not possible, WHO should be informed immediately at the contact address below.

Testing should continue every 3 months until the expiry date of each lot of RDTs.

**Recording and Reporting Results**

The line intensity should be rated using the colour intensity rating scale provided. The colour chart closest to the colour of the RDT test lines should be selected, and used throughout. Note that the intensity rating is not intended to compare visibility of lines between products, but to allow a reduction in intensity over time to be noted.

Results should be communicated to WHO every 3 months by email attachment in the format provided, to mal-rdt@wpro.who.int.
Blood Reference Standard (Obtaining, and use of)

The reference standard is a culture-derived parasite *P. falciparum* parasite sample (Nigeria XII) which forms part of the phase 1 testing panel of the product testing programme and is the standard used for the thermal stability component of the product testing underway at CDC in Atlanta, GA, USA.

CDC will provide sufficient aliquots of the parasite sample to test two lots of the products of each manufacturer, using the blood volume specified in the manufacturer's product instructions. Blood aliquots should be discarded after use, and not re-frozen.

The samples are provided as 50 µL aliquots in dilutions of 200 parasites/µL and 2000 parasites/µL. If the 200 parasites/µL dilution does not produce a positive test result on initial testing, this dilution may be excluded from the subsequent test at 3 monthly intervals, and the dilution of 2000 parasites/µL alone used.

The blood sample must be obtained from US CDC at the manufacturers expense. The samples must be transported frozen (on dry ice, -78°C) and stored at least at -20°C after receipt.

Please note that the samples should be transported from CDC by engaging a courier experienced in the transport of blood samples on dry ice, and in the relevant customs clearance involved in such transport. The integrity of the samples during transport and on receipt, and the process of clearing through customs and obtaining relevant national regulatory approvals, is the responsibility of the manufacturer (consignee). WHO and CDC will assist with necessary documentation on request.

The consignment contains dead malaria parasites in human blood:
- **Category B Biological Substance - UN 3373**
- Human Blood Samples (Frozen) containing dead malaria parasites
- Quantity: XXX* vials containing 50 µL
- For Laboratory Testing Only
- Human material containing no animal material and not of tissue culture origin.

The shipping agent (courier) **MUST** contact CDC before hand to arrange pick-up of samples and relevant documentation. CDC has the discretion to vary the preferred pick-up date.
Contact details for obtaining blood samples to be used in stability testing at the manufacturing site:

<table>
<thead>
<tr>
<th>Contact by email:</th>
<th>Lucian Marts: <a href="mailto:gko1@cdc.gov">gko1@cdc.gov</a></th>
<th>cc: <a href="mailto:mal-rdt@wpro.who.int">mal-rdt@wpro.who.int</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Consigner address for courier</td>
<td>Lucian Marts Centers for Disease Control and Prevention Malaria Branch, Division for Parasitic Diseases Bldg 109, Room 1118.6, MS F-36 4770 Buford Hwy NE Chamblee, GA 30341 ph: 770-488-3771 fax 770-488-4454 Email: <a href="mailto:gko1@cdc.gov">gko1@cdc.gov</a></td>
<td></td>
</tr>
</tbody>
</table>

The parasite-negative blood sample necessary as a control in the testing process should be provided by the manufacturer.

Further Information

Information updates are placed on [http://www.wpro.who.int/sites/rdt/call_for_testing.htm](http://www.wpro.who.int/sites/rdt/call_for_testing.htm)

Enquiries should be addressed to:

David Bell  
Malaria, other Vector-borne and Parasitic Diseases  
World Health Organization – Regional Office for the Western Pacific  
P.O. Box 2932  
Manila, Philippines  
Ph: +63 2 5289756  
FAX: +63 2 5211036  

belld@wpro.who.int and mal-rdt@wpro.who.int
SOP 2.2.d: Stability Assessment at Manufacturing Site

PURPOSE
This SOP describes the procedure for stability testing of a Rapid Diagnostic Test at the manufacturing site, using standards provided by the WHO-FIND malaria rapid diagnostic test evaluation programme.

SCOPE
This procedure applies to manufacturers participating in the malaria RDT product testing programme of WHO and FIND with the US Centers for Disease Control and Prevention.

TESTING PROCEDURE (Refer to Figure 1)

1. General

a) Testing should occur at Day = 0, using 24 RDTs from each of 2 lots (total 48).
   - Test 8 RDTs against 200 parasites/µL
   - Test 8 RDTs against 2000 parasites/µL
   - Test 8 RDTs against negative sample

b) Store sufficient RDTs in the incubator /environmental chamber to allow testing of 40 RDTs (20 per lot) every 3 months for the remaining duration of the shelf-life designated for that product by the manufacturer, and sufficient RDTs at 4°C (2-8°C) to allow testing of RDTs (12 per lot) every 3 months for the remaining duration of the shelf-life.

c) Every three months, test 20 RDTs from each lot stored in the incubator and 12 from each lot stored at 4°C:
   - From 4°C storage:
     - Test 4 RDTs against 200 parasites/µL
     - Test 4 RDTs against 2000 parasites/µL
     - Test 4 RDTs against negative sample
   - From incubator:
     - Test 8 RDTs against 200 parasites/µL
     - Test 8 RDTs against 2000 parasites/µL
     - Test 4 RDTs against negative sample

d) Incubated RDTs should be stored at the maximum storage temperature recommended by the manufacturer.

e) Allow a minimum 3 days to calibrate incubators prior to conducting baseline testing.

f) Incubator temperatures and refrigerator temperatures should be recorded daily on a chart attached to the incubator or closely accessible

g) Mark days when testing is due at 3 month intervals for remainder of shelf life.
h) RDTs should be stacked in incubators in their normal packaging (boxes / kits), allowing air circulation against at least 2 sides of box, and not in direct contact with walls or floor of incubator.

i) All documentation should be readily accessible if manufacturer site inspections occur.

Table A: Number of RDTs required per lot for STABILITY TESTING.

<table>
<thead>
<tr>
<th>Test</th>
<th>RDT required per lot at storage temperature</th>
<th>RDT required per lot at 4°C</th>
<th>Total RDTs (both lots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>24</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>Each 3 months</td>
<td>20</td>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>Expiry date</td>
<td>20</td>
<td>12</td>
<td>64</td>
</tr>
</tbody>
</table>

Example for 2 year shelf-life, 3 month old lot when testing commences:

<table>
<thead>
<tr>
<th>Test</th>
<th>RDT required per lot at storage temperature</th>
<th>RDT required per lot at 4°C</th>
<th>Total RDTs (both lots)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>24</td>
<td>48</td>
<td>64</td>
</tr>
<tr>
<td>6 x 3 months</td>
<td>120</td>
<td>72</td>
<td>384</td>
</tr>
<tr>
<td>Expiry date</td>
<td>20</td>
<td>12</td>
<td>64</td>
</tr>
<tr>
<td>Spare</td>
<td>20</td>
<td>12</td>
<td>64</td>
</tr>
</tbody>
</table>

**Total per lot** 184 96 560

2. Preparation of product and sample

a) Blood samples to be used as the positive standard for testing are supplied from WHO / CDC (manufacturer must arrange and fund courier), in aliquots of 200 parasites/microL and 2000 parasites/microL, and should be stored at ≤-20°C.

b) Test in air-conditioned (low humidity) environment, at ≤25°C with good lighting.

c) Thaw sufficient aliquots of blood samples designated for RDT stability testing for 30 minutes at room temperature (<30°C) (Each aliquot is approximately 50 microL, so for RDTs requiring 5 uL blood, it is recommended to thaw 3 samples)

d) Store samples aliquots at 4°C after thawing

e) All blood samples must be used within 8 hours of thaw. Do not re-freeze.

f) Withdraw the correct number of RDTs of each lot from storage (incubator and 4°C – Figure 1) and allow to reach room temperature before opening envelope.

g) Test the correct number of RDTs from each lot (diagram below), against aliquots of 200 parasites/microL and 2000 parasites/microL and parasite-negative samples. (If all RDTs have failed at 200 parasite/microL at a previous testing interval, this aliquot may be removed from the testing procedure at future testing intervals. Follow manufacturer’s RDT preparation procedure for each product, using pipette or manufacturer’s blood transfer device to obtain correct blood volume.

h) If both lots of a product fail to detect the 200 parasites/microL sample at the initial test, the 200 parasite/µL sample may be excluded from future testing and subsequent tests at 3 month intervals conducted using the 2000 parasites/microL sample only.

3. Reading and reporting results

a) Read result within the time period specified by manufacturer, rating line intensity 0-4 against colour intensity chart provided (Use colour closest to colour of positive line).
WHO-FIND-CDC Malaria RDT Product Testing Methods Manual (Version 1)

b) Record on the record sheet provided in hard copy and electronic copy.

c) Submit results to WHO at the specified intervals.

d) Submission of results may cease once all RDTs have failed at any testing interval.
Stability Evaluation at Manufacturing Site

Select sufficient RDTs of each of 2 lots to test: 24 RDTs initially, 32 RDTs at 3 month intervals throughout shelf-life, and at end of shelf life. It is advised to add at least 64 additional RDTs to allow for errors in process.

Figure 1: Stability Test Flowchart
**Form 036a: STABILITY TEST: MANUFACTURER’S RESULT SHEET**

Manufacturer:  
Product:  
Lot:  
TIME OF READING (INTERVAL IN MONTHS) (‘0’, or ‘3’, ‘6’ etc):  
Technician (Name …………………………………….)  
Colour chart used:

<table>
<thead>
<tr>
<th>Parasite density:</th>
<th>Time of preparation</th>
<th>Time of reading</th>
<th>Result: Rate colour intensity 0-4 using colour chart provided</th>
<th>Interpretation (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative, 200, 2000 para/µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
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<td>Negative</td>
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<tr>
<td>Negative</td>
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<tr>
<td>200 para/µL</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>200 para/µL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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Result should be recorded according to intensity of line, referenced against colour rating chart provided.  
'Control': Control line. 'Line 1 -3': Test result lines, as appropriate for the product.  
Signed: ……………………………….