Global **Malaria** Programme

Procedures for product testing and lot testing. Information for RDT manufacturers and procurers

WHO–FIND Malaria Rapid Diagnostic Test (RDT) Evaluation Programme

World Health Organization

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GLOSSARY

The definitions given in this glossary apply to the terms used in the context of the WHO–FIND Malaria Rapid Diagnostic Product Testing and Lot Testing programmes; they may be used differently in other contexts.

Anomaly. Deviation from the expected appearance of a test after completion, detected by direct observation; may include a red background, incomplete clearance due to failure to flow, shifting or misplacement of a strip, ghost lines, diffuse test lines or patchy broken test lines.

Equivalence of performance. Demonstration that the performance of a modified product is equivalent to or better than that of the product previously submitted for formal assessment by the WHO–FIND Product Testing Programme.

False-positive rate. Percentage of all tests of a product that gave a positive result when it should have been negative after the manufacturer's recommended reading time.

Invalid rate. Proportion of tests declared invalid due to the absence of a control line.

Lot (of malaria RDTs). In the context of the WHO–FIND Product Testing and Lot Testing programmes, a production run with a particular, uniform batch of monoclonal antibodies, nitrocellulose and other essential components. Denoted by unique numeric or alpha-numeric codes; its definition must be compatible with current ISO 13485:2003 or United States Food and Drug Administration 21 CFR 820.

Panel detection score. A score between 0 and 100, representing the proportion of times a malaria RDT gives a positive result in all tests of both lots tested against samples of parasite panels at a specific parasite density, i.e. four tests at 200 parasites per microlitre and two at 2000 parasite per microlitre. It is a composite index that accounts for inter-test and inter-lot variation as well as positivity rates. Invalid tests are excluded from the analysis.

Product. A unique RDT product defined by a unique identifier or product code. Product testing results should be applied only to a specifically defined and labelled product. Similar but re-labelled products from various manufacturers should generally be considered different products but may be considered the same product if specifically indicated by the manufacturers concerned.

Product resubmission, compulsory. Resubmission to the WHO–FIND RDT Product Testing Programme is required for products that have not been re-tested within 5 years.

Rapid diagnostic test (RDT). Immunochromatographic lateral flow device for the detection of malaria parasite antigens.

INTRODUCTION

WHO recommends parasitological confirmation of malaria in all settings by a quality-assured diagnostic method before treatment is started (*1*). Treatment solely on the basis of clinical suspicion should be considered only when a parasitological diagnosis is not available within 2 h of presentation of a patient for treatment. A diagnosis of malaria can be confirmed rapidly by good-quality microscopy or with a good-quality malaria antigen-detecting RDT for *Plasmodium falciparum* and non-falciparum infections. In most countries, both diagnostic methods are required, as microscopy and RDTs often play different roles, depending on the clinical situation and the setting (*2*).

Malaria RDTs are complex biological products made up of several components, which are often produced by different manufacturers. Both the material and the manufacturing process of each component are often subject to change, e.g. in the quality of antibodies, the pore size of the nitrocellulose membrane or the composition and viscosity of the buffer solution, all of which can affect the correct performance of an RDT (*3*).

To minimize the intrinsic vulnerability of an RDT, the WHO Global Malaria Programme, in collaboration with a number of partners, has established a comprehensive quality control system for malaria RDTs, in two programmes:

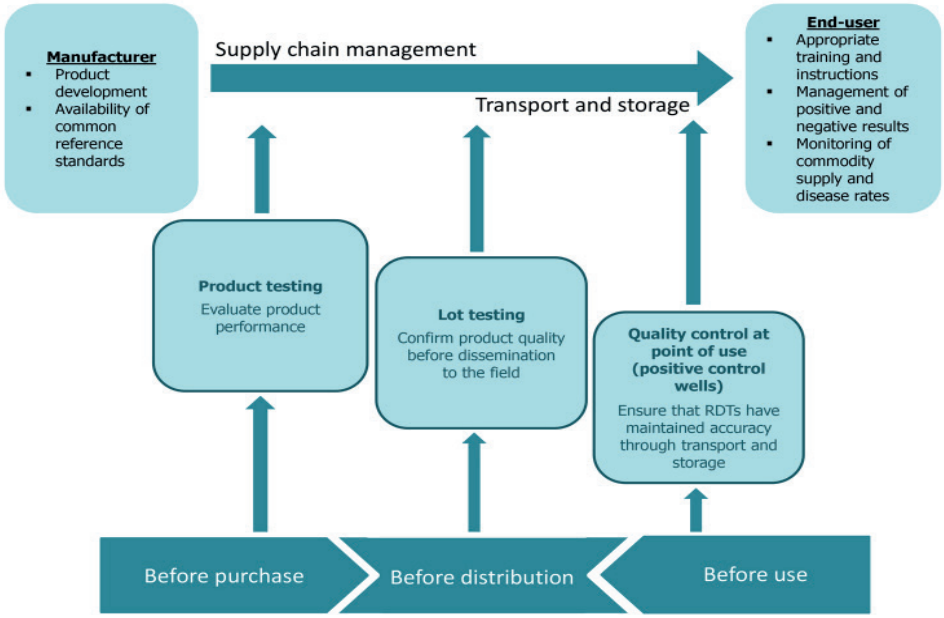
- the **Malaria RDT Product Testing Programme**, for periodic pre-purchase assessment of diagnostic performance (see section 1) to provide guidance for procurement; and
- the **Malaria RDT Lot Testing Programme**, for post-purchase evaluation of diagnostic performance, either before or after shipment (see section 2).

In these two programmes, as illustrated in Fig. 1, WHO undertakes a comprehensive assessment of the diagnostic performance of malaria RDTs, based on an evaluation of either products submitted by manufacturers (Product Testing Programme) or samples of RDTs submitted by purchasing entities or other interested parties (Lot Testing Programme). In both programmes, RDTs are assessed by standardized procedures, and the results are used by the WHO Global Malaria Programme to provide WHO and other United Nations agencies with advice on the performance of RDTs and their suitability for procurement. This document details the eligibility requirements and procedures for the two programmes.

NOTE

The WHO Programme for Prequalification of Diagnostics and Medical Devices uses the results of the Malaria RDT Product Testing Programme as the laboratory evaluation component of the prequalification process for malaria RDTs. These data are also used to set priorities for dossier review and manufacturing site inspection. Although prequalification is not currently a requirement for WHO procurement, manufacturers are encouraged to apply for it, as it may become mandatory in the future. A list of prequalified diagnostics, including malaria RDTs, is available at http://www.who.int/diagnostics_laboratory/evaluations/PQ_list/en/.

FIG. 1.
WHO–FIND programmes for testing malaria rapid diagnostic test products and lots



1. WHO–FIND MALARIA RDT PRODUCT TESTING PROGRAMME

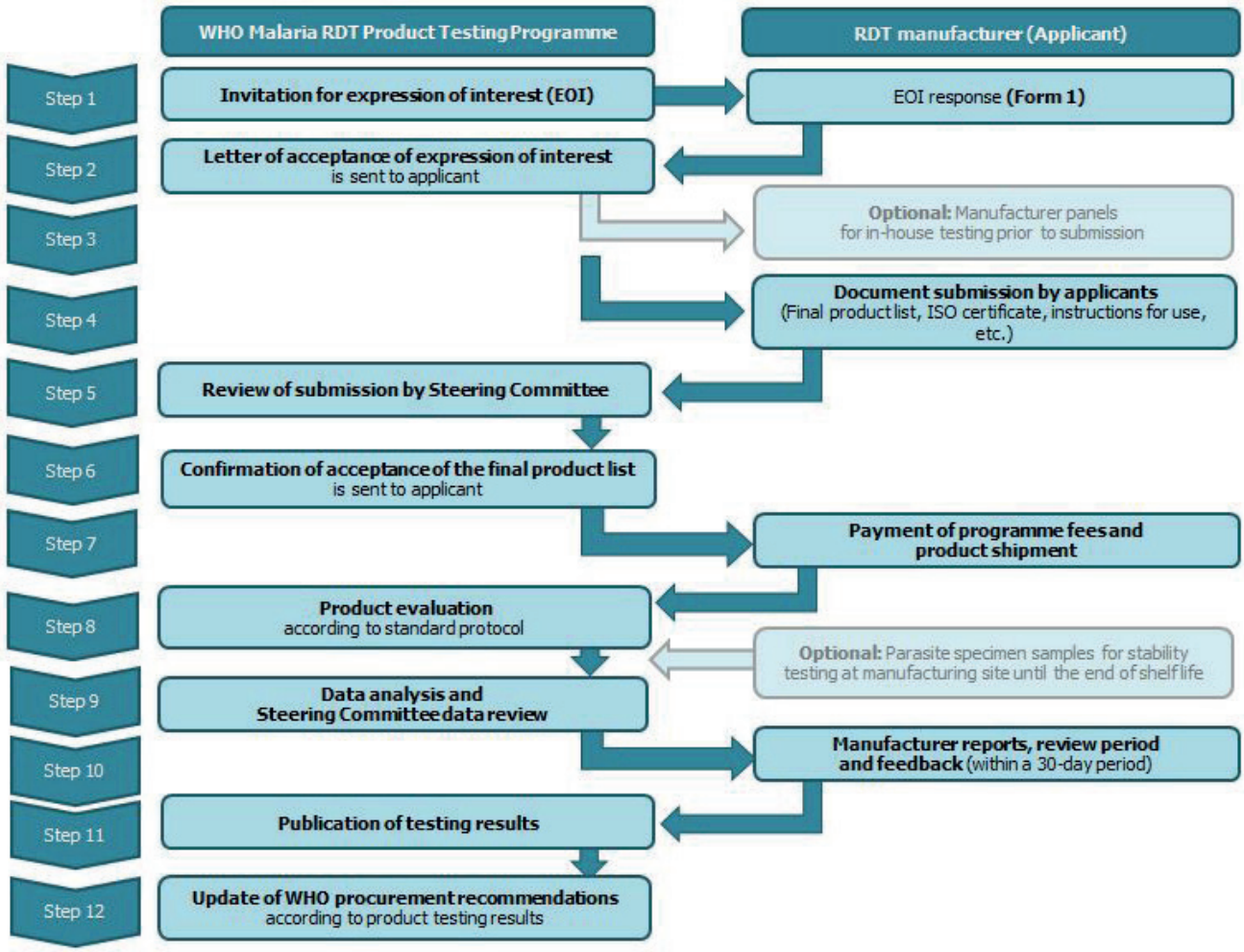
The heterogeneous diagnostic performance of the more than 200 malaria RDTs currently available on the market can undermine the confidence of health professionals in their accuracy. The WHO–FIND Malaria RDT Product Testing Programme, coordinated by the WHO Global Malaria Programme and the Foundation for Innovative New Diagnostics (FIND) and executed in collaboration with the United States Centers for Disease Control and Prevention (CDC), is designed to provide comparative data on the performance of RDTs submitted for evaluation.

The diagnostic performance of antigen-detecting malaria RDTs is assessed by a standard protocol against a panel of cryo-preserved malaria parasite samples from the global specimen bank. Since 2008, the results of product evaluation have been published in regular reports, and the performance results form the basis of the WHO *Information note on recommended selection criteria for procurement of malaria rapid diagnostic tests* (*4*), which guides procurement of RDTs by WHO and other United Nations agencies, national health authorities and other interested parties.[1]

The procedural steps of the Malaria RDT Product Testing Programme are outlined in Fig. 2 and explained in detail in steps 1.1–1.11 below. Further information on specimen collection, characterization and evaluation procedures can be found in the documents listed below (also available at www.finddiagnostics.org) or can be obtained by sending an email to malaria_rdt@who.int:

- Methods manual for laboratory quality control testing of malaria rapid diagnostic tests, version 7. Geneva: World Health Organization; 2014 (*5*)
- Methods manual for product testing of malaria rapid diagnostic tests, version 6. Geneva: World Health Organization; 2014 (*6*)

FIG. 2.
WHO–FIND Malaria RDT Product Testing Programme: step-by-step procedure



Step 1.1. Invitation for expressions of interest[2]

As the initial step of each round, the WHO Global Malaria Programme invites RDT manufacturers that are compliant with ISO 13485:2003 to express their interest in participating in the upcoming round of product testing.[3] Interested manufacturers (applicants) submit completed form 1, giving detailed product information for the RDTs being proposed for assessment. form 1 must be submitted electronically, followed by a hard copy, to the address indicated in the EOI by the specified deadline for submission.

Applicants may submit **commercially available antigen-detecting lateral flow products in any format and for any malaria-specific target antigen**. RDTs with the same product name but different formats (e.g. cassette and dipstick) are considered two different products and require separate submission and assessment. Cassettes are preferred to dipsticks for field use in malaria-endemic countries.

The EOI is not binding for either party. Only those products that the applicant lists on form 1 will be considered for potential inclusion in the next round of product testing. If the total number of products proposed by all applicants who respond to the EOI is beyond the capacity of the Programme for testing in a single round, WHO reserves

the right to limit the number of accepted products per applicant. In the past, the number has been restricted to one to three products per manufacturer.

Product resubmissions. (1) Resubmission of a product for a new assessment by the Programme is **compulsory** at the latest every 5 years, in order for it to continue to be included in relevant publications and to stay on the list of WHO-recommended RDTs for procurement. With the EOI invitation for each round, the Programme issues a list of products that are due for such compulsory resubmission. (2) **Voluntary** resubmission, i.e. earlier than 5 years, is accepted, depending on the Programme capacity. In such cases, the results of previous rounds will be replaced in the performance report by the results obtained in subsequent rounds to which the product is submitted.

Step 1.2. Letter of acceptance of expressions of interest

Applicants' submissions will be reviewed by the Programme for completeness and accuracy as well as for product relevance according to Programme needs and testing capacity. If an applicant's submission is considered acceptable, the Programme will send the applicant an acceptance letter, inviting the applicant to submit the full set of required documents (see step 1.4) for in-depth review by the Steering Committee.

Step 1.3. Optional: Manufacturer panels

Before submitting products to the Product Testing Programme, manufacturers have the option of obtaining panels for in-house quality control testing, consisting of:

- a subset of the product testing panel prepared from cultured *P. falciparum* malaria parasites for their own quality control testing and for stability testing at the manufacturing site and
- an eight-well strip dilution series of dried-down recombinant histidine-rich protein-2 and aldolase, including two negative controls.

The cultured parasites and recombinant antigen panels are available at no charge; however, manufacturers are responsible for the courier costs (from CDC, USA, and Microcoat, Germany, to the manufacturing site) and any associated costs of transport or importation.

Step 1.4. Document submission by applicants

Once the applicant has received the acceptance letter, the applicant is invited to complete the submission by sending the documentation listed below to the Programme, first as scanned copies by e-mail, followed by hard copies by courier to the addresses listed in the EOI:

- **Final product list.** With the EOI response (see step 1.1, form 1), the applicant, on a non-binding basis, proposes particular products for inclusion in product testing.[4] In the EOI acceptance letter, the Programme advises the applicant about the maximum number of products that can be accepted for assessment, according to Programme capacity and needs. For the final product list (see step 1.6, form 3), the applicant will select those products

listed on form 1 that they wish to have assessed, in line with the maximum number. No other new products can be accepted at this time.

- **ISO certificate.** A valid ISO 13485:2003 certificate should be sent for each site at which the product(s) to be submitted for testing is or are manufactured. The authenticity of all ISO certificates will be verified with the issuing accreditation company. Manufacturers that submit invalid certificates will not be accepted for evaluation.

- **Instructions for use.** The applicant should ensure that the instructions for use sent by e-mail, hard copy and with the RDT shipment are accurate and identical; in the case of any discrepancies, the instructions accompanying the RDT shipment will apply.

- **Heat stability protocol.** An acceptable heat stability protocol used for internal quality assurance (Annex 1) should be provided.

- **Confidentiality agreement.** Two original signed copies of the confidentiality agreement (form 2) and acceptance of the conditions for product testing and of publication of results should be submitted.

- **Optional:** Request for a temperature monitor for the duration of RDT transport.

Step 1.5. Review of submission by the Steering Committee

The WHO–FIND Malaria RDT Evaluation Programme Steering Committee[5] is mandated to provide recommendations to WHO and FIND on the following:

- the development and modification of SOPs for specimen collection and use;
- the collection and characterization of specimens and maintenance of the specimen bank;
- the policy on access to the specimen bank;
- protocols for laboratory testing of the accuracy and stability of malaria RDTs, including product testing and lot testing; and
- review and interpretation of the results of product testing, prior to publication.

The Committee is composed of the following members:

- WHO Global Malaria Programme (two members),
- FIND (two members),
- CDC (two members) and
- Médecins sans Frontières (one member)
- Hospital for Tropical Diseases (one member)
- Army Malaria Institute (one member)
- Rotating representatives from at least 2 specimen collection sites (one African, one non-African)

The Steering Committee reviews the EOIs to determine the appropriateness of the products proposed for evaluation and, if necessary, to determine the number of

products per manufacturer on the final list of products. Subsequently, the Committee reviews the completeness of the submitted documentation, as listed under step 1.3, and rules on special requests and on the acceptability of modifications to the application.

Step 1.6. Confirmation of acceptance of the final products

After the Programme has reviewed and agreed on the final product list proposed by the applicant (see step 1.4), the applicant will receive confirmation of acceptance of the list (form 3) from the Programme. At this time, the applicant will be requested to make a payment (see step 1.7) as per the instructions in the confidentiality agreement (form 2). Bank details will be communicated to the applicant with confirmation of acceptance of the final product list.

Step 1.7. Payment of programme fees and product shipment

The applicant pays a fee of US$ 8000 for each product accepted and finally submitted for assessment.[6] This fee contributes to the costs of the current product evaluation scheme and creation of sustainable quality assurance procedures. The fee is due only **after** the applicant has received confirmation of acceptance of the final product list (see step 1.6) and **before** shipment of the products.

A specified number[7] of RDTs, packaged with the standard kit contents (i.e. multi-use buffer bottle, specimen transfer devices, lancet, alcohol swab), from each of two separate lots is required to be sent to the Programme. The applicant is responsible for the cost of the RDT product, courier costs and any other associated costs of transport of RDTs to the CDC in Atlanta, Georgia. Temperature monitors for the duration of transport can be obtained from WHO free of charge upon request. All RDTs must be received at the CDC within the specified time in order to be accepted for product testing.

Step 1.8. Product evaluation

Upon receipt at the testing laboratory, all products are stored in an air-conditioned, temperature-monitored room until actual product testing. The product-testing site will determine the order in which testing is conducted.

Testing is conducted according to a standardized protocol, described in detail in the *Methods manual for product testing of malaria rapid diagnostic tests*, version 7 (*5*).

Phase 1 of testing is performed against a panel of cryo-preserved preparations of cultured P. falciparum parasites and 20 clean-negative samples. **Phase 2** is performed against a panel of diluted cryo-preserved preparations of wild parasites (*P. falciparum* and *P. vivax*) and parasite-negative samples. If a product does not show sufficient performance against the phase-1 panel, the lot will not be tested against the phase-2 panel. Sufficient phase-1 performance is defined as a panel detection score ≥ 80% against samples with 2000 parasites/µL, and ≤ 50% false-positive rate against 20 clean-negative samples. Both lots are also tested for heat (thermal) stability evaluated after 2 months' storage at room temperature, 35 °C and 45 °C. Assessment of the heat stability of products for detecting *P. vivax* has been included since round 6. An ease-of-use description is completed in a standard assessment format, and common RDT anomalies are recorded. The Steering Committee monitors the testing and all the results.

Evaluation panel samples. The WHO malaria specimen bank (currently at CDC) is the repository of characterized samples against which panels are tested. It includes culture-derived and wild-type malaria parasites, parasite-negative samples and recombinant antigens. The wild-type parasites are collected from geographically diverse sites in Africa, Asia and South America and prepared according to standard protocols (*5*). The specimens are characterized at the Hospital for Tropical Disease, United Kingdom, and the Army Malaria Institute, Australia.

Product performance is expressed as:

- **Panel detection score:** a score between 0 and 100, representing the proportion of times a malaria RDT gives a positive result in all tests with both lots tested against parasite panels at a specific parasite density (i.e. four tests at 200 parasites per microlitre and two at 2000 parasites per microlitre). It is a composite index that accounts for inter-test and inter-lot variation as well as positivity rates. Invalid tests are excluded from the analysis.
- **False-positive rate:** percentage of all tests of a product that gave a positive result when it should have been negative after the manufacturer's recommended reading time.
- **Invalid rate:** proportion of tests declared invalid due to the absence of a control line.

Moreover, the Programme assesses **heat stability, ease of use** and, since round 5, the type and frequency of RDT **anomalies seen in the production lots**.[8]

In round *7*, **adherence to international standards and best practices for in vitro diagnostic labelling, packaging and instructions for use** will be assessed (*7*, *8*). In addition, product performance against recombinant malaria antigens will be determined to establish product-specific baseline limits of detection (LODs). Countries and manufacturers will eventually have access to the same recombinant antigen materials and can use the LODs to monitor trends in RDT reactivity between lots, prior to field deployment.

Optional stability testing at the manufacturing site. Manufacturers may request parasite specimen samples from the CDC at steps 8 and 9 to conduct their own stability testing at the manufacturing site. Results should be submitted to the Programme every 3 months until the end of the specified shelf life of the product.

Step 1.9. Data analysis and Steering Committee data review

Once CDC has finalized RDT product testing, including data entry, the data are analysed, and the Steering Committee is convened to review the results.

Subsequently, individual or product-specific reports are prepared – so-called **manufacturers' reports** – which contain the results that will be included in the final published report; sometimes, as applicable, additional comments or observations from technicians are added.

Step 1.10. Manufacturers' reports, review period and feedback

In accordance with the terms of the signed confidentiality agreement (form 2), manufacturers' reports on the performance of the assessed product(s) are sent to each applicant. The manufacturers are invited to review and comment on the testing results of their products over a 30-day period, before the results are published in the final Programme report (see step 1.11). All source documents and electronic records of data from the study are maintained in secure storage until conclusion of the evaluation, data analysis and publication of the report. Raw data are made available to manufacturers upon request.

Step 1.11. Publication of testing results

The Malaria RDT Product Testing Programme publishes a list of evaluated products and their performance data in various formats,[9] including:

- a dedicated WHO web page,[10]
- a dedicated FIND web page,[11]
- electronic[12] and hard copies of the testing results for each product per testing round, and
- electronic[13] and hard copies of the compiled results of all previous rounds of testing.

The manufacturer of a product listed in these publications for which the product specifications have been changed (as outlined in Annex 2) is requested to inform WHO of such changes before commercial release of the changed product. WHO may remove a product from the above-mentioned publications or require resubmission of a product for performance testing if changes in the product specifications indicate that the RDT should be considered a new product. The same applies if performance data obtained from the Lot Testing Programme (see section 2) in the field are considered to be consistently outside those of the product testing programme published by WHO. WHO will remove the names of products from the online interactive database and the summary results and tables if manufacturers: fail to comply with compulsory resubmission, fail to confirm the commercial availability of the products evaluated or submit an invalid ISO 13485 or 9001 certificate.

NOTE

Participation in WHO–FIND Malaria RDT Product Testing and publication by WHO of the testing results may not be used by the manufacturers and suppliers concerned for commercial or promotional purposes. With respect to the manufacturers' or suppliers' participation in the Product Testing Programme, under no circumstances is a manufacturer or supplier authorized to refer to WHO and/or FIND, the publication of the testing results by WHO and/or FIND 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.

Step 12. Updating of WHO procurement recommendations

The results of each round of the Malaria RDT Product Testing Programme guide WHO in making recommendations on RDTs. The guidance is published in a WHO *Information note on recommended selection criteria for procurement of malaria rapid diagnostic tests*, accessible at http://www.who.int/malaria/publications/atoz/rdt_selection_criteria/en/. Accordingly, eligible products should meet the minimum criteria recommended by the Malaria Policy Advisory Committee in early 2012 (*9*):

- For the detection of *P. falciparum* in all transmission settings, the panel detection score against P. falciparum samples should be at least 75% at 200 parasites/µL.
- For the detection of *P. vivax* in all transmission settings, the panel detection score against *P. vivax* samples should be at least 75% at 200 parasites/µL.
- The false-positive rate should be < 10%.
- The invalid rate should be < 5%.

NOTE

The list of submitted products does not 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 furthermore does not warrant that: (1) the list is complete and/or error free; and/or that (2) the products listed are of acceptable quality, have obtained regulatory approval in any country, or that their use is otherwise in accordance with the national laws and regulations of any country, including but not limited to patent laws. Inclusion in the list does not furthermore imply any approval by WHO of the products in question (which is the sole prerogative of national authorities).

FIND's web-based interactive guide for the selection of malaria RDTs. FIND maintains an interactive web-based guide designed to filter and short-list RDTs according to selection criteria that can be defined by the user. The guide is currently based on the performance of the tests in rounds 3–6 of the Product Testing Programme, accessible at: http://www.finddiagnostics.org/programs/malaria-afs/malaria/current-projects/rdt_quality_control/interactiveguide-intro/interactive-guide/index.jsp. The interactive guide allows selection of RDTs on the basis of the following parameters: the target malaria species, the panel detection score for *P. falciparum* at 200 and 2000 parasites/µL, the panel detection score for *P. vivax* at 200 and 2000 parasites/µL, the false-positive rate, the invalid rate, the test format and heat stability. It was extended in 2015 to include specific procedural characteristics, such as test line order, blood volume, buffer volume and reading time.

2. WHO–FIND MALARIA RDT LOT TESTING PROGRAMME

As a complement to the Product Testing Programme (section 1), WHO and FIND offer continued assessment of the diagnostic performance of RDTs in the Malaria RDT Lot Testing Programme. This Programme is designed to detect lots of RDTs that perform poorly before they are sent to and used in the field and sometimes to verify unexpected or unusual rates of negative test results reported from the field. Lot testing reassures procurers that the product they have purchased meets an acceptable standard and gives an incentive to manufacturers to produce consistently good lots and to improve their product quality.

Rationale for lot testing. The performance of individual RDTs is likely to vary by lot over time. Thus, as part of good procurement practice, WHO recommends that all RDT production lots be checked either before or after shipment (*3*). Lot testing ensures that diagnostic products that enter countries meet performance expectations and, if lot testing is conducted after shipment, that RDT performance has not been adversely affected during transport. It guarantees that manufactured RDT lots perform adequately for clinical use and controls for lot-to-lot variation. Lot testing is undertaken until the expiry of the product, i.e. the Programme provides information on the performance of RDTs for the duration of their shelf life, indicating how they can be expected to perform when stored at the temperature recommended by the manufacturer. Moreover, it provides information on anomalies noted during testing, alerting procurers about problems that could be encountered during use of the tests in the field. In the absence of reliable methods for in-country testing, it provides some degree of quality control for procurers and country programmes.

Eligible requests. The Lot Testing Programme responds to requests from procurers of malaria RDTs, from manufacturers or from any group requesting on behalf of these entities, including national malaria programmes, ministries of health and nongovernmental organizations.

Time of lot testing. Routine testing of purchased RDT lots is recommended before or after shipment as a quality control procedure before they are distributed to the field or after distribution to the field in order to evaluate unexpected performance results.

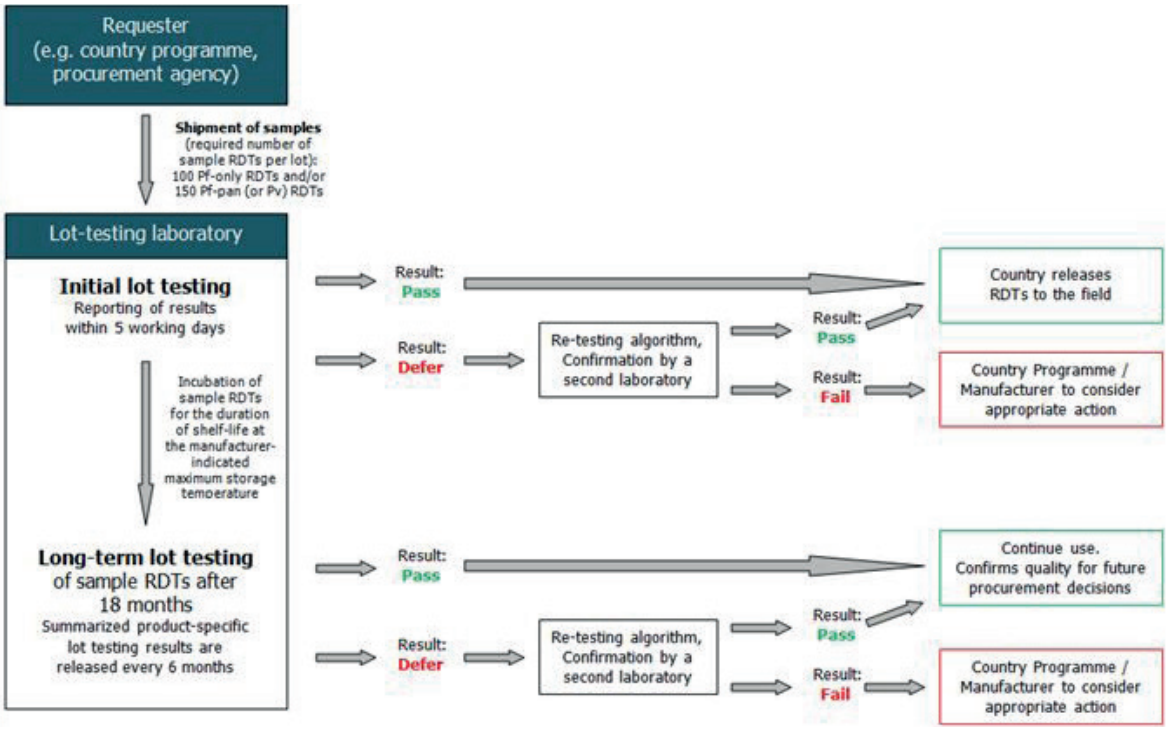
Cost. Lot testing is performed free of charge at the two WHO–FIND lot testing laboratories (see below) when arranged through the RDT Lot Testing Programme. The requesting institution must, however, provide the required number of RDTs (see step 2.3) and cover the costs of door-to-door transport of the RDTs (including taxes and pre-paid duty) to the testing laboratory.

Lot testing laboratories. Currently, lot testing is offered by two laboratories affiliated with WHO–FIND:

- Research Institute for Tropical Medicine, Filinvest Corporate City Compound, Alabang, Muntinlupa City, Philippines 1781. Trunk line nos.: (63-2) 807-2628 to 32. Fax nos. (63-2) 842-2245, 842-2828. Direct line no.: (63-2) 809-7599. Website: www.ritm.gov.ph; and
- Institut Pasteur du Cambodge, 5 Boulevard Monivong, BP 983, Phnom Penh, Cambodia. Phone: +855-23 427 163, 12 218 540. Fax: +855 23 725 606. Website: www.pasteur-kh.org.

Lot testing procedure and reporting of results. The application requirements and procedural steps of the Malaria RDT Product Testing Programme are explained in detail in steps 2.1–2.6 below and illustrated in Fig. 3.

FIG. 3.
Lot testing procedure



Further information on RDT lot testing is available at: http://www.finddiagnostics.org/programs/malaria-afs/malaria/current-projects/rdt_quality_control/lot_testing/. The corresponding forms can be downloaded at http://www.finddiagnostics.org/programs/malaria-afs/malaria/current-projects/rdt_quality_control/lot_testing/forms.html. The Programme can be contacted at the following e-mail addresses: malaria_rdt@who.int and info@finddiagnostics.org.

Step 2.1. Request form

To apply for lot testing, the requester completes a lot testing request form and sends it by e-mail to the lot-testing coordinator (Ms Nora Champouillon, e-mail: nora.champouillon@finddx.org), with a copy to malaria_rdt@who.int. The request form should be submitted to WHO and FIND at least 2 weeks before the RDTs are ready for shipment to the lot-testing laboratory.

Step 2.2. Documentation and shipping

Following receipt of the completed lot-testing request form, the lot-testing coordinator sends a dispatch confirmation to the requester, with details of the procedure, including information on the volume of RDTs required for lot testing (see also step 2.3), shipping instructions and a free-of-charge invoice. The lot-testing coordinator then designates

one of the lot-testing laboratories listed above. The RDT kits will be dispatched to the assigned laboratory only after receipt of this confirmation. The goods should be dispatched by a recognized international courier (e.g. Fedex, DHL) with incoterm delivery duty paid and a copy of the completed lot-testing request. The instructions given by the lot-testing coordinator should be followed carefully to ensure that the shipment is not held at customs.

Step 2.3. Sample RDTs

It is recommended that all purchased lots be tested to ensure that they perform well. The number of sample RDTs required for testing depends on the type of RDT and the expiry date of the product. Usually, a sample of 100 *P. falciparum*-only RDTs or 150 combination *P. falciparum* and pan-specific (or *P. vivax*-specific) RDTs is required from each lot. Random sampling of RDTs from different parts of the pallets by an independent party is the recommended sampling technique.

Step 2.4. Lot testing

RDT lots are tested at one of the two laboratories listed above, both of which undergo annual independent quality assessments to affirm their role as WHO-FIND lot testing laboratories under the WHO-FIND Malaria RDT Evaluation Programme. Upon receipt of RDTs, a rapid initial assessment[14] is made against panels of high and low density parasite-positive and parasite-negative blood. These reference panels are prepared according to the same standard operating procedures and have similar characteristics to the panels used in the product testing programme. The remaining RDTs are stored under controlled conditions at 37 °C and are retested after 18 months.

Step 2.5. Reporting of lot-testing results

A malaria RDT lot-testing quality control report (see sample report) is generated, with a guide for interpreting observations, and is sent confidentially by e-mail to the requester, usually within 5 working days of receipt of the RDTs at the lot-testing laboratory. If test anomalies (see section 1, Step 1.8) are detected and if programme capacity permits, photographs of the test results may be sent with the final report.

Lot testing is performed against a smaller panel of parasite-positive and parasite-negative samples than product testing. Therefore, lot testing is not designed to detect small differences in RDT performance but to detect major deficiencies in a production lot, including the device and/or buffer. Because of the small size and variable antigen concentration in samples of the same parasite density, RDTs that fail initial testing are assessed against reference stock RDTs (high quality) and against another sample. If there are no failures, a "pass" report is issued; however, if any RDT fails testing against a second sample, a "deferred" report is issued, and the RDT is sent to another, affiliated laboratory for confirmatory testing. If the RDT fails confirmatory testing, a "fail" report is issued. Failure reports may also be issued if for any reason the RDT cannot be used according to the instructions for use, e.g. because of insufficient buffer.[15]

- **Pass.** The tested RDTs detected antigens at a threshold sufficient for clinical use in the field. The corresponding RDT lot is considered to have passed the quality control assessment.

- **Deferred.** The tested sample RDTs failed initial and repeated quality control assessment and have been sent to an affiliated lot-testing laboratory for confirmation; a final report is issued upon receipt of confirmatory results. It is recommended that the corresponding RDT lot is retained until a final report is received. Confirmatory testing usually delays final reporting by 7–10 days.

- **Fail.**

    - The tested sample RDTs failed the initial quality control assessment and also failed confirmatory testing at the affiliated lot-testing centre. It is recommended that this particular RDT lot not be used in the field, and that the manufacturer be contacted and advised of the results.
    - An RDT fails if testing cannot be performed because of a defect in the device or components, e.g. insufficient buffer.

A report of the results of long-term testing 18 months after receipt of the RDTs is sent to the requester at the corresponding time. Lot-testing reports and photographs of the testing results cannot be released to a third party without the agreement of the requesting party. Requesters will now be asked if they consent to the results being shared, but, in all cases, only the requesting party can make the report available, not the lot-testing programme.

Step 2.6. Summarized results of product-specific lot testing

The results of malaria RDT lot-testing quality control are released as a summary every 6 months. The updated information can be accessed at http://www.finddiagnostics.org/programs/malaria-afs/malaria/rdt_quality_control/lot_testing/malaria_rdt_lot_testing_results/.

The WHO–FIND programme is not responsible for a final decision by a procurement agent or malaria programme to accept or reject an RDT lot. This decision is to be taken by the requester of lot testing. The aim of the lot-testing programme is to provide data on which this decision can be based.

ENDNOTES

1. Publication of data on product performance does not, however, guarantee that the corresponding RDTs will actually be procured by WHO or any other party.

2. Conditions of entry are based on the recommendations of WHO expert consultations in Geneva, Kisumu and Atlanta in 2006 and their confirmation in subsequent consultations of the WHO-FIND Malaria RDT Evaluation Programme Steering Committee (formerly referred to as the Malaria RDT Specimen Bank Review Committee).

3. Round 7 documentation is available at http://www.who.int/malaria/news/2015/rdt_call_for_testing_round7/en/

4. If the product is available in multiple kit sizes, information for each kit size should be listed. Kits containing RDTs in different formats (cassette or dipstick) are considered different products and must be submitted separately. Kits containing different or varying formats of components, e.g. buffer bottle or single-use buffer vial, are considered different products and may require separate submissions for partial evaluation, with or without other supporting documentation. All product variations should be listed and specified on form 3.

5. Formerly known as the Malaria RDT Specimen Bank Review Committee.

6. This fee is independent of the fees charged for WHO Prequalification of Diagnostics Assessment. For

more information on the WHO prequalification programme requirements, see http://www.who.int/diagnostics_laboratory/evaluations/en.

7. In round 7, a total of 2700 malaria RDTs, consisting of 1350 tests accompanied by standard kit contents from each of two separate lots of each product, is required to be submitted to the Programme.

8. Deviation from the expected appearance of a test, detected by direct observation; may include a red background, incomplete clearance due to failure to flow, shifting or misplacement of a strip, ghost lines, diffuse test lines or patchy broken test lines.

9. Assessment results are published on different web pages, in reports and WHO information notes; however, inclusion in these listings does not imply approval by WHO of the corresponding product, nor does it constitute an endorsement or warranty by WHO of the performance of any product for a particular purpose.

10. Accessible at http://www.who.int/malaria/areas/diagnosis/rapid_diagnostic_tests/en/

11. Accessible at http://www.finddiagnostics.org/programs/malaria-afs/malaria/current-projects/rdt_quality_control/product-testing.html

12. Five rounds of testing have been completed so far. The reports are available at the indicated links.
    - Round 1 (April 2009): http://apps.who.int//iris/bitstream/10665/44120/1/9789241598071_eng.pdf
    - Round 2 (March 2009): http://whqlibdoc.who.int/publications/2010/9789241599467_eng.pdf
    - Round 3 (December 2011): http://www.who.int/tdr/publications/documents/rdt3.pdf
    - Round 4 (December2012): http://www.who.int/iris/bitstream/10665/77748/1/9789241504720_eng.pdf
    - Round 5 (July 2014): http://apps.who.int/iris/bitstream/10665/128678/1/9789241507554_eng.pdf

13. Summary results of WHO Product Testing of Malaria RDTs: rounds 1–5 (2008–2013): http://apps.who.int/iris/bitstream/10665/144780/1/9789241507639_eng.pdf

14. Usually within 5 working days of receipt of the RDTs at the lot-testing laboratory.

15. Details of the protocol can be found in the Methods manual for laboratory quality control testing of malaria RDTs, version 7 (5).

REFERENCES

1. WHO guidelines for the treatment of malaria, 3rd ed. Geneva: World Health Organization; 2015 (http://apps.who.int/iris/bitstream/10665/162441/1/9789241549127_eng.pdf).

2. Universal access to malaria diagnostic testing – an operational manual. Geneva: World Health Organization; 2011, revised 2013 (http://www.who.int/malaria/publications/atoz/9789241502092/en/).

3. Manual on good practices for selecting and procuring rapid diagnostic tests for malaria. Geneva: World Health Organization; 2011 (http://whqlibdoc.who.int/publications/2011/9789241501125_eng.pdf).

4. Information note on recommended selection criteria for procurement of malaria rapid diagnostic tests. Geneva: World Health Organization; 2014 (http://www.who.int/malaria/publications/atoz/rdt_selection_criteria/en/).

5. Methods manual for laboratory quality control testing of malaria rapid diagnostic tests, version 7. Geneva: World Health Organization; 2014 (http://www.who.int/malaria/publications/rdt-lab-quality-manual/en/).

6. Methods manual for product testing of malaria rapid diagnostic tests, version 6. Geneva: World Health Organization; 2014 (http://www.who.int/malaria/publications/rdt-manual/en/).

7. Jacobs J, Barbé B, Gillet P, Aidoo M, Serra-Casas E, Van Erps J, et al. Harmonization of malaria rapid diagnostic tests: best practices in labelling including instructions for use. Malar J 2014;13:505.

8. Harmonization of rapid diagnostic tests for malaria and implications for procurement. Meeting report. Geneva,World Health Organization, 2015 (http://www.who.int/malaria/publications/atoz/9789241509978/en/)

9. Malaria Policy Advisory Committee and Secretariat. Inaugural meeting of the Malaria Policy Advisory Committee to the WHO: conclusions and recommendations. Malar J 2012;11:137 (http://www.malariajournal.com/content/11/1/137).

ANNEXES

Annex 1. Internal heat stability protocol at manufacturing site (example from round 6)

INTERNAL HEAT STABILITY PROTOCOL AT MANUFACTURING SITE

As evidence of stability testing, WHO will accept protocols submitted by manufacturers that comply with the points listed below. However, based on the recommendations of the WHO Malaria Specimen Bank Committee in February 2010 (Bangkok, Thailand), WHO will continue to supply manufacturers participating in WHO Product Testing with cultured parasites but **manufacturers are not required to submit results of stability testing to WHO.**

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

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 programme 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:

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,

References

1. Hornback, L. A. Stability testing for IVDs. *IVD Technology* **10** (2004).

Annex 2. Changes in malaria RDT manufacture and corresponding assessment of required product performance

AREA OF MODIFICATION	PRODUCT CHANGE	RESUBMIT TO WHO PRODUCT TESTING AS NEW PRODUCT	REQUIRES EQUIVALENCE TESTING AT AN INDEPENDENT LABORATORY	NOTIFICATION REQUIRED
TEST KIT				
Format	Type (e.g. cassette, dipstick)	X		
	Length/size		X	
	Number of wells	X		
	Placement of wells		X	
Monoclonal antibodies	Target	X		
	Source (clone)	X		
	Source (manufacturer)			X
Dye conjugate	Type (material)	X		
	Source			X
	Size			X
Nitrocellulose	Composition	X		
	New nitrocellulose manufacturer			X
Buffer	Change in constituents			X
	Change in concentrations			X
Blood transfer device	Type			X
PROCESS				
Timing	Steps		X	
	Reading		X	
Volumes	Blood		X	
	Buffer			X

Abbreviations

CDC	United States Centers for Disease Control and Prevention
EOI	expression of interest
FIND	Foundation for Innovative New Diagnostics
ISO	International Organization for Standardization
RDT	rapid diagnostic test (for the purposes of this document, immunochromatographic lateral flow devices for the detection of malaria parasite antigens)