



WORLD HEALTH ORGANIZATION

The Use of

Malaria Rapid Diagnostic Tests



Roll Back Malaria

The Use of
**Malaria
Rapid
Diagnostic
Tests**



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Misdiagnosis of malaria results in significant morbidity and mortality. Rapid, accurate and accessible detection of malaria parasites has an important role in addressing this, and in promoting more rational use of increasingly costly drugs, in many endemic areas. Rapid diagnostic tests (RDTs) offer the potential to provide accurate diagnosis to all at-risk populations for the first time, reaching those unable to access good quality microscopy services.

The success of RDTs in malaria control will depend on good quality planning and implementation. This booklet is designed to assist those involved in malaria management in this task. While this new diagnostic tool is finding its place in management of this major global disease, there is a window of opportunity in which good practices can be established by health services and become the norm.

What is a Malaria Rapid Diagnostic Test (RDT)?

RDT sensitivity

The sensitivity of malaria RDTs is determined by the:

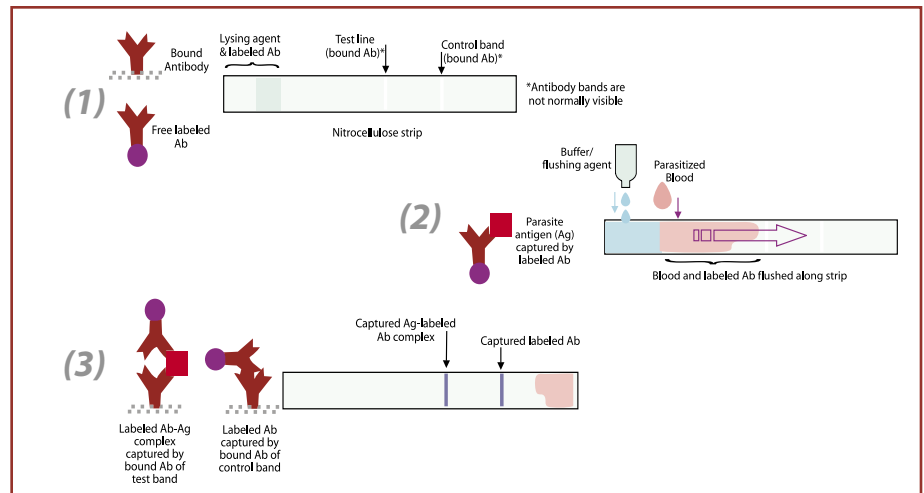
- species of parasite
- number of parasites present
- condition of the RDT
- correctness of technique used to perform the test
- correctness of interpretation by the reader
- parasite viability and variation in production of antigen by the parasite

Mode of action of common malaria RDT format: (1) Dye-labeled antibody (Ab), specific for target antigen, is present on the lower end of the nitrocellulose strip or in a plastic well provided with the strip. Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and either antibody specific for the labeled antibody, or antigen, is bound at the control line. (2) Blood and buffer, which have been placed on the strip or in the well, are mixed with labeled antibody and are drawn up strip across the lines of bound antibody. (3) If antigen is present, some labeled antibody will be trapped on the test line. Excess labeled antibody is trapped on the control line.

Malaria rapid diagnostic tests, sometimes called “dipsticks” or “malaria rapid diagnostic devices” (MRDDs), detect specific antigens (proteins) produced by malaria parasites. These antigens are present in the blood of infected or recently infected people. The RDT signifies their presence by a colour change on an absorbing nitrocellulose strip. Some RDTs can detect only one species (*Plasmodium falciparum*), usually by detecting either histidine-rich protein-2 (HRP2) or parasite-specific lactate dehydrogenase (pLDH). Some detect one or more of the other three species of malaria parasite which infect humans, by detecting various other antigens.

RDTs commonly come in three different formats. The simplest form is a *dipstick*, which is placed in wells containing blood and/or buffer. The nitrocellulose strip may be placed in a plastic *cassette* or on a *card*. Cassettes and cards tend to be more expensive, but simpler to use.

When in good condition, some products can achieve a sensitivity similar to that commonly achieved by microscopy (~100 parasites / μ l). Sensitivity can vary between products. Recommended sensitivity is $\geq 95\%$ at ≥ 100 parasites / μ l for *P. falciparum* (WHO informal consultations, 1999, 2003, see p.19).



When is an RDT Useful?

Potential uses for malaria RDTs

- Diagnosis by health workers distant from good microscopy services.
- Remote diagnosis in organized workforces in malaria-endemic areas (e.g. military or mining companies).
- Outbreak investigation and malaria prevalence surveys.
- Self-diagnosis by trained individuals or groups.
- 'After-hours' diagnosis in hospital laboratories or clinics.

When used correctly, malaria RDTs can provide a useful guide to the presence of clinically significant malaria infection, particularly when good quality microscopy-based diagnosis is unavailable. However, management decisions should not be based on the RDT result alone.

RDTs can offer significant **benefits** in malaria management if:

- a clear benefit will occur in health outcomes;
- demonstration of parasitaemia will allow more rational use of anti-malarial drugs;
- a clear plan of action is developed to deal with positive and negative results;
- good health worker training and monitoring is maintained;
- the accuracy of RDTs is monitored (quality control);
- they are protected from high temperature; and
- they are affordable.

There may be situations in areas of very high malaria prevalence in which demonstration of parasitaemia does not contribute significantly to malaria management. In these situations, parasitaemia may be present without causing illness. There may be so few people with no parasitaemia that, despite the increased cost of diagnosis, cost savings on drugs will be minimal.

Malaria RDT use by small organizations and individuals

Malaria RDTs are frequently marketed to individuals, such as travellers, and to small organizations in endemic areas. They may form a useful adjunct to medical care in these situations *providing* the user is well trained in RDT preparation and interpretation, and understands the limitations of the tests.

A trained health worker should be consulted wherever possible when purchasing and when symptoms occur. When health advice may not be



immediately available (e.g. remote travellers), a plan of action should be prepared beforehand in consultation with an appropriate health worker, and further consultation sought as soon as possible.

Malaria RDTs in the private sector

Because much treatment of malaria occurs in the private health sector, it is appropriate that malaria RDTs are used in this sector under similar conditions to those recommended for public services. Control over RDT storage and transport, and monitoring of RDT accuracy, will frequently be more difficult. The onus is on manufacturers and distributors to ensure that instructions for storage and handling, and for RDT preparation and interpretation, are clearly stated in a language or format that the end-users can be expected to understand. Public health authorities should take steps to disseminate knowledge on correct care and use of RDTs to providers and consumers in the private sector.



RDTs in Malaria Management

Things to remember when using an RDT

- Prior instruction in the use and interpretation of the particular product is vital.
- A management plan for results must be in place.
- Blood-safety precautions should be followed.
- Product instructions should be strictly followed.
- The RDT should be discarded if the envelope is punctured or badly damaged.
- The test envelope should be opened **only** when it has reached ambient temperature, and the RDT used immediately after opening.
- The result should be read within the time specified by the manufacturer.
- An RDT can not be re-used.

Like microscopy, the accuracy of an RDT is dependent on the care and expertise with which it is prepared and interpreted.

Performing the test

If preparation is delayed after opening the envelope, humidity can damage the RDT. Test lines may become 'positive' several hours after preparation: read only within the time limit specified by the manufacturer (see column to left).

Interpreting the result

The result of a malaria RDT should always be interpreted in the light of the patient's clinical state, taking into account the fallibility of the test. The technician must understand what each line indicates. This varies between products. A control line **must** be present for the result to be valid, but the presence of a control line does **not** prove that the RDT result is accurate. Repetition of the test after 1 to 2 days may therefore be indicated if illness persists or if the patient's condition deteriorates.

Acting on the result

Treatment algorithms and health worker training should allow anti-malarial treatment of severe cases in which the RDT result is negative. Appropriate further investigation of all fever cases with negative RDT results is essential. The possibility of concomitant non-malarial illness in cases in which parasites have been demonstrated should also be considered.

In summary, diagnosis must take into account both RDT results and clinical assessment, including history and examination. Where skilled clinical assessment is not available, a plan of management (algorithm) should be developed beforehand with the guidance of a health professional skilled in the management of malaria.

Sometimes RDT results may mislead...

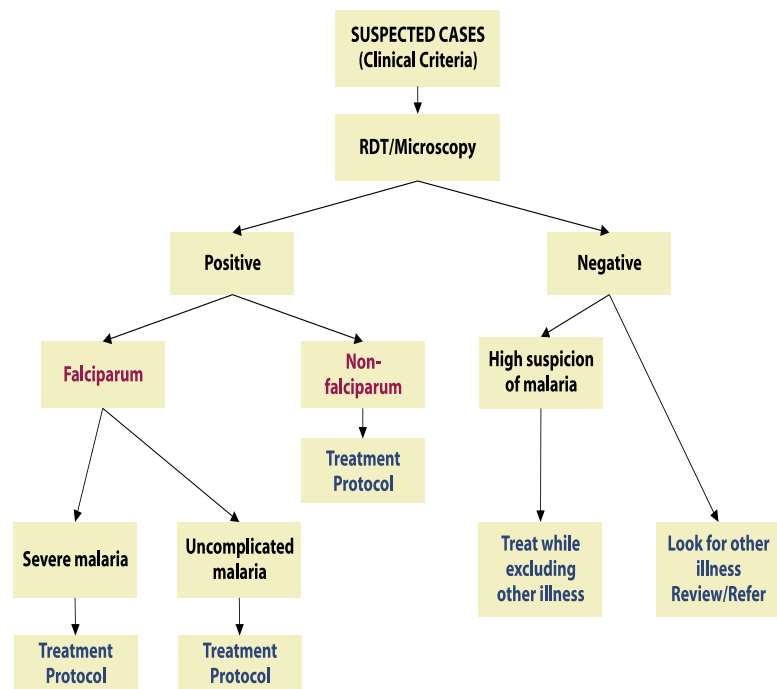
A **negative** test result does *not always* exclude malaria with certainty as:

- there may be *insufficient parasites* to register a positive result
- the RDT may have been *damaged*, reducing its sensitivity
- illness may be caused by *another species* of malaria parasite which the RDT is not designed to detect

A **positive** result does *not always* signify malaria illness because:

- antigen may sometimes be detected after the *infecting parasites have died* (i.e. after treatment) or due to the persistence of malaria *gametocytes* which do not cause illness
- the presence of *other substances* in the blood may occasionally produce a false-positive result
- the presence of parasites does not always signify malaria illness in individuals with high immunity as there may be *other causes of fever*

Sample decision chart for treatment of malaria in remote areas, based on the results of a malaria RDT



Choosing an appropriate RDT

Considerations for choosing an RDT product include:

- *plasmodium* species to be detected (*P. falciparum* only, or pan-specific)
- shelf-life and temperature stability in intended conditions of storage and use
- ease of use, including format of the test (e.g. cassette, dipstick, card)
- requirement for post-treatment testing of patients
- cost (including transport, training, and quality control)
- sensitivity

Malaria RDT products and manufacturers

At present WHO does not certify or otherwise recommend any specific malaria RDT brand or product, but supports the use of RDTs in the management of malaria following the guidelines outlined in this document. For a list of major manufacturers and distributors known to WHO, contact mal-rdt@wpro.who.int

Relative sensitivities of commercially available RDT products are difficult to assess from published literature, and are likely to be influenced by conditions of storage and use. Good quality assurance processes after purchase are likely to be of greater importance.

Some malaria treatment programs require testing of patients after treatment to confirm treatment effectiveness. This requires an RDT which detects antigens which do not persist in the host circulation after death of the parasites. At present, pLDH-based RDTs may achieve this but results can be affected by high densities of gametocytes.

In humid tropical conditions, it is strongly recommended that RDTs be individually packaged in moisture-proof envelopes. Ease of use (e.g. number of preparation steps, blood transfer method, and need for accurate timing) will influence test accuracy, and influence the extent of training and supervision required.

Longer shelf lives reduce the pressure on the supply chain and the probability of wastage of expired tests; a minimum of 18 months (e.g. at least 15 after purchase) is recommended in remote, poorly resourced areas.

Retail prices of RDTs generally vary with the size of the order, and the location. RDTs detecting *P. falciparum* generally range upward from approximately US\$0.65 per test. RDTs detecting all species range upward from approximately US\$1.00. Prices should be checked with individual manufacturers.

Tendering and the availability of product information

Together with considerations of the sensitivity, species of parasite detected, and cost of a product, it is useful to know the quality of manufacturing processes and the stability of a manufacturer. The long-term viability of a company and consistency of production will influence the ability to

Integrating malaria RDTs into health services

Prior to purchase of RDTs for wide-scale use, procedures should be prepared for:

- quality control testing of a designated sample of the product
- 'cool chain' for transport and storage
- health worker training and monitoring
- clear guidelines on action to follow the results (diagnostic and treatment algorithm)

Budgets for RDT use should include provision for the following:

- purchase and shipping
- post-purchase Quality Control (QC) testing
- storage and in-country shipping
- peripheral-level QC testing
- end-user training and supervision

replace a product should the received lot fail, and will ensure long-term supply of a product to minimize the need for re-training.

Purchasers should request the following information from manufacturers during the tendering process:

1. real-time temperature stability data on the product, and accelerated data on the purchased lot;
2. evidence of successful operational use, or good quality field data on the product;
3. long-term viability of manufacturer (to ensure continuity of supply);
4. availability of product support;
5. provision of sample products for assessment and testing for ease of use;
6. agreement for replacement of products which fail agreed quality control procedures (see above); and
7. box sizes appropriate to the rate of use of tests in the intended area, to minimize storage time in poor conditions and limit the need to split boxes.

Point 3 implies that the place of manufacture of RDTs should be disclosed to the purchaser if RDTs are re-labeled.

Purchasers should also consider evidence of good manufacturing practice (e.g. GMP or ISO certification; ISO13485:2003 is a standard specific for medical devices).

Clarity of packaging of the end product is essential to allow identification of product type, production lots and expiry date.

Maintaining a 'cool chain'

Most manufacturers recommend RDT storage between 2°C and 30°C. Expiry dates are generally set according to these conditions. If kits are stored at temperatures exceeding the recommended limits, it is likely that the shelf life of the RDTs will be reduced and sensitivity lost prior to the expiry date.

Trials on commercially available malaria RDTs have revealed low sensitivities in products that have performed well previously. Exposure to high temperatures is probably a major contributor to poor performance, particularly during transport and storage. Transfer from the manufacturer, and road transport within a country, are particularly vulnerable times. High humidity can rapidly degrade RDTs, including prolonged exposure to humidity after removal from the envelope or if the envelope is damaged.

The development of a “cool chain” for shipment of RDTs is essential. Transport of RDTs from manufacturers and within countries should be monitored as follows:

Shipping from manufacturers

1. Before shipping, the manufacturer contacts consignees with details of air waybill numbers, airline carrier, flight number, numbers of containers, and expected arrival time. These details should be sent by e-mail and followed up by facsimile.
2. The shipper (air carrier) is notified of temperature storage requirements by the manufacturer in writing and by clear markings on cartons and related documents. (Stowage of the shipment close to the skin of some aircraft may result in freezing.)
3. The manufacturer initiates shipment *only* when the consignee confirms the shipping notification is received.
4. Consignees then arrange to have customs agents or other personnel on site to receive materials – shipments are moved immediately to moderate temperature storage (less than 30° C if possible). Avoid leaving materials on airport tarmacs, in customs sheds or in vehicles.



Ground transportation

5. Ground transportation during any stage of delivery is carried out without delay and with attention to ambient temperature while the vehicle is moving and if parked. *Avoid leaving RDTs in vehicles parked in the sun.*

Storage

6. Storage at central and final field facilities should be within the manufacturer's specifications (usually $\leq 30^{\circ}\text{C}$).
7. Maximize the time RDTs are stored in centralized, controlled conditions; minimize uncontrolled storage in remote areas. Smaller box sizes may help achieve this.
8. Select a cool peripheral storage location; thatch roofing may be cooler than iron, maximize shade, consider evaporative cooling cabinets.

Transport and storage at temperatures above 30°C is sometimes unavoidable, as in many remote locations where RDTs are intended for use. Monitoring of sensitivity of RDTs at appropriate intervals is therefore essential. WHO is developing recommendations for quality assurance to address these issues.



Organizing national Quality Assurance programmes for RDTs

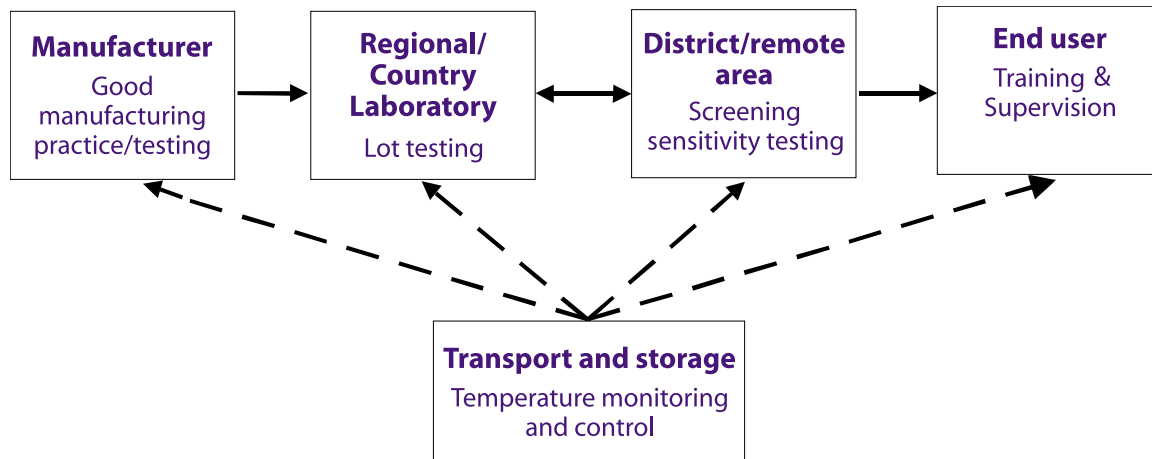
A system for ensuring the continued accuracy of diagnostic services is essential in any large programme determining treatment for a severe disease. Good Quality Assurance (QA) includes careful purchasing, handling and training, in addition to testing of the product and user. QA should be an integral part of RDT budgets and implementation plans in the same way that it forms an important part of microscopy-based diagnosis. Monitoring should extend from testing at the time of purchase to testing and supervision at a peripheral level, and include monitoring of transport and storage. Responsibility for overseeing QA processes should be clearly defined and coordinated from a central level.

The sensitivity of RDTs should be checked at a central laboratory on receipt from the manufacturer, and periodically throughout the recommended shelf life.

Monitoring of sensitivity at a peripheral level and adequate training and supervision of end-users should be integrated as far as possible into existing health worker training and quality assurance schemes. Instructions for RDT preparation and interpretation should be clear and concise in local languages. Health workers using the tests should be trained and assessed, and systematically monitored on test preparation and interpretation. As RDTs must be read soon after preparation, and technique is important, monitoring should be done with real cases rather than by review of previously prepared tests.



Outline of requirements for an adequate Quality Assurance System for maintaining accuracy of malaria RDTs



More details on recommendations for quality assurance can be found in the 2003 Meeting Report: *'Malaria Rapid Diagnosis: Making It Work'*. WHO can assist with arrangements for laboratory quality assurance.



Further information:

A website containing further information on malaria RDTs, including a list of known manufacturers and major distributors, can be found at www.wpro.who.int/rdt Reports of WHO consultations on the subject are detailed in *'Malaria Diagnosis: New Perspectives'*, WHO 2000; and *'Malaria Rapid Diagnosis: Making It Work'*, WHO 2003, available on the website above.



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