MALARIA DIAGNOSIS: A GUIDE FOR SELECTING RAPID DIAGNOSTIC TEST (RDT) KITS - 1st edition

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Overview

Plasmodium falciparum, P. vivax, P. ovale and P. malariae are the four main species of malaria parasites that infect humans, with the first two species causing the most infections worldwide. *Plasmodium falciparum* malaria is prevalent in Africa, whereas *P. vivax* is present in greater proportions in parts of Asia and Latin America.

Rapid, accurate and accessible detection of malaria parasites is important in the prevention and treatment of malaria. Malaria morbidity, mortality and transmission can be reduced if prompt diagnosis and adequate treatment is available. Rapid diagnostic tests (RDTs) offer the potential to provide accurate and timely diagnosis to everyone at risk, reaching those previously unable to access good quality microscopy services. In malaria-endemic regions, the use of RDTs is very helpful for the effective use of anti-malarial drugs as treatment is based on parasite diagnosis and not just fever alone. In these regions, a considerable proportion of these drugs have been wasted on patients with non-malarial disease due to lack of prompt and accurate laboratory diagnosis.[1]

Parasitological confirmation of the diagnosis of malaria through microscopy, is part of good clinical practice and should always be part of malaria case management. However, the following exceptions apply:

- Children under the age of 5 years in high prevalence areas. There is no evidence yet that the benefits of parasitological confirmation outweigh the risk of not treating false negatives.[2]
- Cases of fever in established malaria epidemics where resources are limited.
- Where good quality microscopy is not feasible.

Objective

This guide is meant to act as a guide in the decision making process for procurement of rapid diagnostic tests. It also provides information that is useful for supply chain planning, including product shelf lives and storage requirements. Some product specific information on RDTs currently available through UNICEF Supply Division is also included.

What is a malaria rapid diagnostic test (RDT)?

A malaria RDT, sometimes called “dipstick” or “malaria rapid diagnostic device detects specific antigens (proteins) produced by malaria parasites. These antigens are present in the blood of infected or recently infected people. The RDT works through the lateral flow or Immunochromatographic Strip (ICS) method and signifies the presence of antigens by a colour change on an absorbing nitrocellulose strip.

RDTs detecting anti-malaria antibodies also exist, but have indications other than case management. For example, they can be used for screening donated blood in blood banks to prevent transfusion-induced malaria.

Types of malaria rapid diagnostic tests

RDTs commonly come in three different formats. The simplest form is a dipstick (test strips), which is placed in wells containing blood 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.
The three main groups of antigens detected by commercially available RDTs are:

- Histidine-rich protein 2 (HRP-2), specific to *P. falciparum. It is an abundant soluble, heat stable antigen that is present in the cytoplasm and membrane of infected erytocytes.*
- Parasite specific plasmodium lactate dehydrogenase (pLDH), currently available as *P. falciparum*-specific, pan-specific, and *P. vivax*-specific pLDH antibodies.
- Aldolase (pan-specific). These two antigens are conserved major enzymes in the glycolytic pathway of malaria parasites, they are abundant and are soluble in the parasite.

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1 Evaluating Diagnostics, Ensuring Quality and Access for Malaria v Diagnosis: how can it be achieved? Nature Reviews September 2006.

2 Technical Consultation to Review the Role of Laboratory Diagnosis to Support Malaria Disease Management: Focus on the Use of RDTs in Areas of High Transmission Deploying ACT Treatments (25-26 October 2004).

Target antigens for commercially available RDTs:

Antigen Species	HRP 2	pLDH	Aldolase
P. falciparum specific	√	√	
Pan-specific (all species)		√	√
P. vivax specific		√	

Most commercial products include antibodies to:

- HRP2 alone (*P. falciparum*)
- HRP2 and vivax-specific pLDH (distinguishing *P. falciparum/P.vivax*)
- HRP2 and pan-specific pLDH (distinguishing *P. falciparum* and other *Plasmodium species*)
- HRP2, vivax-specific pLDH and pan-specific pLDH
- HRP2 and aldolase (distinguishing *P. falciparum*/mixed infection from non *falciparum* alone)[3]
- Falciparum-specific pLDH
- Falciparum-specific pLDH and vivax-specific pLDH (distinguishing *P. falciparum/P.vivax)*
- Falciparum-specific pLDH and pan-specific pLDH (distinguishing *P. falciparum*/mixed infection from non-falciparum alone)
- Pan-specific pLDH (distinguishing all four Plasmodium species: *P. falciparum/P. vivax/ P. malariae/ P. ovale)*

RDTs detecting both *falciparum*-specific and non-*falciparum* (or pan-specific) target antigens are commonly called combination or 'combo' tests. Pan-specific means that the RDT detects all the four types of plasmodia that infect humans.

Although pLDH tests detecting *P. falciparum* and *P. vivax* (species-specific) are commercially available, their prices are much higher than versions of the same tests using pan-specific pLDH.

The products come in a number of formats:

- Plastic cassette
- Card
- Dipstick
- Hybrid cassette-dipsticks

Cassettes tend to be simpler to use than dipsticks, and this is likely to affect test accuracy[4].

Appropriate use of RDTs

Malaria rapid diagnostic tests can effectively be used for several purposes:

- Diagnosis - to identify, confirm or rule out malaria in symptomatic patients.
- Case management – to guide accurate prescription of therapeutic interventions and to monitor treatment.
- Epidemiology - to detect and monitor the incidence or prevalence of malaria for targeting prevention and evaluating health programmes.

The specific performance requirements of a test will vary depending on the intended use or uses. When considering whether or not to use a malaria rapid diagnostic test in a particular setting, it is important to consider their strengths and plan how to manage the challenges.

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[3] As products are commonly more sensitive to HRP2 than aldolase in P. falciparum-only infections, these will commonly appear as an HRP2 line only if the parasite density is low.

[4] Unpublished report of WHO, Quality Assurance Project (USA), Research Institute for Tropical Medicine (Philippines) and Centre for Malaria, Parasitology and Entomology (Cambodia) at http://www.wpro.who.int/RDT/

Strengths of malaria RDTs	Challenges of malaria RDTs
Relatively easy to use with minimal training required	Costs per test may exceed those of microscopy
Relatively rapid, giving timely results	Short shelf-life, requiring efficient procurement, transportation, storage and distribution systems
Little or no manipulation of sample required, can be performed in places without laboratories	Most tests are qualitative (i.e. gives a yes or no answer). Any quantification of parasitemia will require further laboratory-based tests
Most of the RDTs do not require refrigeration, hence tests can be performed where there is no power supply	Intensity of test band varies with amount of antigen present at low parasite densities-this may lead to reader variation in test results
Uses whole blood (prick or venous blood-prick preferred)	In many cases, they are less sensitive (and less specific) than laboratory-based tests

Choosing a malaria RDT

Important considerations in choosing an RDT include:

- The *plasmodium* species to be detected.
- Accuracy (sensitivity and specificity).
- Shelf-life and temperature stability during transport, storage and use.
- Ease of use, including format of the test (e.g. cassette, dipstick, and card).
- Cost.

The Plasmodium species to be detected

The appropriateness of *P. falciparum*-specific, pan-specific and non-*falciparum* RDTs varies with the relative prevalence of the different human malaria species in the intended area of use. These areas are categorized by WHO as:

- **Zone 1**. *P. falciparum* only, or with non-*falciparum* species occurring almost always as co-infections with *P. falciparum* - most areas of sub-Saharan Africa and lowland Papua New Guinea.
- **Zone 2***. Falciparum* and non-*falciparum* infections occurring commonly as single-species infections - most endemic areas in Asia and the Americas as well as isolated areas in Africa, particularly the Ethiopian highlands.
- **Zone 3**. Areas with non-f*alciparum* malaria only - mainly *vivax*-only areas of East Asia and central Asia and some highland areas elsewhere.

Accuracy (sensitivity and specificity)

A test method is said to be accurate when it measures what it was supposed to measure. In technical terms, this means its ability to measure a substance's true value of concentration in a sample. As a test is performed on patient specimens, its accuracy is routinely monitored with a "control specimen." The control has a known test value and is analyzed alongside patient specimens, revealing whether the test measurement process is correct. Accuracy of RDTs is expressed through several measures, the most used being sensitivity and specificity. These measures compliment each other.

Sensitivity

Sensitivity is the ability of a test to correctly identify individuals who have a given disease or disorder. For example, a certain test may have proven to be 90 per cent sensitive. If 100 people known to have a certain disease are tested with that method, the test will correctly identify 90 of those 100 cases of disease. The other 10 people who were tested will have the disease, but the test will fail to detect it. For that 10 per cent, the finding of a "normal" result is a misleading false-negative result. The sensitivity of a test becomes particularly important when you are seeking to exclude a dangerous disease. *The more sensitive a test is, the fewer "false-negative" results it produces. A false-negative result fails to expose disease states that may be present.*

The sensitivity of a malaria RDT means that it will produce a True Positive result when used in a population infected with malaria as compared to the reference malaria test gold standard of microscopy. In other words, it measures how often the test is positive when malaria is present.

The sensitivity of an RDT for detecting malaria parasitaemia (or recent parasitaemia) depends on the concentration of circulating antigens in the patient's blood, and the ability of the labelled antibody on the RDT to bind the antigen and accumulate to form a visible line. In good conditions, some products can achieve sensitivity similar to that commonly achieved by microscopy (~100 parasites /µl). Sensitivity can vary between products.

Specificity
Specificity is the ability of a test to correctly exclude individuals who do not have a given disease or disorder. For example, a certain test may have proven to be 90 per cent specific. If 100 healthy individuals are tested with that method, only 90 of those 100 healthy people will be found "normal" (disease-free) by the test. The other 10 people will not have the disease, but their test results seem to indicate they do. For that 10 per cent, their "abnormal" findings are a misleading false-positive result. When it is necessary to confirm a diagnosis that requires dangerous therapy, a test's specificity is one of the crucial indicators. *The more specific a test is, the fewer "false-positive" results it produces. A false-positive result can lead to misdiagnosis and unnecessary, possibly life-altering, diagnostic procedures and therapies.*

The specificity of a malaria RDT means that it will produce a True Negative result when used in a population not infected with malaria as to the reference malaria test gold standard of microscopy. In other words, it measures how often the test is negative when malaria is absent.

Both sensitivity and specificity are influenced by product storage and use conditions. In general, it is recommended that at least 95 per cent of *P. falciparum* infections should be detected at 100 parasites per microlitre, and at higher parasite densities[5], which is probably similar to good field microscopy.

Shelf life and stability
To ensure that a product will retain its quality, it should be stored and transported within the transporting requirements and necessary precautions as outlined under *transport and storage*. The details of these requirements can be found in the package insert which accompanies the RDT. Longer shelf-life reduces the pressure on the supply chain and the probability of wastage of expired tests. A minimum of 18 months (e.g. at least 15 months after purchase) is recommended in remote, poorly resourced areas.

Ease of use
The intended conditions of use must be considered when choosing an RDT. If the RDTs are to be used in a remote area without temperature-controlled storage, stability (shelf-life) will be of great importance, compared to storage and use in temperature-controlled laboratories. If RDTs are to be used by isolated volunteer health workers, an easy-to-use format will be of greater importance than in a laboratory setting.

Cost
RDTs are often more costly than microscopy and this should be borne in mind when deciding purchase quantities and level of use in a health care system.

Cassette format RDTs are usually 10-20 per cent higher priced than dipstick RDTs, although dipsticks sometimes require the procurer to provide wells, resulting in a similar total cost. When used by health workers, cassette RDTs are probably more reliable than dipstick RDTs, and so may provide savings through improved diagnosis.

[5]WHO informal consultations, 2000, 2003.

Other considerations

RDT specific

- HRP2-detecting tests are likely to have greater sensitivity than pLDH- and aldolase for detection of current *P. falciparum* infection in most environments.
- When stored and used in temperature-controlled environments, pLDH detecting RDTs are likely to have greater sensitivity than aldolase in detection of non-*falciparum* infections. Microscopy is generally the preferred tool for this purpose.
- pLDH and aldolase-detecting RDTs are likely to be less temperature stable than HRP2, and will therefore loose sensitivity more rapidly in uncontrolled storage.
- RDTs detecting non-*falciparum* species offer little advantage in terms of case management in areas where *P. falciparum* predominates and non-*falciparum* species nearly always occur as co-infections. In such situations, particularly where tests will be stored without temperature control, RDTs detecting HRP2 offer advantages in terms of sensitivity, stability, cost and format.
- Cassettes are preferable to dipsticks in remote areas due to relative simplicity of preparation and use.

Programmatic

- All large batches of RDTs should be tested after procurement and monitored throughout their shelf-life.
- Evidence of good manufacturing practice (GMP) and good field experience of manufacturers should be considered during procurement. Standard specifications for procurement should be followed[6].

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

- Quality control testing of a designated sample of the product.
- Cold chain for transport and storage.
- Health worker training and monitoring.
- Clear guidelines on action to follow the results (diagnostic and treatment algorithm).

This will ensure smooth integration of malaria RDTs into health systems/services without any undue burden.

Transport and Storage

Exposure to extreme temperatures is a major contributor to poor performance of RDTs, particularly during transfer from the manufacturer, and transport within a country as well as storage. High humidity can rapidly degrade RDTs, including prolonged exposure to humidity after removal from the envelope or if the envelope is damaged.

In order to maintain quality and performance, the following indications should be followed:

- The consignee must be informed well in advance of the shipment.
- Goods should be dispatched in such a manner that no shipment arrives on a Friday afternoon (the day preceding a weekend) or during the weekend and public holidays.
- Consignees must facilitate prompt clearance so that shipments are moved immediately to moderate temperature storage (less than 30ºC if possible) and materials are not left on airport tarmacs, in customs sheds or in vehicles parked in the sun.
- Ground transportation during any stage of delivery must be carried out without delay and with attention to ambient temperature while the vehicle is moving and if parked.
- Storage at central and final field facilities should be within the manufacturer's specifications (usually 30ºC) to maintain a cold chain.

[6] WHO 2004; WHO-WPRO 2005.

Quality

UNICEF procurement is currently limited to RDTs included in the WHO bulk procurement tenders. The list is based on evidence[7] of quality manufacturing, through provision of evidence of independent certification of compliance with ISO13485:2003 (or US FDA 21 CFR 820) and a stability testing protocol provided by the companies to WHO. Whilst requiring this evidence from manufacturers as a pre-requisite for listing of products, WHO (and thus UNICEF SD) cannot guarantee the accuracy of the information.

For more information, please contact UNICEF Supply Division in Copenhagen at supply@unicef.org.

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