

GUIDE FOR THE INTERPRETATION OF OBSERVATIONS NOTED DURING LOT TESTING OF MALARIA RDTs

This document is intended to guide the requester/user in the interpretation of observations noted in the lot-testing reports. The comments on anomalies noted on malaria RDTs that undergo lot-testing at laboratories of the WHO-FIND malaria RDT evaluation programme are reported according to a standard format, designed to be consistent between technicians and laboratories.

The lot-testing process

The evaluation carried out by the lot-testing reference laboratories is conducted according to the testing protocol provided by the manufacturer (testing procedure), and the Standard Operating Procedures (SOPs) of the Methods Manual¹.

See: http://www.finddiagnostics.org/programs/malaria-afs/malaria/rdt_quality_control/lot_testing/ and http://www.wpro.who.int/malaria/sites/rdt/who_rdt_evaluation/lot_testing.html

Interpretation of results during lot-testing

RDTs must detect parasite-positive panels at 200 parasites per microliter of blood in order to pass the quality control evaluation. This is considered close to the minimum density likely in a clinically-significant malaria infection.² Any visible line is considered a positive result. Very faint lines observed at such low density does not mean that the same result will be noted during testing in the field since parasite density is likely to be higher, and the test band correspondingly more intense.

The following comments are observations that are intended to bring to the attention of the procurer issues that may sometimes affect field use and make interpretation more difficult, or require emphasis in training. The importance of these observations needs to be considered in the light of their frequency, and the intended use of the RDTs.

The absence of comments in the report means that the result is good and the anomalies were not detected (i.e. clear test bands, no red background, no incomplete clearing etc).

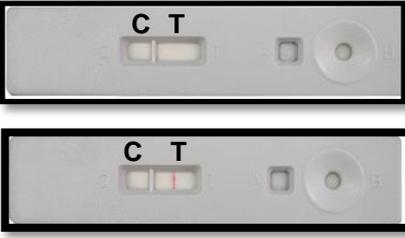
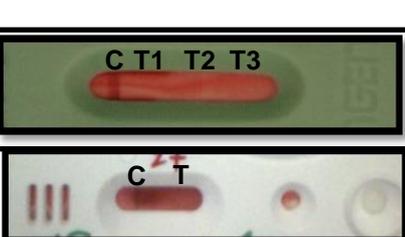
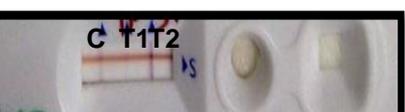
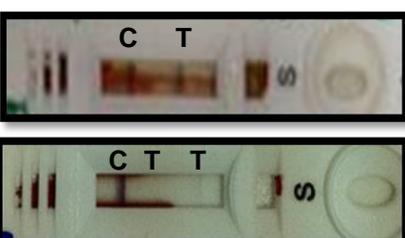
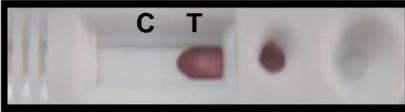
When possible, photos of the testing are attached to the report so that the requesters can see the results of the RDTs tested.

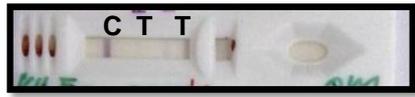
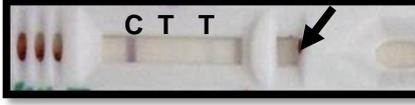
A test result is positive as long as the test line is visible, irrespective of the intensity of the line.

A test result is negative when test line is not visible.

¹ WHO-TDR-FIND (2010). Methods Manual for Laboratory Quality Control Testing of Malaria Rapid Diagnostic Tests, Version Six. Geneva, World Health Organization.

² WHO (2010). Parasitological confirmation of malaria diagnosis - Report of a WHO technical consultation GENEVA, 6–8 October 2009. Geneva, World Health Organization.

ILLUSTRATED EXAMPLES	OBSERVATION/ EXPLANATION	INTERPRETATION OF RESULTS	REPORTED COMMENTS (in lot testing reports)
	<p>Clear control (C) line and clear test (T) line, clean background</p>	<p><i>Positive test result</i></p>	<p><i>No comment (Positive test result)</i></p>
	<p>Clear control line, but no test line, with clean background.</p>	<p><i>Negative test result</i></p>	<p><i>No comment (Negative test result)</i></p>
	<p>Absence of the control line.</p>	<p><i>Invalid test result: the test is repeated with the same sample again.</i></p>	<p>Invalid</p>
	<p><i>A red background, if intense, may obscure weak positive test lines, causing false negative results. Faint background staining is relatively common. In this example, the result is negative since test line is not visible.</i></p>	<p><i>If the test line is not seen (obscured) with a parasite positive sample, this is noted as a negative RDT result. If the test line is seen, this is noted as a positive RDT result.</i></p>	<p>Red background</p>
	<p><i>Poor clearing of blood with a clear blood streaking line. Poor clearing of blood may obscure weak positive test lines, causing false negative results. In this example, the result is positive since test line is visible.</i></p>	<p><i>If the test line is not seen (obscured) with a parasite positive sample, this is noted as a negative RDT result. If the test line is seen, this is noted as a positive RDT result.</i></p>	<p>Incomplete clearing with streaking blood</p>
	<p><i>Poor clearing of blood may obscure weak positive test lines, causing false negative results. In this example, the result is positive since test line is visible</i></p>	<p><i>If the test line is not seen (obscured) with a parasite positive sample, this is noted as a negative RDT result. If the test line is seen, this is noted as a positive RDT result.</i></p>	<p>Incomplete clearing</p>
	<p><i>Blood and buffer did not run the length of the strip</i></p>	<p><i>This is noted as 'invalid' (no control line), and the RDT is repeated as per the standard procedures.</i></p>	<p>Failure to flow</p>
	<p><i>White lines on a stained background. In this example, the result is negative since test line is not dark thus not visible.</i></p>	<p><i>This is noted as a negative RDT result.</i></p>	<p>Ghost test lines</p>

	<i>The test line is visible but interrupted (broken).</i>	<i>Visible test lines are noted as positive, even if incomplete.</i>	Patchy broken test line(s)
	<i>Faint test line results will be noted as a comment and are considered as a positive test result.</i>	<i>Visible test lines are noted as positive, even if faint.</i>	Faint test line(s)
	<i>Test line wider than control, without clearly-defined edge.</i>	<i>Visible test lines are noted as positive, even if diffuse.</i>	Diffuse test line(s)
	<i>Strip can only partially be seen in the results window.</i>	<i>NA (RDT cannot be used for testing).</i>	Strip misplaced in the cassette
	<i>Normally, the colour of the conjugated antibody can be seen in the sample window (commonly purple, pink or blue).</i>	<i>NA (RDT cannot be used for testing).</i>	Specimen pad not seen in sample window

C = Control line, T = Test line(s)

FURTHER COMMENTS NOTED IN THE LOT TESTING REPORTS

ON THE BUFFER BOTTLE

- ❖ Container does not puncture
- ❖ Empty bottle
- ❖ Insufficient volume
- ❖ Discoloured buffer

ON THE TEST ENVELOPE OR PACKAGING

- ❖ Not easy to pull tab on test
- ❖ Missing essential test accessories
- ❖ Damaged sachet of desiccant
- ❖ Wrong package insert or labelling

NB. This list is not exhaustive. These comments are based on problems encountered by the lot testing laboratories during lot testing.

THE USE OF COMMENTS RECEIVED IN LOT-TESTING REPORTS

Malaria RDTs are essentially a qualitative test that must reliably detect the presence or absence of malaria parasites in a febrile patient whose illness is the result of malaria parasitaemia. Due to the nature of the tests, they will not always detect very low density (asymptomatic) parasitaemia. The WHO product testing programme recommends that RDTs should reliably detect the global evaluation panel based on samples diluted to 200 parasites/ μ L (with a 'parasite detection score' of $\geq 75\%$)³. Similar panels are used in lot-testing. This is expected to provide a high sensitivity for detection of symptomatic malaria in nearly all clinical situations, as the parasite panel set at 200 parasite/ μ L reflects the low-end of parasite density that will be associated with symptoms (i.e. most symptomatic infections are much more easily detected). A 'Fail' result in lot-testing indicates a low confidence that the tests will detect infections at the lower end of expected parasite density in clinical malaria infections.

The anomalies listed in this information document therefore need to be taken in this context. Relatively faint lines may sometimes be seen at low parasite density as only a low concentration of detectable antigen is present. Anomalies such as background blood staining may also be acceptable if at a low frequency, and not likely to obscure positive test lines. Training of health workers should also address these issues, both in requirement for reporting of frequent anomalies in the field, and in advice to repeat tests where test lines may be obscured. A high frequency of anomalies noted in lot-testing could indicate deficiencies in manufacturing or in the manufacturer's quality control, and so should be considered when making procurement decisions. It is recommended that procurement agents and national programmes use the above principles to formulate a policy of accepting and rejecting batches, and to inform future procurement decisions.

General notes

The lot testing programme is an independent evaluation programme coordinated by WHO and FIND, and funded by UNITAID and other donors. Lot-testing is carried out according to a Methods Manual (SOP) on which all lot-testing is based. This is found at:

http://www.wpro.who.int/malaria/sites/rdt/who_rdt_evaluation/lot_testing.html

http://www.finddiagnostics.org/programs/malaria-afs/malaria/rdt_quality_control/lot_testing/

Lot-test reports are confidentially emailed to the designated recipient of the reports (as specified in the lot testing request form) and cannot be released to any third party unless agreed with the recipient of the reports. In this case, the report is to be requested from the recipient of the report, not from the programme. However, summarized results are released every 6 months at the following URLs:

http://www.wpro.who.int/malaria/sites/rdt/who_rdt_evaluation/lot_testing.html

http://www.finddiagnostics.org/about/what_we_do/successes/malaria_rdt_lot_testing_results/

Photos of the testing are provided with the report when the testing workload allows it and comments (as listed above) are provided to illustrate observations noted during this testing. Any requests for such photos are to be raised with the manufacturer/report recipient.

The programme is not responsible for deciding the acceptance or rejection of an RDT lot by a procurement agent or malaria programme. This decision is to be taken by the requester of the lot-testing. The lot-testing programme aims to provide data on which this decision can be based.

³ http://www.finddiagnostics.org/programs/malaria-afs/malaria/rdt_quality_control/product_testing/