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# MALARIA

# Diagnostics Technology Landscape

## SEMI-ANNUAL UPDATE

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**UNITAID Secretariat**
**World Health Organization**
**Avenue Appia 20**
**CH-1211 Geneva 27**
**Switzerland**
**T +41 22 791 55 03**
**F +41 22 791 48 90**
**unitaid@who.int**
**www.unitaid.eu**

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## Overview

The Malaria Diagnostics Technology Landscape is published annually and is prepared as part of a broad and on-going effort at UNITAID to understand the technology landscape for malaria diagnostics. This document is a semi-annual update, focused on updates to the diagnostic pipeline first described in the Malaria Diagnostics Technologies Landscape (available at http://www.unitaid.eu/resources/publications/technical-reports).

## Methods

The Malaria Diagnostics Technology Landscape Update is compiled by Jennifer A. Daily with support from UNITAID. The updates in this document were provided by the developers of these diagnostic technologies. If technologies that appear in the Malaria Diagnostics Technologies Landscape do not appear in this update, it is either because the developer did not provide an update or indicated that there were none at this time.

## Update on Technologies in the Market

### *LAMP Malaria Diagnostic Kit (Eiken Chemicals, FIND, HTD)*

Eiken Chemicals launched a reaction kit for LAMP (Loop-mediated isothermal amplification) in July 2012. LAMP, developed in partnership with FIND and HTD, is a bench top platform using isothermal DNA amplification technology, whereby parasite DNA is amplified at stable temperature and the bi-products of amplification detected using a real time turbidimeter or visually by fluorescence.

The product launched comprises reaction tubes containing primers and reagents for amplifying parasite DNA, along with positive and negative controls. Although various LAMP methods for detecting malaria have been published in the literature, this is the first commercially available malaria kit for LAMP. The product is CE marked and two clinical evaluations have been completed and are pending publication.

The malaria reaction kits, which detect *P. falciparum* and *Plasmodium* genus (i.e. pan-malaria), may be purchased directly from Eiken. Pricing for high-burden countries is approximately $5/per reaction tube (ex-works; varies with volume, shipping destination, and exchange rates).

In addition to reaction tubes, LAMP requires the following:

- Sample preparation: Several DNA extraction methods are possible. Sample processing kits, the PURE Method kit, are available from Eiken for approximately $5/patient. FIND has validated an alternative DNA extraction method, a boil and spin method requiring a centrifuge and taking less than 20 minutes, and standard operating procedures for both methods are available on the FIND website. Alternative conventional DNA extraction methods are also effective.
- Amplification requires a heating block. These are available from Eiken for $4,000-$10,000, or conventional incubators (e.g., PCR thermocyclers) can be adapted.

- Detection: After 40 minutes reaction time, detection is performed through visual or automated methods. Most commonly, this step involves visual detection of fluorescence using a UV or blue LED light; or, to eliminate the subjectivity involved in visual detection, an incubator that also measures turbidity (turbidimeter, available from Eiken) may be used.

Currently, several operational studies are underway to look at various applications for the LAMP malaria diagnostic kit. FIND is also working to simplify sample preparation and has received funding from the German Government to modify the current LAMP format for high-throughput testing. FIND expects the high throughput version to be available in 2-3 years.

### *Fio-net (Fio Corporation)*

Fio launched Fionet in mid-2012. Fionet comprises the Deki Reader, a mobile universal reader of commercially available RDTs, equipped with software to guide clinical workflow and capture patient and health worker data; airFio, a secure cloud database that aggregates data transmitted by Deki Readers over the mobile phone network; and Spiri, a portal for accessing data reports and analysis tools. The system aims to improve the quality of diagnostic test processing and case management at the point of care, and provide health managers with real-time data for infectious disease surveillance, remote quality control of diagnostic testing and remote monitoring of adherence to clinical protocols. For sites using microscopy, Deki Phones are available to guide clinical workflow and support data capture.

In early 2012, Fio completed trials in Colombia and Tanzania which demonstrated >98% concordance between the Deki Reader's interpretation of RDTs and that of expert RDT technicians. The pricing model is similar to prepaid cell-phone plans, with no upfront capital cost, and pricing based on the volume of data transmitted. The rate includes Deki units, airFio storage and data aggregation, and information services through Spiri. Several Fionet deployments are in the process of scaling up.

Fio is ISO 13485 certified and the Deki Reader is CE marked for use with malaria RDTs; additional disease applications are planned for release in 2013.

## Update on Technologies in the Pipeline

No additional technologies have been launched. Below are updates on some of the products in the pipeline.

### *Urine Malaria Test (Fyodor Biotechnologies)*

Fyodor Biotechnologies is developing a urine-based test for the diagnosis of malaria in individuals with fever. Fyodor's urine test is a one-step dipstick assay that uses immunochromatographic technology to detect malaria proteins that are shed in the urine of persons with fever.

The first generation product will detect *P. falciparum* only; a second generation product that will detect both *P. falciparum* and *P. vivax* malaria is in development. Fyodor expects the cost of the first generation device to be comparable to the price of current blood-based rapid tests.

Originally developed at Johns Hopkins University, Fyodor licensed the technology in 2008, and expects to launch its first generation product in 2014. Fyodor is currently implementing a series of clinical validation studies in Africa, and a final large-scale clinical trial is planned for 2013. Currently, the company expects to submit its malaria RDT to the WHO Prequalification of Diagnostics Program in 2013.

Since the publication of the Malaria Diagnostics Technology Landscape in December 2011, several updates to the operational characteristics of the Urine Malaria Test have become available; an updated table is provided in Appendix 1.

### *Fluorescent Rapid Diagnostic Tests (Access Bio Inc.)*

In response to the need for improved sensitivity and limit of detection (LOD) in malaria RDTs, Access Bio Inc. is developing a lateral flow test that generates a fluorescent signal. Since the publication of the Malaria Diagnostics Technology Landscape in December 2011, more detailed information on this technology has become available and is presented below and in a table in the appendix to this document.

Access Bio's fluorescent RDTs use fluorescence to improve the RDT's detection system by making even minute quantities of parasite antigen (bound to the monoclonal antibody and captured on the signal line) detectable. The technology is similar to traditional RDTs, except that monoclonal antibodies (HRP-II or pLDH) are coated onto tiny particles that contain europium instead of being attached to colloidal gold. Europium is a metal that fluoresces when viewed with an ultraviolet light. In order to read the results, the RDT must be inserted into an RDT reader that converts the fluorescent signal into a digital read out.

Based on laboratory evaluations using cultured and clinical specimens, the fluorescent RDT technology may be 100 times more sensitive than traditional RDTs, and it may be possible to detect malaria proteins not only in the blood but also in the urine. Access Bio expects the fluorescent RDTs to be available in 2013. Prices have not yet been established, however Access Bio is forecasting that the RDT will cost slightly more than traditional RDTs and that the reader will cost $ 500–$ 1,000 depending on the level of customization (e.g., printer attachments, WiFi enabled etc.) Access Bio is also developing fluorescent RDTs to detect HIV antibodies or Chlamydia antigens in urine, which are not possible to detect in traditional RDTs.

See Appendix 1 for more information on this technology.

### *Truelab™ micro PCR platform (Tulip Group and Bigtec Labs)*

Molbio Diagnostics (P) Ltd., a Joint Venture between the Tulip Group and Bigtec Labs have developed a POC PCR platform and malaria assay. The system comprises an analyzer, (Truelab™ Uno - real time micro PCR analyzer), a sample preparation device and kit (Trueprep™ MAG), and a chip-based test for *P. falciparum* (Truenat™ Malaria Pf). The developers expect to launch the platform, along with the *P. falciparum* assay and a TB assay, by the end of 2012. The malaria test has been registered in India and is undergoing the CE marking process. A *P. falciparum/p. vivax* duplex test has also been developed and expected to be launched in the first quarter of 2013, along with several assays for other diseases.

Since the UNITAID Malaria Diagnostics Technology Landscape was published, the developers report one change to the product specifications: the blood sample volume required is now 100 µl. The analyzer and sample preparation device together will be priced at $6,000. The Truenat Pf chip will cost $12-15 during the launch phase; prices are expected to decrease as production scales over the years.

### *Nucleic Acid Lateral Flow Immunoassays (MALACTRES Consortium)*

The MALACTRES consortium is developing PCR assays that include several simplifications to traditional PCR methods: (i) the assay is a direct PCR, meaning it uses whole blood and does not require any sample preparation; (ii) after performing traditional PCR amplification, detection of DNA is done using a disposable lateral flow test device, called a nucleic acid lateral flow immunoassay, or NALFIA; and (iii) a commercial kit will contain all of the necessary primers, reagents, and the lateral flow device required to run the test.

MALACTRES reports that the assay development is progressing well; after successful published lab evaluations and field evaluations in Burkina Faso and Thailand,[1] it is now being further studied in Nigeria and Kenya. The product launch date is now contingent upon funding for manufacturing and further commercialization of the product.

### *Malaria FISH Assay (ID-FISH Technology)*

ID-FISH Technology Inc. expects to launch its malaria fluorescent in-situ hybridization (FISH) assay in 2013. FISH technology takes advantage of fluorescent probes that bind with parasite RNA causing malaria-infected cells to fluoresce when viewed under a fluorescent microscope. In-country evaluations of the assay are underway, and ID-FISH expects to undergo the FDA 510(k) clearance process in 2013.

1 See: Mens, P.F., et al. (2012). Direct blood PCR in combination with Nucleic Acid Lateral Flow Assay for the detection of *Plasmodium* species in malaria endemic settings. Journal of Clinical Microbiology, in press. And Mens, P.F et al. (2012). Development, validation and evaluation of a rapid PCR-Nucleic Acid Lateral Flow Immuno-Assay for the detection and differentiation of *Plasmodium* species. Malaria Journal: 279.

### *SpectraWave and SpectraNet (Claro Scientific)*

Claro Scientific is developing SpectraWave and SpectraNet, a reagent-less POC diagnostics system based on optical profiling technology. In response to the need for improved malaria and anemia diagnosis and management, Claro is developing a malaria diagnosis and complete blood count assays for its system, and these are expected to be available in 2014–2015

Claro reports progress including completion of market and form factor studies in Africa. These studies, conducted in collaboration with PATH, focused on function, cost, test menus, and user acceptance, and resulted in small modifications to the current system design. Claro has also made progress in developing its sample processing system for whole blood, which is based on acoustics and microfluidics. The sample processing system is currently working both for malaria diagnosis and for complete blood counts, where it achieves the necessary blood separation levels for measuring subtypes of hemoglobin and albumin. Finally, Claro reports favorable results from a clinical proof of concept study, focusing on red blood cell measurements.

### *Spectraphone (Quantaspec)*

Quantaspec is a Research and Development company developing a POC molecular detection system for malaria using infrared spectroscopy. Currently, it is working to miniaturize the technology into a handheld device, which will be called Spectraphone. The device will be capable of running multiple assays, including malaria. Quantaspec reports that it has secured funding to continue development of the Spectraphone platform and confirms the launch date of 2014.

## Technologies Added since the Previous Edition

Since the release of the annual report, additional technologies have been introduced to the pipeline as described below. Additional detail on these technologies will be provided in the 2013 edition of the Malaria Diagnostic Technology Landscape.

#### *Amplino*

A team of three Dutch scientists is developing Amplino, a low-cost quantitative PCR instrument and a malaria assay. The technology is currently in prototype form and has been designed with simplicity and low cost in mind. The team is targeting $250 for the device and < $2 for each test. The expected launch date is mid 2014.

#### *Nanomal (Nanomal Consortium)*

Nanomal is an EU-funded project to develop a POC handheld device to both diagnose malaria and to detect drug resistance. The device will analyze a finger-stick blood sample using PCR and sequencing technologies. The development consortium includes: St George's, University of London, QuantuMDx Group, The Karolinska Insititute, and Tubingen University. The device is in early development, with clinical trials expected to begin within three years.

# Appendix 1: Operational Characteristics of Malaria Diagnostic Platforms

Only tables that have been updated are included here. For a comprehensive catalog of tables, please see UNITAID Technical Report: Malaria Diagnostics Technology Landscape, December 2011 (available at: http://www.unitaid.eu/resources/publications/technical-reports).

### *Urine Malaria Test (Fyodor Biotechnologies)*

| Platform Characteristics | |
|---|---|
| **Type of technology** | Disposable one-step urine dipstick based on immunochromatographic detection of malaria parasite proteins in urine. |
| **Output** | First generation product is a two-line test that will differentiate "fever due to *Plasmodium falciparum* malaria" from fever due to some other cause. The results are visible: one line indicates fever not due to not malaria, two lines is positive for malaria.<br>Second generation product (2015) will be a three line test that detects both *P. falciparum* and *P. vivax* malaria. |
| **Performance** | The test is designed to detect the presence of malaria proteins present in urine during fever. Interim analysis of ongoing field trials shows that the test achieves > 90% sensitivity and 90% specificity for the detection of *P. falciparum* malaria. The urine test has a limit of detection of 125 parasites/µl blood. |
| **Turnaround time/ Capacity** | Test results are available in 20 minutes. |
| **Sample needed/ Stability** | The device requires about 5 drops (100–200 µl) of urine.<br>The test is a real-time test and intended to be performed immediately after sample collection. |
| **Environmental requirements** | Stability studies of the urine test have not been completed, however it is being designed with stability in mind and is anticipated to have a 12 month or longer shelf life and recommended storage conditions of 25-30° C. |
| **Testing protocol** | The urine malaria test is a one-step test with no requirement for sample preparation. The testing protocol is: (i) collect urine sample; (ii) open packaging and dip test into sample; (iii) allow test to dry for 20 minutes; (iv) read results. |
| **Cost/Test** | Comparable to cost of current rapid blood tests. |
| **Cost/Instrument** | No instrument. |
| **Power requirements** | None |
| **Training/Technical sophistication** | Test procedure is simple, no sample preparation, no blood draws, or buffers are required. It is designed for point-of-need use by lay people. |
| **Durability/ Maintenance** | N/A; disposable test. |
| **Infrastructure requirements** | No infrastructure required; test is designed for point-of-need use at all levels of the health system. |
| **Result display and storage** | Results appear as visible lines on the test strip. No sample or results storage required. |
| **QA/QC** | Fyodor intends to submit the product to the WHO Pre-Qualification programme, and product manufacturing will be done only at manufacturing sites which qualify and operate under the internationally recognized International Standards Organization (ISO) 13485:2003 standards. |
| **Availability** | Expected in early 2014. |

## *Fluorescent Rapid Diagnostic Tests (Access Bio Inc.)*

| Platform Characteristics | |
|---|---|
| **Type of technology** | Disposable rapid diagnostic tests based on time resolved fluorescence technology to detect malaria antigens in samples, RDT results are read using a portable RDT reader.<br><br>Time resolved fluorescence (TRF) RDTs are similar in terms of components and reactions to traditional malaria RDTs except in their signal detection system: instead of using gold conjugate as the signal and reading the results visually, fluorescent RDTs utilize europium particles as signals. These particles fluoresce when viewed with an ultraviolet light.<br><br>A portable RDT reader equipped with a UV LED reads the results and converts the fluorescent signal to a digital readout for the user. The reader incorporates a time resolving function to improve sensitivity. The approximate dimensions of the RDT reader are: 14 cm x 21 cm x 14 cm.<br><br>  |
| **Output** | Qualitative and quantitative results for *P. falciparum*, *P. vivax* and pan-malaria. Several types of RDTs will be available, (e.g., 2 line, 3 line) to detect *P. falciparum*, *P. vivax*, pan-malaria and HRP II deleted *P. falciparum*. |
| **Performance** | Pre-clinical studies suggest that the technology may be >100 times more sensitive than traditional RDTs. |
| **Turnaround time/ Capacity** | Test results are available in 15 minutes. |
| **Sample needed/ Stability** | For a blood sample: 5 µl whole blood from finger prick or venipuncture. For a urine sample: Sample quantity TBD, likely 2-3 drops. |
| **Environmental requirements** | The RDTs are expected to have a 24 month shelf life with recommended storage temperature of 4-30°C. |
| **Testing protocol** | The testing protocol is: (i) collect blood sample; (ii) transfer 5 µl of blood to RDT; (iii) add 2 drops of buffer; (iv) wait 15 minutes for reaction to occur; (v) insert test into RDT reader and view results. |
| **Cost/Test** | Targeting $.75-1.00 for a single line test |
| **Cost/Instrument** | Targeting $500-1,000 per instrument |
| **Power requirements** | RDT reader is battery operated with a charger |
| **Training/Technical sophistication** | Designed to be performed by low skilled health workers, <1/2 day of training required for new test operator. |

| | |
|---|---|
| **Durability/ Maintenance** | RDTs are disposable. RDT reader is expected to last at least three years under normal operations. Non-functioning readers will be swapped out. |
| **Infrastructure requirements** | Appropriate for health facilities at all levels. |
| **Result display and storage** | Results appear on the RDT reader's screen. Capacity for printing, storage of results in the device, and wireless transmission of results may be built into the device. |
| **QA/QC** | AccessBio plans to submit the product to the WHO Pre-Qualification program. |
| **Availability** | 2013 |

## Appendix 2: Glossary of Terms and Acronyms

| | |
|---|---|
| **ACT** | Artemisinin-based combination therapy |
| **CE/CE marking** | A mark placed on products in the European Economic Area that indicates the product conforms with requirements of EU directives. CE stands for Conformité Européenne (European Conformity). |
| **DNA** | Deoxyribonucleic acid |
| **FDA** | Food and Drug Administration (USA) |
| **FIND** | Foundation for Innovative New Diagnostics |
| **FISH** | Fluorescent in-situ hybridization |
| **Hemozoin** | A malaria parasite produces hemozoin crystals as a byproduct of its metabolism of hemoglobin: After infecting a person, the parasites enter red blood cells and feed on hemoglobin, an iron-bearing molecule that plays a key role in supply of oxygen throughout the body. The parasite is unable to use the iron-containing part of hemoglobin, and sequesters it in the form of tiny crystals called hemozoin. The presence of hemozoin in a patient is a strong indication of malaria infection. |
| **HIV** | Human immunodeficiency virus |
| **HRP** | Histidine-rich protein |
| **HTD** | Hospital for Tropical Diseases (UK) |
| **ISO** | International Standards Organization |
| **KIT** | Koninklijk Instituut Voor de Tropen/Royal Tropical Institute (The Netherlands) |
| **LAMP** | Loop-mediated isothermal amplification |
| **LED** | Light-emitting diode |
| **LOD** | Limit of detection |
| **Microfluidics** | Microfluidics involves the reduction of the volume of reagents and samples required to perform a test and the movement of these small amounts of fluids in miniature channels integrated into a device. In diagnostic assays, microfluidics cartridges are designed to move a small volume of the sample being analyzed through a series of channels and chambers. Among the advantages of microfluidics is a substantial reduction in the volume of sample and reagent required to perform an assay, often allowing for reduced time to result and lower overhead costs. |
| **MOT** | Magneto-optical technology |
| **NIH** | National Institutes of Health (USA) |

| | |
|---|---|
| **PCR** | Polymerase chain reaction. A laboratory method developed in the mid-1980's that allows for a particular segment of nucleic acid to be copied limitlessly. This copying (or amplification) makes it easier to detect minute quantities of nucleic acid in a sample. |
| **POC** | Point of Care |
| **QA/QC** | Quality assurance/Quality control |
| **RDT** | Rapid diagnostic test |
| **RNA** | Ribonucleic acid |
| **Thermal cycler** | Laboratory instrument used to achieve the rapid changes of temperature required for PCR. The thermal cycler contains a thermal block with holes for tubes containing the samples. The thermal cycler is programmemable to precisely control temperature increase/ decrease steps, the length of time that a reaction is held at a particular temperature, and the number of cycles that are completed. |
| **TRF** | Time resolved fluorescence |
| **Turbidimeter** | A laboratory instrument for measuring the loss in intensity of a light beam through a solution due to particle formation. |
| **WHO** | World Health Organization |