



Developing new diagnostics
and bringing them to
the community

Annual Report
2010



Our vision is of a world where everyone will have equitable access to high quality diagnosis

Our mission is to drive the development and implementation of accurate and affordable diagnostic tests that are appropriate to patient care in low-resource settings

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Message from the Chairman of the Board and the Chief Executive Officer

The year 2010 saw the consolidation of FIND's strategy, with an increased focus both on developing platform technologies to detect multiple diseases and on ensuring that our technologies are producing an impact in disease endemic countries. Seven years following its inauguration, FIND was recognized by the Swiss Government as an [Other International Organization](#), demonstrating how we have become an increasingly important player in the diagnostics and laboratory strengthening arenas.

On the technological side, the key highlight of the year was the [WHO endorsement](#) in December of the [Xpert® MTB/RIF test](#), developed by FIND, Cepheid and the University of Medicine and Dentistry of New Jersey. An accurate, easy-to-use rapid diagnostic tool for tuberculosis (TB) and drug-resistance, Xpert MTB/RIF has the potential to revolutionize TB control and management for the populations most affected by the disease.

The endorsement rewards years of work by all those involved in the project. FIND initially started by scouting the landscape for what new TB diagnostics were needed and where. We then identified partners, provided necessary financing, worked together with our partners to refine the test and organized the various in-country trials. Our negotiations then brought the product price as low as possible, so that the test would be available to the largest possible number of patients.

FIND's job didn't end here. Through knowledge sharing and technology transfer, we helped create the necessary conditions for sustainable and effective roll-out and implementation of our co-developed diagnostic tests. We were particularly gratified in September to be awarded a multi-country 5-year Cooperative Agreement by the US Centers for Disease Control and Prevention to work towards [enhancing local capacity for the diagnostic testing](#) of tuberculosis, malaria and HIV through laboratory strengthening.

2010 also saw an extension of the [EXPAND-TB](#) project, which is funded by UNITAID and is a collaboration between FIND, WHO and the Stop TB Partnership's Global Laboratory Initiative and Global Drug Facility. This extension signalled a renewed commitment by all partners to support access to novel diagnostic technologies and the laboratory services needed to diagnose multidrug-resistant TB.

With regard to our in-country presence this year, [FIND India](#) is particularly proud of the approval of a \$25 million sub-grant from the Global Fund to Fight AIDS, TB and Malaria for laboratory strengthening activities throughout the [country](#). [FIND Uganda](#) has continued to play a critical role both in the demonstration of new tools and in advising the government on malaria diagnostic strategies.



*Dr. Gerald Möller
Chairman of the Board*



*Dr. Giorgio Roscigno
Chief Executive Officer*

This has also been an exciting year for FIND's work on other technologies and other diseases, including significant strides in the development of the manual molecular platform [LAMP](#) for the detection of TB, malaria and sleeping sickness Human African Trypanosomiasis (HAT). This progress highlights FIND's ability to pool technological know-how and resources to address the needs of multiple diseases.

Other new tools are also in the pipeline. For instance, in February, FIND signed an agreement with Standard Diagnostics to develop a [rapid point of care test](#) for the screening of HAT.

In malaria, we are particularly proud to have published in 2010 the results of the second round of the WHO-FIND malaria [RDT evaluation project](#), which rigorously assesses the comparative performance of commercially available RDTs against prepared blood panels. The results of this programme now inform the procurement decisions of UN agencies and the Global Fund to Fight AIDS, TB and Malaria.

FIND is also exploring innovative ways to support partners in the effective roll-out of diagnostics programmes. To this end, we have been using a novel [SMS-based data collection and communications](#) system, RapidSMS, to monitor malaria indicators and commodity stocks in two districts in Uganda, in collaboration with the Ministry of Health, the Earth Institute at Colombia University and WHO. In response to the initial positive experience of using this system, the Ugandan Ministry of Health is in discussion with key stakeholders to extend RapidSMS nationwide.

Finance and organizational changes

The period of consolidation, begun in 2009, continued in 2010 as projects in late phase demonstration studies in the latter part of 2009 reached completion. As a result, analytical and project expenditure was down to \$21.9 million compared with \$22.9 million in 2009, a drop of 4%. This consolidation was reflected also in the decrease in total expenditure to \$25.7 million, down from \$26.3 million in 2009. Tight controls held support and infrastructure costs to levels consistent with previous years and enabled project spending to be maintained at 86% of the total, compared with 87% in 2009, 88% in 2008 and 85% in 2007.

Accurate and prompt reporting is a key feature for our funding partners. This not only plays a major role internally in helping project leaders to manage their project portfolios, but also safeguards the efficient project management practices that are a hallmark of FIND's work with partners. With the objective to further improve these standards and to offer a wider range of services to users, we have decided to upgrade our systems to handle higher volumes and more complex reporting algorithms. The main phase of this work is expected to be completed by the end of 2011.

Accounting standards for revenue recognition provide for contributions from donors to be recognized over the full term of each grant agreement. In 2010 revenue thus determined plus sundry income amounted to \$27,588,000 (2009 - \$24,629,000), which resulted in an excess of income over expenditure of \$1.9 million. This was added to previous surpluses to give a total of \$2.4 million at the end of 2010. Further information on FIND's financial results in 2010 can be found in the Auditors' Report, Financial Statements and Notes elsewhere in this report.

As ever, FIND's vision could not be implemented without support from our funding partners and we would like to thank them for their continued financial contributions and strategic advice: the Bill and Melinda Gates Foundation, the UK Department for International Development, the European Union, the Government of the Netherlands, the US National Institutes of Health, UNITAID and the US Centers for Disease Control and Prevention.

Introducing fast and accurate tests
where patients seek care



Tuberculosis Programme

While tuberculosis remains one of the deadliest global health threats, the main detection method in many parts of the world remains sputum microscopy, which is over 100 years old and fails to diagnose the majority of worldwide cases. Lack of proper diagnosis costs patients and their families valuable time and money, delays treatment and leads to continued TB transmission. Mounting drug resistance and a growing number of patients co-infected with TB and HIV have highlighted the urgent need for better diagnostic tests.

FIND's goal is to improve the accuracy, affordability and speed of TB diagnostic tests, making them accessible throughout disease endemic countries, at all levels of the health system. Together with our partners, we are improving the standard of diagnostic tools and targeting them to all levels of the health system. Thus far, four technologies co-developed by FIND have been endorsed by WHO for use in developing countries. These include liquid culture and drug susceptibility, rapid speciation, the line probe assay and fluorescence microscopy, which are already being rolled out in laboratories in disease endemic countries. In order to facilitate the rapid uptake of new tools and their integration into national diagnostic algorithms, FIND also undertakes laboratory strengthening and scale up projects, mainly through the UNITAID-funded EXPAND-TB project.

However, there is still a pressing need for continued innovation. FIND's highest priority is for a simple qualitative case-detection tool for point-of-care testing at the lowest level of the healthcare system, where diagnosis is currently based on clinical signs and symptoms only. This is where the majority of patients first seek care, and where early treatment and transmission interruption could have the greatest public health impact. The second priority is laboratory-based assays that are more sensitive and/or less laborious than microscopy, for use at the next level of the health system where sputum microscopy is currently the mainstay of TB diagnosis.

Key accomplishments

- demonstration of use and impact of Xpert MTB/RIF and its endorsement by WHO STAG-TB;
- further advances in research towards the development of a point-of-care platform with potential technologies identified for feasibility testing or field trials;
- evaluation projects started at four sites and demonstration protocol drafted for three countries on the manual loop-mediated isothermal amplification (LAMP) assay;
- expansion of EXPAND-TB duration, scope and funding;
- further expansion of downstream activities, including award of a TB REACH grant to reach inaccessible patients in Lesotho;
- award of a multi-country 5-year cooperative agreement by CDC to enhance local capacity for diagnosis of multiple diseases.

TB antigen discovery

- Partners: The Forsyth Institute (Netherlands), Harvard Proteomics Lab (USA), MorphoSys AG (Germany), Natural and Medical Sciences Institute (NMI, Germany), National University of Singapore (NUS), Avanti Polar Lipids, Inc. (USA)
- FIND project leader and/or coordinator: Gerd Michel
- Project start: 2007
- Resume of project: Significant progress has been made in the development of essential reagents to be used in the development of assays using TB peptide and lipid biomarkers.

Description

Direct pathogen detection of TB infection remains a challenge. While there have been many advances in DNA detection methods, these are technically not suited for point-of-care assays to be used at the community level. This project was set up to validate candidate antigen biomarkers for TB and discover new marker candidates, with a view to integrating these into new tests.

Four *M. tuberculosis* proteins described by the Forsyth Institute as being present in patient urine have been selected for validation studies. All antigens have been produced in *E. coli* and are available as purified antigens. Antibodies developed based on an established commercial phage library were generated against the first target antigen, and assay development is ongoing. Similar work is also in progress for the remaining targets.

In addition, 10 new peptide targets were identified by mass spectrometry in patient urine and are awaiting validation.

In a separate project with NUS, we have identified several TB-specific lipids that can differentiate between active TB and non-TB. This project has now turned into a comprehensive reagent development programme.

Key accomplishments

- Several published *M. tuberculosis* antigens are in validation studies.
- Reagent development has yielded antibodies for assay development.
- Novel peptide and lipid biomarkers were discovered and have entered the reagent development phase.

TB antibody detection

- Partners: Antigen Discovery, Inc. (USA), Public Health Research Institute (USA), NMI (Germany), AbaSci LLC (USA), University of California, Irvine (USA)
- FIND project leader and/or coordinator: Gerd Michel
- Project start: 2006
- Resume of project: The majority of the discovered serological protein targets have been expressed and purified on a large scale and a reference assay (Luminex) has been developed.

Description

FIND and partners have produced the first ever whole proteome chip array for *M. tuberculosis*. Using this novel tool, several hundred patient and control sera were tested for IgG sero-reactivity to establish the “TB reactome”, i.e. the IgG-reactive proteins of the TB pathogen. Two innovative independent bioinformatics approaches were applied for data analysis resulting in 13 proteins that can sufficiently differentiate between active and non-TB or latent disease. Another 47 proteins were added as potential additional targets (although these were less strongly reactive) as a safeguard.

The high yield expression and purification of target proteins were partially achieved, but are still ongoing. Some of the targets (mainly membrane proteins) turned out to be difficult to produce at a larger scale. Efforts aimed at these targets production are underway.

Using the available purified proteins, supplemented by proteins from commercial providers, a Luminex bead assay was developed. This assay is being used as a reference method for ongoing experiments in order to reduce the number of targets to as few as possible. Respective validation experiments are supported by data from purified proteins printed on chip arrays. By combining serology data from different platform technologies we

will be able to obtain more reliable information to determine the final set of antigens for use in a point-of-care platform for field evaluation.

Key accomplishments

- First systematic definition of the TB immuno-proteome.
- Strong candidate serological targets selected.
- Target expression and purification partially completed.
- Working Luminex and planar array assays established for target validation.

Lateral flow immunoassay (LFI) sensitivity increase

- Partners: Chembio Diagnostic Systems Inc. (USA), Response Biomedical Corp. (Canada), Nanogen Inc. (USA), TbDiaDirect (Sweden), Deutsches Krebsforschungszentrum (Germany), Chimera Biotech GmbH (Germany), PriTest (USA)
- FIND project leader and/or coordinator: Gerd Michel
- Project start: 2006
- Resume of project: The project was successfully completed, with the identification of several point-of-care (POC) technologies suitable for product development once appropriate biomarkers have been chosen.

Description

It is assumed that, if TB antigens are present at all in body fluids other than sputum, their concentration is very low. They will therefore require highly sensitive detection methods.

This project aimed at a) selecting appropriate POC technologies suited to the most sensitive analyte detection, and b) identifying ultrasensitive immunoassay platforms to serve as reference methods for POC test development. LAM antigen detection was used as a model case throughout. Reagents had been pre-evaluated and had shown excellent performance.

The project compared the detection limits of products based on various assay formats and using different signal detection approaches (colorimetric, fluorescent, chemo-luminescent). Our data indicate that analytical detection limits in the picomolar to low nanomolar range are reachable by at least three of the evaluated POC platforms. This confirms and exceeds LFI sensitivity limits reported by FIND in an earlier literature survey. Moreover, we established a reference method able to determine LAM at an unprecedented concentration of several hundred molecules (10^{-14} to 10^{-13} mol/l).

This project achieved its goals. We are now in the position of selecting the most appropriate detection technology depending on the sensitivity requirements and performance criteria of a given biomarker across disease areas.

Key accomplishments

- Most sensitive POC detection technologies evaluated and identified.
- Unprecedented detection limit reached in reference assay.
- Established database enabling FIND to quickly and flexibly select platform technology for biomarker detection.

Automated TB DNA Detection

- Partners: Cepheid (USA), University of Medicine and Dentistry of New Jersey (USA)
- FIND project leader: Ranauld Sutherland
- Project start: May 2006
- Resume of project: Development, evaluation, demonstration and WHO endorsement of the Xpert® MTB/RIF assay on the GeneXpert® platform, a semi-quantitative PCR assay in a single cartridge system that detects pulmonary tuberculosis and associated rifampicin resistance in adult patients.

Description

Xpert MTB/RIF is a new cartridge-based assay used in a multi-disease testing platform (GeneXpert) that dramatically simplifies molecular testing by fully integrating and automating the three processes required for real-time PCR-based molecular testing: sample preparation, amplification, and detection.

The Xpert MTB/RIF cartridge contains lyophilized reagents, buffers and washes for real-time, hemi-nested detection with molecular beacons, allowing the test to be performed with no ancillary equipment. Testing requires only the addition of a sample reagent to the sputum – which inactivates any live *M. tuberculosis* present – followed by the transfer of the treated sample into the device. All other steps are automated. Tuberculosis and rifampicin resistance are detected in less than two hours.

The assay can be performed directly from a clinical sputum sample or from a decontaminated sputum pellet. The cartridges and sample reagent can be stored at room temperature.



Photo Cepheid

Key accomplishments

- Finished demonstration projects at 6 sites (India, South Africa (2), Peru, Azerbaijan, Germany).
- Positive recommendations from Expert Group of WHO Strategic and Advisory Group for TB (STAG-TB), which was presented with aggregated data on 1 September 2010.
- STAG-TB met on 27-29 September 2010 and endorsed the Expert Group recommendations and draft WHO policy guidance.
- Global Consultation was called by WHO on 30 November - 2 December 2010.
- 8 December 2010 Press Release “WHO endorses a new rapid tuberculosis test”.
- Agreed pricing with Cepheid and published special pricing for the FIND Target Markets.
- Started projects in India, Brazil (with the Bill and Melinda Gates Foundation or BMGF), China (with BMGF & PATH).

Line probe assay (second line)

- Partners: Centers for Disease Control and Prevention (CDC,USA), International Tuberculosis Research Center (Korea), University of Cape Town Lung Institute (South Africa)
- FIND project leader and/or coordinator: Rick O'Brien
- Project start: April 2010
- Resume of project: Evaluation of the MTBDRsI test for diagnosing drug-resistant TB

Description

The current capacity for the diagnosis of drug-resistant TB, in the great majority of countries where drug resistance is common, is quite limited. Recent calls for strengthening and expanding TB laboratory capacity, coupled with increased funding for TB laboratory services, are leading to a modest scale up of culture and drug susceptibility testing (DST). The WHO endorsement in June 2008 of line probe assays (LPAs) for rapid screening for multidrug-resistant TB (MDR-TB) has resulted in implementation of this technology at the reference laboratory level in many countries. However, in most settings there is no capacity for diagnosing resistance to second line anti-TB drugs, including extensively drug-resistant TB (XDR-TB), MDR strains with resistance to fluoroquinolone antibiotics and at least one of the second-line injectable drugs (kanamycin, amikacin and capreomycin).

The recent availability of the MTBDRsI assay - a LPA for XDR-TB, which can detect genetic mutations associated with resistance to fluoroquinolone antibiotics, amikacin and capreomycin - suggests the possibility of diagnosing XDR-TB rapidly in laboratories that have established the LPA for MDR-TB screening. However, there has been only one published study on the performance of this second-line test.

The MTBDRsI assay is a molecular LPA that uses PCR technology with reverse hybridization, with binding on nitrocellulose strips to wild type and mutant probes to the *gyrA* and *rrs* genes associated with resistance to fluoroquinolones and the injectable aminoglycoside and cyclic peptide antibiotics. Results can be read visually. The process involves DNA extraction from culture isolates or AFB smear-positive respiratory specimens, PCR amplification, hybridization and test reading.

This project aims to evaluate the test in a large sample of TB isolates, including approximately 250 XDR strains, comparing test performance to MGIT DST for fluoroquinolones, amikacin and capreomycin. Testing will be done in three laboratories that have established the LPA and MGIT DST for second-line drugs: CDC, University of Cape Town, and the US NIH-supported International TB Research Center (ITRC) in Korea.

FIND presented results of the CDC and ITRC studies, as well as other published and unpublished studies, to a WHO Expert Group in September 2010. The studies indicated good performance of the test for fluoroquinolone resistance (sensitivity 90-95%, specificity 95-100%) and for resistance to the second-line injectable agents (sensitivity 75-90%, specificity 90-95%) on culture isolates.

It is envisioned that this test would be used for TB patients diagnosed with MDR-TB by either LPA or conventional DST in order to guide individualized therapy.

Key accomplishments

- Memoranda of Understanding were signed with participating partners in April 2010.
- CDC and ITRC provided FIND with study results in August 2010, while UCT faced problems with test performance. These were ultimately determined to be due to inadequate DNA extraction.
- The Expert Group concluded that there was sufficient evidence to recommend the test for use on culture isolates, but insufficient data for performance on smear-positive sputum specimens.

- Rather than issue a recommendation that would have limited utility, WHO decided to defer seeking STAG endorsement for use of the test until it could review additional performance data for direct testing on sputum specimens. Such data are expected to be available from UCT and ITRC, as well as from testing at the NHLS laboratory in Cape Town, South Africa, in early 2011.

Manual TB DNA detection

- Partners: Eiken Chemical Company Ltd. (Japan), 42 Technology Ltd (UK)
- FIND project leader: Randal Sutherland
- Project start: July 2005
- Resume of project: Development, evaluation, demonstration and WHO endorsement of the manual LAMP-based assay format that could replace microscopy at peripheral clinics in the developing world.

Description

LAMP (Loop-mediated Isothermal Amplification) technology has been used to detect a number of infectious agents, including malaria (Poon *et al.* 2006), African trypanosomiasis (Kuboki *et al.* 2003), SARS (Poon *et al.* 2004) and influenza (Poon *et al.* 2005). Recently, specific primers have been developed, targeting the *gyr B* gene of mycobacteria. This allows detection of the *M. tuberculosis* complex directly in sputum specimens or in culture isolates. Results of the first study were very promising and showed a high specificity, efficiency and rapidity (Iwamoto *et al.* 2003).

Since then, Eiken Chemical Co., Ltd., in a joint development agreement with FIND, has modified the LAMP technique and transformed it into a more convenient kit format (Loopamp™), providing reaction tubes coated with a lyophilized master mix and simple visual readout of amplification result.



Visual detection of amplified product by fluorescence

The reaction is carried out at constant temperature (67°C), and amplification and detection of the target gene can be completed in a single step. Detection of the amplified product is based on calcein fluorescence. A UV lamp is incorporated in the heating block and used to detect the fluorescence at the end of amplification.

The advantages of LAMP are: 1) no special reagent required, 2) no sophisticated temperature control device required, 3) visual detection of the amplified product by fluorescence, and 4) minimal hands-on training.

Key accomplishments

- Started evaluation projects at 4 sites (Vietnam, Peru, Brazil, South Africa).
- Interim evaluation report available October 2010.
- Drafted demonstration protocol for Peru, Uganda, India.
- Country of origin Japanese registration filed.
- Agreed pricing with Eiken based on price-volume relationship.
- Started projects in China (with Bill and Melinda Gates Foundation and PATH).
- Demonstrated proof-of-concept for a bacteria concentration process.

Latent TB infection diagnosis

- Partners: London School of Hygiene and Tropical Medicine (UK), ZAMBART (Zambia), Stellenbosch University (South Africa), University of Cape Town (South Africa), St. John's Research Institute (India), John's Hopkins University (USA), Centers for Disease Control and Prevention (CDC, USA), Aeras Global TB Vaccine Foundation (USA)
- Other collaborating institutions: South African Tuberculosis Vaccine Initiative (South Africa), The Gade Institute, University of Bergen and Haukeland Hospital (Norway), Statens Serum Institute (Denmark), University of the Witwatersrand (South Africa), Chris Hani Baragwanath Hospital (South Africa), Cho Ray Hospital (Vietnam), Denver Public Health Department (USA), The Methodist Hospital Research Institute (USA)
- FIND project leader and/or coordinator: Rick O'Brien
- Project start: August 2006
- Resume of project: Evaluation of the use of IGRAs for the diagnosis of latent tuberculosis infection, particularly in high risk groups.

Description

Interferon-gamma release assays (IGRAs) are now being used widely in developed countries for the diagnosis of latent tuberculosis infection (LTBI). Two IGRAs are commercially available and offer considerably improved operational performance characteristics compared to the tuberculin skin test (TST), the traditional test for LTBI diagnosis.

However, at the time this project was initiated, there were few published data on the use of IGRA tests for LTBI screening of high-risk groups (e.g., HIV infected persons and childhood household contacts of infectious TB patients) in low-income countries. There were virtually no data on the predictive value of IGRAs for future active TB in these groups in high TB incidence settings. While it was postulated that IGRAs might be useful as a diagnostic aid in paediatric TB, no data on this question were available. Therefore, FIND partnered with Cellestis, Ltd. on evaluation studies of their IGRA, the QuantiFERON Gold In-Tube (QFT-IT) assay to address these questions.

The QFT-IT assay is a whole blood cytokine assay that measures, using ELISA, plasma levels of interferon-gamma following stimulation of sensitized lymphocytes by three *M. tuberculosis* specific antigens (ESAT-6, CPF-10 and TB7.7). Sensitivity for LTBI as determined in patients with active TB is as high as or higher than that of the TST. IGRAs correlate much better with TB exposure than do the TSTs. Furthermore, the specificity of IGRAs for LTBI (as determined in studies of individuals at low risk of TB infection) is much greater than the TST, particularly in individuals with prior BCG vaccination.

FIND's evaluation studies included a large study of 8,000 TB patients and household contacts followed over a 3-year period in Zambia and South Africa (ZAMSTAR), paediatric contact studies in Johannesburg and Cape Town, evaluation of the QFT-IT in screening young children with suspected TB in South Africa and India, a longitudinal study of HIV-infected persons in South Africa, and a study in Vietnam of adults and children screened for TB prior to immigration into the United States.

Key accomplishments

- All of the FIND-supported studies, excepting the ZAMSTAR study and the paediatric contact study were completed in 2010.
- The two studies evaluating the QFT-IT test in over 1,000 young children evaluated for suspected TB in India

and South Africa found that the test was not useful as a diagnostic aid. The longitudinal study of over 1,000 HIV-infected adults in South Africa screened for TB prior to entry into a study for anti-retroviral therapy and isoniazid preventative therapy (IPT) found that a single QFT-IT test did not improve the ability of current tools to discriminate between individuals with culture positive TB disease and those who were culture negative.

- The study in Vietnam of persons being screened for TB prior to immigration into the United States found that the QFT was as sensitive as TST in the detection of TB, with greater specificity for LTBI. Preliminary results of the ZAMSTAR study suggest a four-fold higher correlation between the QFT and the probability of active TB compared with the TST, although the rate of TB among QFT-IT-positive persons (39/1,000) -does not suggest that the test would be useful for selecting persons for IPT.
- In September 2010, FIND submitted a comprehensive report on its QFT-IT studies to the WHO for review by an Expert Group considering recommendations on the use of IGRAs in low- and middle-income settings. This group also reviewed detailed meta-analyses of studies of IGRAs in various risk groups. The group concluded that there was insufficient evidence to support a recommendation on the use of IGRAs in these settings. This conclusion was then endorsed by WHO STAG-TB, a body within the organization that advises on tuberculosis policy and practice.

China laboratory strengthening

- Partners: PATH (USA and China), Hong Kong Tuberculosis Reference Laboratory (Hong Kong)
- FIND project leader and/or coordinator: Rick O'Brien
- Project start: May 2009
- Resume of project: Demonstration projects of three FIND technologies to guide decisions of the China Ministry of Health concerning scale-up.

Description

FIND, with assistance from PATH/China and the Hong Kong TB Reference Laboratory (TRL), is providing technical assistance and diagnostic supplies and instruments to the China National TB Reference Laboratory (NTRL) for demonstration projects. These form one component of a larger project funded by the Bill and Melinda Gates Foundation to improve TB control in China. FIND's two and a half year project was intended to demonstrate Zeiss iLED fluorescence microscopy in six microscopy centres in three provinces, the Hain LPA (MTBDRplus) test in four hospitals in four provinces and the Eiken LAMP assay in six microscopy centres in three provinces. Data from the demonstration projects are to guide subsequent decisions by the Ministry of Health on the scale-up of these technologies.

For this project, a four-part MOU (FIND, PATH, Hong Kong TRL, and China CDC/NTRL) was signed in May 2009. A project management team and an oversight committee were formed, and detailed milestones were prepared for the three demonstration projects.

Key accomplishments

- The iLED demonstration projects were completed in late 2010. The results indicated the expected gain in sensitivity for detecting AFB smear-positive cases (13%) and decreased reading time (about 50%). The total laboratory costs of iLED were slightly lower than for conventional ZN light microscopy.
- The validation phase of the LPA project was completed with acceptable performance, although 2 of the 4 sites experienced significant amplicon contamination that took some time to correct. However, because of a significant and unexpected delay in State Food and Drug Administration registration of the LPA in China, the

project management team decided to substitute a locally produced molecular assay for TB and rifampicin/isoniazid resistance detection (the GeneChip assay from CapitalBio) at the four project sites. Instrument installation and initial laboratory technician training on the assay took place in December 2010.

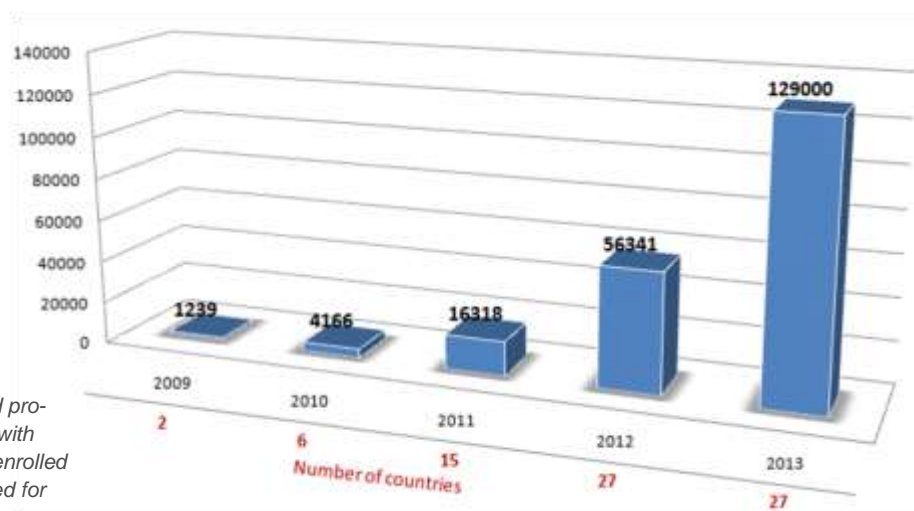
- Culture capacity was established and validated at the six peripheral laboratories where the LAMP assay was to be demonstrated. However, because it is not expected that this assay will be registered by SFDA for use in China before the end of 2011, it was decided in late 2010 to validate the assay in only two project sites in order to gain some experience with the use of this test. The Cepheid Xpert MTB/RIF system is to be validated in the other four project sites. All of the FIND-supported studies, excepting the ZAMSTAR study and the paediatric contact study were completed in 2010.

EXPAND-TB

- Partners: UNITAID, WHO, Stop TB Partnership's Global Laboratory Initiative (GLI) and Global Drug Facility (GDF)
- FIND project leader and/or coordinator: Eric Adam
- Project start: January 2009
- Resume of project: Expanding access to new diagnostic technologies; establishing appropriate laboratory settings and knowledge-base at a country level.

Description

Lack of diagnostic capacity is a critical barrier to the scale-up of diagnosis, treatment and control needed for an effective response to the dramatic increase in multi-drug resistant tuberculosis (MDR-TB), which has been further complicated by the convergence of the TB and HIV epidemics. In 2008 and 2009, the UNITAID Board approved a five year project covering in total 27 countries including India, aiming to expand access to new diagnostics for patients at risk of multi-drug resistant tuberculosis (MDR-TB) and coordinated by WHO and GLI in collaboration with FIND and GDF. The EXPAND-TB Project aims to address one of the key obstacles to the scale-up of MDR-TB care, by accelerating access to new and rapid diagnostic technologies within appropriate laboratory services at country level, accompanied by the necessary know-how for technology transfer, and ensuring that these new technologies are properly integrated within TB control programmes.



MDR-TB cases detected and projected to be detected, along with number of countries having enrolled MDR-TB patients and planned for enrollment until 2013.

FIND's role

FIND is the main implementing agency and responsible for project management, including support for procurement and logistics, according to ISO 13485 and 9001 certified project management standards. As part of technology transfer to countries, FIND shares manuals, guidelines and operating procedures that have been developed to assist with the introduction of new diagnostics and their proper use.

Key accomplishments

- Since the inception of the project in 2009, initial visits, laboratory need assessments and mapping of partners at country level were undertaken in 22 countries overall.
- Political commitment was confirmed by signature of agreements with national health authorities in 18 countries overall, and agreements are under review in another 3 countries (Belarus, Tanzania and Vietnam).
- EXPAND-TB is fully "owned" by the Ministries of Health of the recipient countries and works on a model of best-practices, learning-by-doing and optimizing resources for laboratory strengthening at country level. In 2010, 6 countries in total out of 27 have enrolled MDR-TB patients. The details of these 6 country projects totalling 4,166 MDR-TB cases are detailed below.

Country: India

FIND's focus on India as a priority high burden TB country for facilitating access to new and improved tools for the diagnosis of TB, MDR-TB and drug-resistant TB (DR-TB) is starting to bear fruit. Since 2008, FIND has provided expertise and support to the Indian Revised National TB Control Programme (RNTCP) through laboratory strengthening, including the implementation of the WHO-endorsed, FIND co-developed diagnostic tools. This was done in close coordination with the WHO and other international partners, such as PATH, the Union and the Clinton Health Access Initiative (CHAI). In particular, over the course of 2010, India's Central TB Division consulted FIND extensively in the development of a strategy to expand culture and DST services which meet the requirements of the Indian National Response Plan to drug-resistant TB. Over the course of 2010, the FIND India office increased its technical, financial, HR, and support staff capacity to better support the Expand-TB and Global Fund projects.

The Expand-TB Memorandum of Understanding with the Ministry of Health's National TB Programme was signed with a provision to strengthen 40 LPA laboratories and 30 liquid culture laboratories through 2014, including both state and national level laboratories.

To accelerate the introduction and scaling up of newer technologies in the identified laboratories, a proposal was submitted by FIND India and the EXPAND-TB team to the Ministry of Health to establish a specialised training facility. Approval was received, and the International Center for Excellence in Laboratory Training (ICELT) was set up at the National TB Institute, Bangalore, which is also one of the National Reference laboratories in India. The construction of ICELT was completed in a 6-month time span and a formal inauguration has been planned for January 2011. A visit was made to ICELT by the French Cour des Comptes when the first batch of LPA trainings was in progress, within the context of the French Government's review of UNITAID activities in India. In addition the UNITAID Executive Secretary and FIND's CEO visited EXPAND-TB support sites to review the UNITAID-funded projects.

In 2010, a total of 26 culture & drug susceptibility testing (DST) sites were assessed by the FIND India team. It is expected that at least 9 of these sites will be using LPA for patient care activities by the end of 1st Quarter 2011. The Central TB Division was assisted by state level TB officers, WHO and EXPAND-TB partners in preparing a plan of action for the scale-up of diagnosis and treatment of MDR-TB cases. As per the government's instructions, layouts of five sites were provided for the establishment of LPA lab infrastructure by PATH. As of

December 2010, 1,792 LPA results were available for the programme, 740 patients were identified as MDR-TB cases and 480 patients from 7 sites were started on treatment with second line drugs.

Country: Myanmar

The equipment installation at the National TB Reference Laboratory in Yangon and the regional laboratory in Mandalay was successfully completed at the end of January through collaboration with the WHO country office and the Ministry of Health.. The on-site FIND EXPAND-TB consultant took an active role in coordinating the activities and facilitating the visits of the contracting company's engineers. The laboratory areas were refurbished for the installation of bio-safety and ventilation equipment. A hands-on training programme on liquid culture and species identification for the diagnosis of TB and MDR-TB was organized by FIND and CDC at the National TB Reference Laboratory in Bangkok. BD (Becton Dickinson and Company) provided technical support and the reagents and the LPA training material developed by FIND was used for the training. Negative pressure and other bio-safety measures are currently in place both at Yangon and Mandalay laboratory facilities. These state of the art laboratory facilities were formally dedicated by the Minister of Health, Government of Myanmar in July 2010.

90 MDR-TB cases have been reported since then. Until these systems were put in place, MDR-TB suspects were hospitalized and treatments were given only after having results of solid culture.



In August 2010 took place the inauguration ceremony of the refurbished NTRL housed at the Aung San TB Hospital Compound in Yangon.

Country: Lesotho

To date, the laboratory continues with enrolment for the external quality assurance programmes, with SNRL-MRC, Pretoria, and NHLS, Johannesburg. The molecular LPA for detection of MDR-TB is an ongoing success story thanks to the active participation of clinicians at the MDR-TB hospital, Bots'belo. Plans for offering the LPA services at two regional laboratories at Leribe and Mafeteng are being pursued. Early infant diagnosis (EID) of HIV DNA from dried blood samples was introduced in the lab to have an integrated molecular platform for TB and HIV.

The intra-laboratory validation has shown 100% proficiency in performing the assay among laboratory technologists. In March 2010, an external quality assurance programme was introduced through the Global AIDS Program (GAP-CDC), with the support from CDC in Lesotho. The programme consists of global proficiency testing by GAP and is conducted quarterly.

Around 87 laboratories participate in the Proficiency Testing globally and the Lesotho laboratory has scored 100% concordance with the expected test results in 2 consecutive quarters. At present, the laboratory is conducting testing for the district of Maseru. A scale-up plan has been drawn by the laboratory services to cover all the districts of the country in a phased manner, taking over the testing from the National Institute of Communicable Diseases (NICD) in Johannesburg, where the testing is presently being done for other districts. The in-country testing is designed to be cost-effective and would provide results to patients within one week, thus facilitating early initiation of treatment. The supplies and logistics required for expansion of the services to the entire country have been worked out with support from the Clinton Health Access Initiative (CHAI) in Lesotho and NICD. FIND and the Global Fund's Round 8 have provisions for additional funds for kits and consumables.

158 MDR-TB cases were reported in 2010 under the EXPAND-TB project, and 273 cases overall since the inception of the project in 2009. This represents an excess of MDR-TB patient enrolment versus the initial target of detecting 176 MDR-TB patients over the course of the project.

Country: Ethiopia

Regional laboratory constructions were completed in Adama, Bahir Dar, Mekele and Awasa, but were pending in Jima and Harar laboratories. Furthermore, the training of technicians from new regional laboratories was initiated.

At the Ethiopian Health and Nutrition Research Institute's laboratory, the implementation of new diagnostic methods has progressed well. To date, there are six well trained technicians working in the lab under the laboratory supervisor. Specimens are currently processed daily as the National TB Prevalence Survey is also ongoing. This daily processing has reduced the contamination rate to acceptable levels. Since 2009, 318 MDR-TB cases were detected at EHNRI, by either Löwenstein-Jensen (LJ), liquid culture or LPA DST.

At the St. Peter's Hospital, a total of 125 MDR cases were detected since project inception. The TB laboratory has moved to a new site, which was assessed by the EHNRI along with Johns Hopkins University (USA) and FIND. Presently, 4 trained technologists are working the laboratory, and it is processing 28 samples per day as the other hospitals in Addis Ababa have also started sending samples. Negative pressure was installed in 5 regional laboratories and procurement of basic equipment and supplies is on-going with the help of CDC Ethiopia and university partners.

Country: Uzbekistan

An initial replenishment order of commodities for Uzbekistan has been prepared early in 2010, including equipments and reagents for the National Reference Laboratory in Tashkent, which is collaborating with the

Supranational Reference Lab in Gauting, Germany. The regional setting in Samarkand was prepared and the German development bank KfW, provided most of the equipment for the upgrade of the laboratory. WHO recommended that bio-safety measures be implemented by EXPAND-TB. The necessary equipment needed to certify bio-safety cabinets (BSCs) was delivered by KfW, and 3 local engineers identified by the Ministry of Health were trained to use it. The training included theory, mainly focused on airflow velocities in different parts of BSCs of different types, principles of construction, differences and advantages of each type. The setting was assessed and measures of the laboratory rooms involved were taken to plan the installation of negative air pressure.

In total, 961 MDR-TB patients were identified in 2009, and an additional 1,548 cases in 2010. This represents already 33% of the target number of MDR-TB cases to be detected under the EXPAND-TB project.

Country: Uganda

In August 2010, a Memorandum of Understanding was signed between FIND and the Government of Uganda in support of the EXPAND-TB project implementation. An initial visit was organized by the project team to assess the existing NTRL building, as well as the building under renovation to enable use of liquid culture. The project team also met with the relevant partners working on TB in the country (CDC, AMREF, IMF, FIND, NTRL, and WHO among others) to present the project scope. The implementation of new diagnostic technologies under EXPAND-TB should take place at the national TB reference laboratory for liquid culture and LPA, and at Mulago hospital, as part of the NTRL complex, for the LPA only. The LPA activities carried out at present by the NLTP at the FIND research lab will be relocated to this new facility in due course.

All the partners working on TB laboratory strengthening in Uganda now have a common plan and are coordinated by a well specified unit to avoid any overlap. Qualified human resources are available to apply the new diagnostic technologies in the laboratories where they will be implemented. All the patients at risk of developing MDR-TB benefit from early and free of charge diagnosis and treatment. The NTRL meets and sustains bio-safety requirements according to international norms and standards. The diagnostic algorithm using new and rapid diagnostic technologies for MDR-TB are integrated in the global strategy of the National TB Programme plan. 110 MDR-TB cases were already reported in 2010 under the EXPAND-TB project representing 17% of the project target.

Contributing to elimination through improved tests



Photo: WHO

Human African Trypanosomiasis (HAT) Programme

Human African trypanosomiasis (HAT) or sleeping sickness is a deadly, neglected tropical disease that affects impoverished rural communities in sub-Saharan Africa. It is transmitted by the bite of the tsetse fly and about 60 million people in 36 countries are thought to be at risk. There are no clinical signs that are characteristic of the disease, which makes it difficult to diagnose. If infected people are not treated, they eventually die. The diagnosis of sleeping sickness currently relies heavily on trained health workers using a screening test to identify suspects, and then using microscopes to detect parasites in the blood and other body fluids.

If not diagnosed and treated early, sleeping sickness inexorably progresses to a stage where the parasites enter the central nervous system, making treatment more difficult and the likelihood of irreversible neurological damage more likely. Early diagnosis is therefore critical and will help avoid exposing patients to dangerous and expensive drugs.

FIND has been working with partners to develop diagnostic tests for HAT that are cheap, easy to use, sensitive and specific enough not only to accurately detect cases of sleeping sickness but also to determine the stage of disease, confirm cure after treatment, and detect relapses after a failed treatment.

Key accomplishments

- Phase 1 development of LAMP for HAT was completed;
- Multi-site field evaluation of LED fluorescence microscopy was started;
- Lead candidate antigens for detection of an immune response were selected and two prototypes of a lateral-flow rapid test that could be included in large-scale screening programmes were developed;
- Biomarkers were identified in cerebrospinal fluid presenting great potential for identification of HAT patients in second stage and for monitoring of treatment.

HAT LAMP

- Partners: Eiken Chemical Company (Japan), Makerere University (Uganda), Institute of Primate Research (IPR, Kenya), Murdoch University (Australia), Obihiro University (Japan), World Health Organization (WHO), Biomedical Proteomics Research Group (BPRG, Switzerland)
- FIND project leader and/or coordinator: Joseph Ndung'u
- Project start: July 2006
- Resume of project: Investigation of potential use of LAMP for the diagnosis of HAT and confirmation of cure after treatment.

Description

The parasitological tests in use for the diagnosis of HAT have low sensitivity, and current serologic tests have inadequate specificity. Detection of trypanosomal DNA in a patient's blood, urine or saliva could be a significant improvement on parasitological examination.

Loop-mediated isothermal amplification (LAMP) of DNA is a promising molecular technique that shows high sensitivity and specificity and amplifies target DNA under isothermal conditions, which means that the test can be carried out with minimal equipment. Furthermore, positive samples are identified visually by fluorescence. LAMP can be performed by individuals with minimal experience in molecular biology. This test may also be useful for confirming cure in treatment follow-up.

Key accomplishments

- In 2008, FIND signed an agreement with Eiken Chemical Company Ltd for the development of a LAMP test for HAT. Phase 1 development was completed in early 2010. The test detects trypanosomes belonging to the sub-genus *Trypanozoon* (*Trypanosoma brucei gambiense*, *T.b. rhodesiense* and *T.b. brucei*), *T. evansi* (which causes disease in camels) and *T. equipardum* (which causes disease in horses).
- The optimization of sample preparation methods for HAT LAMP has been carried out at Makerere University using experimentally infected rodents. The sensitivity of LAMP is significantly improved by combining the analysis of red blood cells in samples followed by centrifugation, then LAMP.



Reading LAMP results with an LED light source.

- Studies using blood samples collected on FTA classic cards have revealed that this method of sample collection could be very useful in the remote rural areas where the disease occurs.
- FIND has also partnered with the BPRG and the Institute of Primate Research to explore the feasibility of using LAMP as a test of cure, and for predicting relapses when treatment is not successful. After treatment is completed, patients are followed up for a period of 24 months to confirm that they have been cured.

Advocacy

The project has been publicized through a programme of advocacy for HAT, being implemented in 11 African countries that are endemic for HAT, in collaboration with the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Collaboration with WHO has provided the linkage that is necessary for the eventual introduction of the test in the diagnostic algorithm of HAT, and adoption into global policy. The head of the programme has made a series of presentations on this and related projects at international fora.

HAT staging markers

- Partners: Biomedical Proteomics Research Group (BPRG - Switzerland), Makerere University (Uganda), Institute of Primate Research (IPR, Kenya), World Health Organization (WHO), Institute of Tropical Medicine (ITM, Belgium), Institut de Neurologie Tropicale (INT, France), Karolinska Institutet (Sweden), Institut National de Recherche Biomedicale (INRB, D.R. Congo), Instituto de Combate e Controlo das Tripanossomíases (ICCT, Angola) and St. George's, University of London (UK)
- FIND project leader and/or coordinator: Joseph Ndung'u
- Project start: December 2007
- Resume of project: the development of less invasive and more accurate tests for the staging of HAT, both before selection of appropriate treatment, and after treatment to assess level of success.

Description

Diagnosis of HAT involves case detection followed by staging, a procedure that is crucial in guiding decisions on the most appropriate treatment. In Stage 1 HAT, the infection is confined to the haemo-lymphatic system. In Stage 2, the parasites have penetrated the central nervous system (CNS) and, depending on the duration of the infection, the CNS will have been damaged to varying degrees. To distinguish between the two stages, a lumbar puncture has to be performed. The cerebrospinal fluid (CSF) is examined for the presence of parasites, and white blood cells (WBC) are counted. These parameters suffer from insufficient sensitivity and, in the case of WBC count, of specificity as well. Moreover, the two diagnostic approaches are hampered by technical problems, including controversy over cut-off values. Due to these shortcomings, the invasive character of lumbar puncture and toxicity of drugs used to treat the Stage 2, improved markers for staging are desperately needed. Furthermore, after treatment is completed, patients are followed up for a period of 24 months to confirm that they have been cured. Since relapses are mainly of CNS origin, and parasites are often difficult to find in blood, follow-up mainly relies on multiple lumbar punctures and examination of CSF.

FIND is working with partners to determine the feasibility of developing new tests, preferably in a lateral flow format, for the staging of HAT with improved accuracy to guide treatment, and for follow-up to determine treatment success. Special attention is given to speed, simplicity, cost and reliability, as well as reduced invasiveness. The ultimate goal of the project is to develop a dual purpose test that will be used for staging, and to monitor the effectiveness of treatment. The lateral flow test will be accurate and specific, and simple enough to be used without the need for any instrument.



Collecting spinal fluid from a HAT patient.

Key accomplishments

- The CSF concentration of ICAM-1, VCAM-1, IgM, Neopterin, MMP-9, B2MG, CXCL13 and CXCL10 was determined in 600 HAT patients (243 stage 1 and 257 stage 2) from Angola, Chad, the Democratic Republic of Congo (DRC), Malawi and Uganda, who were infected by either *Trypanosoma brucei gambiense* or *T. b. rhodesiense*. Classification of patients as Stage 2 was based on the number of WBC in CSF (> 5 WBC/ μ L) and/or presence of parasites. Ninety eight *T. b. gambiense* patients from the DRC who were followed for two years after treatment were included. Statistical analyses were used to determine the ability of biomarkers to identify a patient's stage of disease and to predict relapses after treatment.
- All the 8 biomarkers were found to be good at differentiating between the two stages of the disease, with high sensitivity and specificity. Three of them can also confirm cure or identify cases of treatment failure less than 6 months after treatment. This has created the opportunity for the development of an RDT with the dual purpose of staging HAT and monitoring the efficacy of treatment.

Advocacy

The project has been publicized through a programme of advocacy for HAT, being implemented in 11 African countries that are endemic for HAT, in collaboration with the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Collaboration with WHO has provided the linkage that is necessary for the eventual introduction of the test in the diagnostic algorithm of HAT, and adoption into global policy. The head of the programme has made a series of presentations on this and related projects at international fora.

HAT advocacy

- Partners: Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) office of the African Union Commission (AUC, Ethiopia)
- FIND project leader and/or coordinator: Joseph Ndung'u
- Project start: November 2007
- Resume of project: Promoting awareness of HAT at the level of governments, communities and medical personnel.

Description

FIND is working with the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) office of the African Union Commission (AU) on a programme of advocacy for African trypanosomiasis, aimed at prioritizing surveillance and control of the disease, and strengthening the capacity of countries to map HAT.

The project involves:

- a. encouraging countries to allocate funds in their national budgets for surveillance and control of the disease,
- b. improving awareness of the disease at the level of medical personnel,
- c. increasing awareness of the disease at the level of the communities that are at risk of infection.

Activities supported by this project can help fulfil both country-specific and regional/global advocacy needs.

Eleven countries that are endemic for HAT have been implementing country-specific work plans through this project, guided by a Strategic Plan on Advocacy for HAT for the 2008-2011 period. In addition, the epidemiological data collected in the countries are being regularly shared with the PATTEC Coordination Office in Addis Ababa, where they have been used to build a centralized database which will be necessary for further regional activities.

Key accomplishments

Implementation of country-specific work plans continued throughout 2010, culminating in the 4th Annual workshop of contact persons in November 2010, in Abuja, Nigeria, to review progress, and agree on strategies for 2011. The countries acknowledged that they fall into three groups:

- One group comprises countries where few to no cases of HAT have been reported over the past 2 to 3 years, even with reasonable surveillance. It is likely that the disease has largely been eliminated from these countries, and further advocacy activities will only be meaningful if supplemented by a strategy for confirming and sustaining elimination.
- The second group includes countries where surveillance and control activities have resulted in a sustained fall in the number of cases reported per year. Advocacy activities in these countries have resulted in a commitment by their governments to sustain the efforts, with a focus on elimination of the disease. The workshop participants agreed that these countries should be assisted in developing specific strategies for elimination of the disease.

- The third group either continues to report large numbers of cases of HAT, or the magnitude of the disease is still unclear. These countries will require intensified advocacy, surveillance and control activities, which will need to be sustained before they can start considering elimination strategies.

LED fluorescence microscopy

- Partners: Institut National de Recherche Biomédicale (D.R. Congo), University of Kinshasa (D.R. Congo), National Livestock Resources Research Institute (Uganda), Makerere University (Uganda), Lwala Hospital (Uganda), Swiss Tropical Institute (Switzerland)
- FIND project leader and/or coordinator: Sylvain Biéler
- Project start: June 2006
- Resume of project: Evaluation of LED fluorescence microscopy using blood smears stained with acridine orange, including sample preparation method to concentrate trypanosomes by lysis of red blood cells and centrifugation.

Description

Confirmatory diagnosis of human and animal African trypanosomiasis relies on the demonstration of trypanosome presence in body fluids by bright field microscopy, which is not very sensitive because the concentration of parasites is usually low. Recent advances in light-emitting diode (LED) technology have enabled the development of cheap fluorescence microscopes that can be used without a dark room, which means that these could be used in remote rural areas where African trypanosomiasis occurs. The goal of this project is to improve the sensitivity and ease of HAT diagnosis by combining LED fluorescence microscopy with a procedure of red blood cell (RBC) lysis, parasite concentration and staining with acridine orange.

Key accomplishments

- Laboratory studies to evaluate the possibility of combining LED fluorescence microscopy with lysis of RBC to improve the sensitivity and speed of detecting trypanosomes were conducted at four centres in Uganda and in the Democratic Republic of the Congo. Detection of parasites was significantly improved by RBC lysis and concentration. The best results were obtained with thin smears prepared using 20 µl of sediment and stained with acridine orange. The time taken to see the first parasite was dramatically reduced when smears were examined by LED fluorescence microscopy, compared to bright light microscopy. In addition, LED fluorescence microscopy was found to be easier and more relaxing on the eye than bright field microscopy. These studies demonstrated an incremental improvement in diagnosis of African trypanosomiasis by combining LED fluorescence microscopy with RBC lysis and concentration.
- Clinical trials to evaluate the performance of LED fluorescence microscopy combined with the RBC lysis and concentration method have been initiated in 3 centres in DRC and in one centre in Uganda.



Improved detection of trypanosomes with the iLED fluorescence microscope.

- Studies to evaluate the possibility of using acridine orange LED fluorescence microscopy to improve the detection of live trypanosomes from infected tsetse flies and from HAT patient lymph node aspirates and buffy coats have been initiated.
- The Primo Star iLED fluorescence microscope that was co-developed by Carl Zeiss and FIND was successfully set up to operate on solar energy in several laboratories in endemic countries.

Advocacy

A scientific meeting was held with various stakeholders (WHO, MSF, Swiss-TPH, ITM-Antwerp) in February 2010 to define the design of the iLED microscopy field trials. Regular communications have taken place at various levels (national control programmes, governments, local administrations, health workers, other organizations) within 11 endemic countries through the HAT Advocacy project. FIND staff have presented the project at various conferences and meetings.

HAT antibody detection

- Partners: Standard Diagnostics Inc. (South Korea), International Livestock Research Institute (ILRI, Kenya), Institute of Tropical Medicine (ITM, Belgium), Cambridge University Enterprises (UK), World Health Organization (WHO), Institut de Neurologie Tropicale (INT, France)
- FIND project leader and/or coordinator: Joseph Ndung'u
- Project start: May 2006
- Resume of project: Research into, and development of, a rapid diagnostic test for HAT.

Description

Parasitological examination for HAT is only carried out on individuals in at-risk populations who demonstrate anti-trypanosome antibodies with the card agglutination test for trypanosomiasis (CATT), the primary screening tool used by control programmes in areas where *Trypanosoma brucei gambiense* is endemic. The specificity of the CATT test is good, but in situations where the prevalence of disease is low, a large number of positive results are false-positive. The CATT comes in doses of 50 tests and, once reconstituted, it has to be refrigerated. The test is not manufactured under IVD-GMP, and involves exposing health workers to human-infective parasites. Furthermore, there is currently no screening test available for *T.b. rhodesiense* HAT.

This project aims to replace the CATT test with a simpler, more sensitive and specific rapid diagnostic test (RDT) for *T.b. gambiense* HAT. A simple, instrument-free, single format test will be very useful for screening people in remote rural areas, and in health posts where the disease occurs. Inclusion of the test in surveillance programmes will also accelerate the process towards elimination of the disease. The ultimate goal is an RDT that will be effective for both forms of HAT.

Key accomplishments

- Seven antigens with potential for diagnosis of HAT were selected through a process that involved screening panels of sera from HAT positive and negative individuals.
- Standard Diagnostics and FIND are co-developing a 1st generation RDT that is specific for *T.b. gambiense*.
- Two prototype RDTs, each with one antigen, were compared to CATT using stored plasma. Indications are that their performance could be as good as CATT.
- Field studies to compare the performance of a prototype RDT that has both antigens on the same strip, using fresh blood samples, will be carried out in 2011.

Advocacy

The project has been publicized through a programme of advocacy for HAT, being implemented in 11 African countries that are endemic for HAT, in collaboration with the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). Collaboration with WHO has provided the linkage that is necessary for the eventual introduction of the test in the diagnostic algorithm of HAT, and adoption into global policy. The head of the programme has made a series of presentations on this and related projects at international fora.

Developing new tools and assuring quality of existing rapid tests



Photo: FIND/D. Bell

Malaria Programme

FIND has been actively engaged with WHO and other partners in advocating for improvements in malaria management, in particular for good practice in diagnostics and increased research and development in these areas. Through projects such as malERA, we have been working with the malaria community to identify priorities for tool and strategy development in malaria diagnostics. We have also been working with key partners to make specific guidance and tools available to national programmes.

The development of rapid diagnostic tests (RDTs) has been an important advance in the point-of-care diagnosis of malaria. Although in use for more than 10 years, their actual impact on malaria control has been impeded due to variations in their sensitivity and thermostability and, in consequence, a lack of confidence in their results. FIND is working with several partners to develop improved quality control for malaria RDTs, identify and apply of technical solutions to overcome obstacles to the implementation, and looking to the next generation of malaria diagnostic devices. The cornerstone of FIND's work on RDTs has been the development, with WHO, CDC and others, of a global evaluation programme. Through the establishment of this land-mark global product-testing and lot-testing programme for point-of-care tests, essential information is now available to guide RDT procurement, and performance of individual purchases can be assured before use in the field. While these evaluations are on-going, emphasis is shifting to development of new streamlined evaluation methods that will allow long-term sustainability and the adoption of common performance standards for national programmes, procurement agencies and industry.

Further solutions to on-the-ground implementation problems include (i) development of a comprehensive malaria RDT implementation guide, (ii) development of a transport and storage guide for personnel handling RDTs in storage and at health clinics; (iii) continued development of a "cool box" which that protects RDTs and drugs from extremes of temperature without the need for electricity; and (iv) development of a repository of product-specific job aids and training materials for RDTs.

As malaria transmission declines in many areas and elimination is considered, new methods and strategies for malaria diagnosis are becoming necessary, and emphasis is also shifting to other infections causing fever and mortality. FIND is working with partners to develop field-ready molecular diagnostics and to identify priorities and methods for diagnosis of other febrile diseases to meet these new challenges.

Key accomplishments

- Detailed comparative performance data over 100 commercially-available RDTs published, and over 40 more currently under analysis.
- Maintenance of lot-testing capacity to ensure adequate quality of RDTs at procurement.
- Development of an accurate, field-capable molecular diagnostic assay (LAMP) for malaria. Development of prototype positive control wells towards the establishment of capacity for point-of-care quality control for malaria RDTs.
- Development of evidence-based job-aids and training materials to improve blood safety and RDT accuracy in the field.
- Demonstration of effective, low-cost electronic reporting of malaria diagnosis from remote locations
- Development through evaluation stage of a new blood-transfer device to improve blood safety and accuracy of RDT preparation.

RDT evaluations

- Partners: WHO/TDR; Centers for Disease Control and Prevention (CDC, USA), Hospital for Tropical Diseases (HTD, UK), Research Institute for Tropical Medicine (RITM, Philippines), Institut Pasteur Cambodia (IPC, Cambodia), WHO Global Malaria Program (WHO/GMP), Army Malaria Institute (AMI, Australia), Ethiopian Health and Nutrition Research Institute (EHNRI, Ethiopia), Institut Pasteur Madagascar (IPM, Madagascar), Institut Pasteur in Bangui (IPB, Central African Republic), Ifakara Health Research and Development Centre (IHRDC, Tanzania), University of Lagos (Nigeria), Kenya Medical Research Institute (KEMRI, Kenya), Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia (UPCH, Peru), Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM, Colombia), Department of Medical Research (Myanmar), Médecins Sans Frontières (MSF, Netherlands)
- FIND project leader and/or coordinator: David Bell
- Project start: 2007
- Resume of project: Detailed comparative performance data on 70 commercially-available RDTs published, and over 50 under analysis. Establishment of lot-testing capacity to ensure adequate quality of RDTs at procurement.

Description

WHO now recommends parasite-based diagnosis in all cases prior to treatment for malaria. Successful implementation of this policy, and effective management of malaria programmes, depends in particular on effective quality control of malaria rapid diagnostic (point-of-care) tests, and accurate data on their performance. The new diagnostics quality assurance policy of the Global Fund, now requiring strict criteria for RDT procurement and recommending lot-testing to confirm quality prior to use, has further raised the importance of this evaluation programme for malaria control globally.

Based on a global specimen bank gathered through a partnership with research institutions in three continents, the global product testing programme continues at CDC, funded predominantly through FIND and conducted in close partnership with WHO/TDR, WHO/GMP and others. The outcomes form the basis for WHO global procurement recommendations as well as, more recently, the basis for procurement and quality control requirements for RDTs procured through Global Fund support.

Current RDT evaluations are performed against reference panels - consisting of highly-characterized wild-type (patient-derived) mono-species parasite samples at specific dilutions and collected from a range of geographical locations - culture-derived panels and parasite-negative panels. The global bank and smaller lot-testing banks are replenished periodically. The collection and evaluation processes are governed by extensive SOPs and overseen by a Steering Committee.

FIND and partners have been actively laying the groundwork for new methods for RDT evaluations over the past few years, involving the selection and testing of recombinant antigens and, where necessary, the synthesis of new antigens. A large bank of recombinant antigens has been evaluated for stability, purity and ease of expression and detection characteristics. Selected candidate antigens are undergoing equivalence testing against the existing parasite panels.

These antigens are being used to develop positive control wells to check RDT quality at the village level (described below), but are also the basis of panels that will allow for the standardization of lot-testing from manufacture to country of use, and put the capacity to perform such lot-testing in the hands of national programmes and regulatory bodies. They will also streamline the global product-testing programme, enabling it to be more readily transformed into a self-funding mechanism, sustainable without large donor support in the

long-term. FIND has developed a 5 year business plan outlining and budgeting the process for development, demonstration and roll-out of these panels, currently under review by strategic partners.

Key accomplishments

- To date, the product testing programme has completed and published detailed evaluations on 70 products, and evaluations of a further 50 are under analysis. A 4th round of testing will commence mid-2011, with a further increase in interest from industry demonstrated in the responses to the Expression of Interest request.
- The lot-testing programme, based currently at RITM in the Philippines and IPC in Cambodia, has had a steadily increasing workload with 232 production lots tested for a range of procurement agencies and national programmes in 2010 alone. The new Global Fund recommendation for lot-testing of RDTs prior to use is expected to increase demand further.
- The constitution of the Global Malaria Specimen Bank and its characterization has led to new insights into the properties of malaria target antigens and their impact on parasite detection. In particular, the lack of HRP2 expression in the Amazon, the subject of further field investigation by FIND and partners, will have important implications for malaria diagnosis on that continent. Previous work provided important knowledge on antigen structural variation, critical to understanding and planning RDT performance assessments and predicting sensitivity in the field.



Preparation of samples for RDT quality control in Senegal

Advocacy

- The methods and outcomes of the RDT Product Testing and Lot Testing, as well as a guide for assisting in RDT procurement choices are available online on the FIND, WHO and WHO-WPRO websites. Annual reports of the RDT Product Testing and brochures of the RDT Lot Testing have been widely distributed.
- Outcomes of malaria antigen research studies have been published, and the lack of HRP2 expression in *P. falciparum* has been communicated to PAHO and Ministries of Health through country-specific meetings.
- Product testing results, and lot-testing results, are published in collaboration with WHO.
- Results, and the evaluation process, have been presented at numerous meetings and conferences on malaria over the past few years.

Evaluation of RDTs in pregnancy

- Partners: WHO/TDR co-funded this study. Institut de Recherche en Sciences de la Santé (Burkina Faso), Makerere University, Faculty of Medicine (Uganda), College of Medicine, University of Lagos (Nigeria), Centers for Disease Control and Prevention (CDC, USA), University of Washington (USA), Malaria Control Programme, Uganda Ministry of Health (Uganda)
- FIND project leader and/or coordinator: David Bell
- Project start: 2010
- Resume of project: Assessment of malaria RDTs in detecting placental malaria.

Description

While the use of RDTs in routine clinical management is now well-established, their utility in screening for malaria in pregnancy remains one of the major gaps in diagnostic knowledge. Pregnant women are at particular risk of malaria infection, which can lead to significant negative effects on the mother's health and on fetal development. As resistance to the anti-malarial drug sulphadoxine-pyrimethamine (SP) becomes more widespread, the current policy of intermittent prophylactic treatment in pregnancy (IPTp) in Africa will become less effective. To replace this with the use of more effective drugs, it will be necessary, for safety reasons, to first demonstrate parasitaemia. Preliminary evidence suggests that detection of circulating parasite antigens by RDTs may give a reliable indicator of placental infections and predict pregnancy outcomes. Therefore, screening with rapid diagnostic tests may offer an accurate and practical way to identify pregnant women who will benefit from targeted therapy for placental malaria infection.

FIND and partners are currently conducting a major multi-centre clinical study to assess the accuracy and utility of malaria RDTs in detecting placental malaria. RDTs detecting both histidine-rich protein 2 (HRP2) and plasmodium lactate dehydrogenase (pLDH) are used for the evaluation, having been initially evaluated at CDC to establish precise thresholds for detection. Women are enrolled at their routine antenatal visits. On the usual antenatal visit schedule, blood is collected from study participants for RDT testing, blood smears are prepared for light microscopy and PCR is conducted. Participants are followed up until delivery, when maternal peripheral and placental blood samples are collected for RDT testing, light microscopy, and PCR, and placental tissue samples are processed for histopathological examination. Measurements of maternal haemoglobin and infant birth weight are obtained. Data collection is expected to be completed in early 2012, with final analysis and results available in mid-2012.

The study is expected to add valuable data to inform future policy for screening and management of malaria infection in pregnancy.

Advocacy

A number of well-respected journals, scientists and public health agencies including the WHO have drawn attention to the urgent need for better methods for prevention and management of malaria in pregnancy. The FIND/TDR study has been designed in conference with other researchers and experts in order to ensure the resulting data are useful and complementary to other studies in this area. In addition, the FIND/TDR study is benefiting from the advice and collaboration of leading experts in laboratory methods related to malaria in pregnancy.

Detection of low-density malaria infections: LAMP for malaria

- Partners: The Hospital for Tropical Diseases (HTD, UK); Eiken Chemical Company Ltd. (Japan)
- FIND project leader and/or coordinator: Iveth J. González
- Project start: 2007
- Resume of project: Development of an accurate, field-capable molecular diagnostic assay (LAMP) for malaria.

Description

Loop-mediated isothermal amplification (LAMP) of DNA is a novel molecular technology, which is highly sensitive and specific, faster than PCR, requires minimal processing and minimal instrumentation and allows result detection with the naked eye. It offers the ability to detect very small quantities of pathogen DNA following minimal tissue sample processing. FIND has been working with Eiken and HTD in the development of a LAMP assay for the diagnosis of malaria. Its characteristics make the LAMP assay a potentially useful tool in the screening and treatment of malaria in the frame of clinical trials, for screening in elimination and very low transmission settings, and as a reference standard against which RDTs and other malaria diagnostics may be evaluated.

The LAMP assay for malaria uses primers targeting different sequences in the mitochondrial DNA of *Plasmodium* parasites to diagnose infection with any of the *Plasmodium* species infecting humans, detecting DNA sequences specific to *P. falciparum* and common to all malaria species. The reaction conditions have been optimized to detect down to 5 parasites per µl of blood in less than 1 hour. Potential samples include fresh or frozen blood, and blood dried on filter paper.

FIND is also working with HTD in the development of LAMP primers for the specific detection of other *Plasmodium* species infecting humans and of high throughput methods for DNA extraction from blood spots on filter paper.

Key accomplishments

- The clinical evaluation of the current LAMP malaria kit started in 2010 with real-time collection and testing of samples from patients with symptoms suggestive of malaria in Uganda (endemic site) and in the United Kingdom (travellers). The purpose of these clinical studies is to determine the performance of the LAMP malaria assay as compared to two reference standards - expert microscopy and PCR - and when using two different simple methods for DNA extraction -the PURE device and a conventional Boil and Spin method requiring sample centrifugation. Preliminary results demonstrate that technicians without molecular training can perform the test in a simple laboratory space without specialized equipment after a short training period. The data collected are currently in analysis and final results will be available during the first half of 2011.

- Primers able to detect *P. vivax* and *P. malariae* at infections over 5 parasites/µl of blood were optimized. The PURE method appears to be the optimal methodology for rapid DNA extraction from filter paper samples for this assay.

Advocacy

Agencies that are currently conducting clinical trials and elimination campaigns have confirmed the potential utility of the LAMP malaria assay and expressed interest in evaluating this tool in their own field projects. However, the analysis of hundreds of samples per day, which would be necessary for these applications, requires the development of a high throughput LAMP assay. FIND is currently exploring the interest of funding agencies in supporting the development and field evaluation for this.

Non-malarial febrile illnesses (NMFI)

- Partners: Microbiology Laboratory of the Mahosot Hospital (Lao PDR), Center of Malariology, Parasitology, and Entomology (Lao PDR), Institut Pasteur Cambodia (Cambodia), National Center for Parasitology, Entomology and Malaria Control (Cambodia), University of Munich (Germany), WHO Western Pacific Region (WPRO), WHO Global Malaria Programme (WHO/GMP), USAID Regional Development Mission for Asia
- FIND project leader and/or coordinator: David Bell / Iveth J. González
- Project start: 2009
- Resume of project: Research and development of tools for diagnosis and management of non-malarial acute fevers.

Description

The current guidelines of the WHO for the treatment of malaria recommend parasitological confirmation of diagnosis in all suspected cases before administration of treatment. With the implementation of improved parasitological diagnosis and the recent increased use of malaria RDTs, it has become apparent that a large proportion of acute fever cases are of non-malaria etiology. However, little is known about the etiology of non-malaria fever in many malaria-endemic regions. Laboratory tools are limited and research in this field is scarce.

A multi-centre study of non-malarial febrile illnesses commenced in 2007 in the Lao PDR and Cambodia with the following primary objectives: 1) to develop the evidence base to guide management of malaria parasite-negative acute fever in malaria-endemic areas; and 2) to assess the need for diagnostic tools applicable in field conditions. FIND joined the project in 2009, specifically providing support for the processing and analysis of pathology samples, as well as for the setting of priorities for the development of point-of-care diagnostics to assist in case management decisions.

This is a cross sectional prospective study to identify the causes of acute fever without any obvious explanation and when negative for malaria by routine malaria tests (microscopy and RDTs). Blood samples from febrile patients from 5 different sites (three in Cambodia and two in Lao PDR) are tested for more than 10 different pathogens and two biomarkers to distinguish between viral and bacterial infection. Throat swabs are also obtained for evaluation of infection with influenza viruses.

Key accomplishments

- FIND has coordinated the last two pathology meetings of the NMFI project (November 2009 in Switzerland and November 2010 in Cambodia) and participated in the Final Review workshop in January 2011 in Lao PDR.

- Samples from almost 3,000 patients have been analyzed and a significant proportion of them (around 50%) remained undiagnosed, suggesting that further pathogens should be tested. Sample analysis is in its late phase and it is expected that final results will be available in May 2011.
- Results of this study are producing new insights on the incidence and epidemiology of malaria and other diseases and are expected to inform management algorithms for febrile illness, prioritization of diagnostic needs. They will also guide the development of further studies on neglected diseases.

Advocacy

Representatives of local Ministries of Health and Malaria Control Programmes, as well as representatives of WPRO, the WHO and some funding agencies (Wellcome Trust, USAID, BMGF, Fondation Mérieux) participated in the Final Review Workshop of the NMFI project in Lao PDR. The general conclusion of this workshop was that additional research activities and protocol refinements are required and that this would only be possible if adequate and sustainable funding is obtained.

Technical improvement of malaria RDTs

- Partners: Royal Tropical Institute (KIT, Netherlands), Queensland Institute of Medical Research (QIMR, Australia); AdAlta Pty Ltd. (Australia), Chembio Diagnostic Systems Inc. (USA), AbD Serotec (Germany), MorphoSys AG (Germany)
- FIND project leader and/or coordinator: Iveth J. González
- Project start: 2007
- Resume of project: identification and application of technical improvements to develop better performing RDTs.

Description

The development of rapid diagnostic tests (RDTs) has been an important advance in the point-of-care diagnosis of malaria. Although in use for more than 10 years, their actual impact on malaria control has been impeded due to variations in their sensitivity and thermostability and, in consequence, the lack of confidence in their results. FIND is working with several partners on the identification and application of technical improvements for better performing RDTs.

The primary objective of this project is to improve the performance of malaria RDTs by identifying novel abundant and stable target antigens and by producing more thermostable detection reagents. FIND and KIT have identified and produced more than 50 specific monoclonal antibodies against the proteins GLURP, DHFR and HDP. Partners at the QIMR have evaluated sequence polymorphisms and blood concentrations of the HRP2 protein and their impact on the sensitivity of RDTs as well as the effect of patient antibody levels and parasite antigen on the performance of RDTs. Furthermore, FIND and other partners such as AdAlta, Chembio and AbD Serotec have evaluated more sensitive test formats and produced highly thermostable antibodies specific for malaria detection.

Key accomplishments

- During 2010, KIT evaluated and selected couples of monoclonal antibodies able to detect GLURP, DHFR, and HDP malaria antigens in a lateral flow assay format. These antibodies immobilized in the test format are currently in evaluation for their thermostability and sensitivity to detect *P. falciparum* and *P. vivax* parasites.
- Chembio evaluated their highly sensitive Dual Path Technology Platform (DPP) with antibodies recognizing specifically the Plasmodium aldolase protein. They have demonstrated that with a series of improvements,

the DPP technology could detect as low as 30 ng/μl of recombinant protein. However, when tested with reference parasite strains at different levels of parasitemias, this assay offered no clear advantage over existing RDTs.

- AbD Serotec screened their human combinatorial antibody (HuCAL) PLATINUM library with the HRP2, *P. falciparum* LDH, and *P. vivax* LDH proteins in order to select specific antibodies. Prior to selection, the library was subjected to stress conditions and in all selections, antibodies could be identified that bound these antigens specifically. More than 47 unique antibodies were isolated and this number was reduced in panning with the library subjected to stress. Currently, AbD Serotec is purifying the selected antibodies and testing them for sandwich pairing and analysis for thermostability.

Improving roll-out and use of RDTs

- Partners: WHO, University Research Company Llc (USA), Malaria Consortium (UK), national malaria control programmes (NMCPs), Earth Institute of Columbia University (USA)
FIND project leader and/or coordinator: Evan Lee
- Project start: 2008

Resumé of project

- Monitoring temperature fluctuations to which RDTs are exposed
- Development of a new blood-transfer device to improve blood safety and accuracy of RDT preparation
- Development of evidence-based job-aids and training materials to improve blood safety and RDT accuracy in the field
- Development and demonstration of best practices in RDT implementation
- Development and implementation of effective, low-cost electronic reporting of malaria diagnosis from remote locations

Temperature monitoring and safe storage

Description

Establishing the typical conditions under which point-of-care tests (and drugs) are transported and stored is important for determining the thermal stability necessary for RDTs to be effective for field use, and to raise awareness of the importance of good logistics management in maintaining diagnostic quality. Together with national partners across a range of climatic conditions, FIND has electronically monitored temperature and humidity continuously over 12 months in central and peripheral RDT storage areas, and as these commodities move through in-country supply chains. The purpose of this study was to gather data on the actual conditions that RDTs are exposed to in a variety of settings. This information can provide feedback to the manufacturers and the wider malaria community on stresses related to temperature and humidity that could potentially affect RDT performance in the field. This study was challenging from a logistic point of view because of the need to coordinate the handling and custody of the log tags among the different individuals who are typically involved in commodity supply chains in the field, and to ensure that recorded data was recovered.

Key accomplishments

- “Static” data, based on temperature and humidity recorded over a twelve month period in a variety of storage facilities, was gathered and received from Burkina Faso, Senegal, the Philippines and Ethiopia.

- Transport data (temperatures and humidity conditions that RDTs are exposed to during their distribution) were collected for several transport chains in Senegal, Burkina Faso and the Philippines.
- In an effort to develop low cost, passively cool storage solutions to protect RDTs from the effect of extreme temperatures in hot tropical areas, FIND has funded further development of evaporative cooler models with the Colorado School of Mines. Having demonstrated a simple and effective design developed from simple materials, the prototype is currently undergoing further refinement.

Blood transfer device

Description

The correct transfer of blood from a patient to a point-of-care test, seemingly simple, is vital to both accuracy of the diagnosis and the safety of the health worker. FIND has coordinated the design and manufacturing of a new blood transfer device, an inverted cup device, based on a previous non-commercialized design. The current device was developed for transfer of blood to RDTs with Ormance S.A. and Althéo Plast-Inject, both in Geneva, Switzerland. This is intended to be used for transfer of blood accurately and safely from finger-prick to point-of-care diagnostic test, with minimal risk of blood exposure for the health worker.

Key accomplishments

- The blood transfer device has been extensively evaluated in the field by partners in Uganda, Nigeria and the Philippines and found to be useful and safe to use by health care workers. It performed more effectively than a range of other devices currently in routine use.
- A number of RDT manufacturers have expressed interest in replacing their current blood transfer device with the new product.

Evidence-based training materials and job-aids

Description

Sets of training materials and job aids (instructions for health workers) developed through a thorough, evidence-based approach in Zambia and elsewhere are now available for widespread use, in versions specific to a range of commonly-used products. Partner agencies and national programmes have also adapted these tools to different language settings. They are designed to improve the accuracy of malaria RDT-based diagnosis and improve user safety.

Key accomplishments

- All materials and job aids are freely available on the FIND website.
- FIND, URC and WHO are providing a service to assist in the modification of the generic materials to the product and language requirements of malaria control programmes.



Job aids training in Zambia

Development and demonstration of best practices in RDT implementation

Description

Over the past few years, FIND has been working closely with the National Malaria Control Programme of Uganda, as well as other country programmes and agencies, to develop and demonstrate best practices in planning and rolling out malaria RDTs. This is an ongoing process and involves knowledge sharing, planning, and collecting further evidence to support the scale up of RDT use. The project also looks at creating feasible solutions to mitigate the risks associated with poor RDT storage and use. FIND has also supported the National Regulatory Authority of Uganda to develop the medical devices regulation guideline, which will be published in 2011.

Key accomplishments

- FIND has contributed to the creation of a malaria diagnostics technical working group in the Ministry of Health of Uganda, which ensures that best practices are integrated into RDT deployment.
- FIND also assisted the Global Fund grant application processes to guide NMCPs on malaria RDT implementation plans and development of quality assurance schemes for supporting RDT rollout. In particular, FIND participated in writing the Global Fund Round 10 grant in Uganda, which was successful. This grant will sustain RDT supply in all target health facilities for the next 5 years. Uganda is currently rolling out RDT-based parasitological diagnosis using round 4 phase 2 funds. FIND is supporting coordination at all stages of implementation to ensure that the rollout is carried out in a quality assured manner.
- On the basis of the experience gained in implementation support, FIND is coordinating the development of a global implementation manual to provide clear guidance and support materials for programmes seeking to introduce and scale up the use of malaria RDTs. This manual, which will also include a suite of existing documents on budgeting, supervision, training, advocacy, is currently being drafted by FIND in partnership with the WHO, RBM Partnership, the Malaria Consortium, Ministries of Health, the CDC, the US President's Malaria Initiative, Roll Back Malaria partnership and other stakeholders. It is due to be finalized by late-2011.

Launch of a disease and commodity monitoring system based on text messaging

Description

FIND partnered with Columbia University's Earth Institute (USA) and the Ugandan Ministry of Health to design and launch a monitoring system based on SMS messaging, which can be used even with the most basic of cell phones. The project aims to facilitate and support rapid reporting of malaria cases, the use of malaria diagnostics, stocks of anti-malarial commodities and disease surveillance by health units located in remote areas in Africa. The system was introduced in Uganda in early November 2009 and covers 127 health workers from 45 health units in Gulu district; and 247 health workers in 113 health units in Kabale district. The choice of SMS for data transmission helps with sustainability, as no extra equipment had to be procured. Ongoing running costs are minimal, consisting of the airtime needed for the SMS transmission and a few hours per week of technical assistance to maintain the server which is hosted locally in Uganda. This last point was another factor which facilitated buy-in by the Ministry of Health.

Key accomplishments

- The SMS-based reporting tool has now been officially adopted by the Government of Uganda as a weekly form of their Health Management Information System. This form will enable a stronger linkage between parasite-based diagnosis and treatment, as well as disease outbreak reporting.

Positive control wells

- Partners: National Bioproducts Institute (NBI, South Africa), Queensland Institute of Medical Research (QIMR, Australia), ReaMetrix (India), MicroMol GmbH (Germany), Hospital for Tropical Diseases (HTD, UK), Centers for Disease Control & Prevention (CDC, USA), WHO/GMP
- FIND project leader and/or coordinator: Iveth J. González
- Project start: 2007
- Resume of project: Development and implementation of a tool to test the quality of products in use at a clinic/village level and to increase the user's confidence in test results. PCWs contain recombinant proteins currently detected by RDTs and would be packaged with RDTs for easy initial and/or retesting of RDTs.

Description

At present, field users have no method of ensuring that the malaria RDTs that they are using are still functioning properly after exposure to heat and humidity during transport and storage. Poor performance has major consequences both for public health, as well as for confidence in, and effectiveness of, malaria control programmes. Providing capacity to test the quality of RDTs at a clinic/village level could overcome several of the current blocks to effective RDT use.

Prototype PCWs, developed with a commercial partner, Reamatrix, in Bangalore, India, have now been developed, consisting of plastic tubes containing lyophilized recombinant proteins HRP2, pLDH and aldolase; these are the main targets of malaria RDTs. These proteins are diluted with a fixed volume of clean (tap) water and then placed on RDTs. If RDTs and PCWs are working correctly, a weak positive band will appear at the test line of the RDT. A failure of the RDT test line to appear indicates that the RDT has lost the required sensitivity to detect malaria parasites in a clinically relevant malaria parasite infection.



The initial product specifications of the PCWs considered the addition of a single pLDH, specifically the one from *Plasmodium falciparum* species (PfLDH), suitable for evaluation of both pan- and Pf-specific bands. However RDTs detecting specifically *P. vivax* LDH (PvLDH) are becoming more common and 8 different RDTs detecting this protein are under evaluation in Round 3 of the RDT Product Testing Programme. Based on this, it was recommended to produce two versions of PCWs, one with PvLDH and another with PfLDH. This required the production of a new recombinant protein (PvLDH) at MicroMol and the transfer of clones and standard methods to ReaMetrix.

PCW format evaluation in Uganda

PCW prototypes are expected to be ready for field trials during 2011, and the same technology will form the basis of the new methods for lot-testing panels described above.

Key accomplishments

- During the last meeting of the Steering Committee for the RDT Product Testing Programme (February 2010), it was recommended that recombinant proteins in PCWs should be at concentrations around the cut-off value equivalent to 50% RDT detection rate of 200 parasites/ μ l, allowing appropriate tolerances for manufacture, storage and preparation.

- In order to select recombinant proteins for prototype PCWs and ensure equivalence to testing against real parasite samples, extensive evaluations of the proteins and PCW prototypes are underway at CDC and at HTD in the UK. These experiments have thus far not only demonstrated that recombinant proteins are very well detected by RDTs and that PCWs performed properly, but have also demonstrated high stability of the prototypes of PCWs, essential for effective use in the field.
- These prototype PCWs were also tested on RDTs and ELISAs at HTD for their performance and thermostability after storage at high temperatures. Recombinant proteins in PCWs were very stable after incubation at 45°C for 3 months.

Financial Statements
for the year ended
31 December 2010 and
Report from the Statutory
Auditor

Report of the statutory auditor

To the Board of the
Foundation for Innovative New Diagnostics (FIND), Geneva

Report on the financial statements

As statutory auditor, we have audited the accompanying financial statements of the Foundation for Innovative New Diagnostics (FIND), which comprise the balance sheet, statement of income and expenditure, cash flow statement and notes for the year ended December 31, 2010.

Foundation Board's Responsibility

The Board of the Foundation is responsible for the preparation of the financial statements in accordance with the requirements of Swiss law and the statutes of the Foundation. This responsibility includes designing, implementing and maintaining an internal control system relevant to the preparation of financial statements that are free from material misstatement, whether due to fraud or error. The Board of the Foundation is further responsible for selecting and applying appropriate accounting policies and making accounting estimates that are reasonable in the circumstances.

Auditor's Responsibility

Our responsibility is to express an opinion on these financial statements based on our audit. We conducted our audit in accordance with Swiss law and Swiss Auditing Standards. Those standards require that we plan and perform the audit to obtain reasonable assurance whether the financial statements are free from material misstatement.

An audit involves performing procedures to obtain audit evidence about the amounts and disclosures in the financial statements. The procedures selected depend on the auditor's judgment, including the assessment of the risks of material misstatement of the financial statements, whether due to fraud or error. In making those risk assessments, the auditor considers the internal control system relevant to the entity's preparation of the financial statements in order to design audit procedures that are appropriate in the circumstances, but not for the purpose of expressing an opinion on the effectiveness of the entity's internal control system. An audit also includes evaluating the appropriateness of the accounting policies used and the reasonableness of accounting estimates made, as well as evaluating the overall presentation of the financial statements. We believe that the audit evidence we have obtained is sufficient and appropriate to provide a basis for our audit opinion.

Opinion

In our opinion, the financial statements for the year ended December 31, 2010 comply with Swiss law and the statutes of the Foundation.


Report on Other Legal Requirements

We confirm that we meet the legal requirements on licensing according to the Auditor Oversight Act (AOA) and independence (article 728 of the Swiss Code of Obligations) and that there are no circumstances incompatible with our independence.

In accordance with article 728a paragraph 1 item 3 of the Swiss Code of Obligations and Swiss Auditing Standard 890, we confirm that an internal control system exists, which has been designed for the preparation of financial statements according to the instructions of the Board of the Foundation.

We recommend that the financial statements submitted to you be approved.

Deloitte SA


Peter Quigley
Licensed audit expert
Auditor in charge


Isabelle Babey

Geneva, July 18, 2011
PBQ/IBA/ahc

Attached : Financial Statements (balance sheet, statement of income and expenditure, cash flow statement and notes)

FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

STATEMENT OF INCOME AND EXPENDITURE FOR THE YEAR ENDED 31 DECEMBER 2010

(all amounts in US dollars)

	2010	2009
INCOME		
Contributions income	27 381 427	24 484 891
Sundry income	206 215	144 238
Total income	<u>27 587 642</u>	<u>24 629 129</u>
EXPENDITURE		
Analytical & Project Work		
Tuberculosis	14 793 823	15 083 050
Human African Trypanosomiasis	3 450 099	4 252 387
Malaria	3 077 095	3 419 806
Human Immunodeficiency Virus	491 728	137 548
Leishmaniasis	56 497	-
	<u>21 869 242</u>	<u>22 892 791</u>
Information & Communication		
Publications production	29 973	50 283
Website	19 971	20 897
Fundraising consultants	4 093	12 748
	<u>54 037</u>	<u>83 928</u>
Governing & Advisory Bodies		
Foundation Board	15 271	11 437
	<u>15 271</u>	<u>11 437</u>
General Administration		
Staff costs and travel	1 137 637	924 331
IT expenses	309 994	247 294
Photocopies, stationery, printing & sundries	60 193	99 006
Rent of premises	691 911	534 976
Repairs & maintenance	168 722	122 679
Telecommunications	128 170	115 176
Office moving expenses	469	204 803
	<u>2 487 096</u>	<u>2 249 255</u>
Finance & Service Expenses		
Staff costs and travel	699 814	595 357
Auditing & accounting	78 979	65 471
Bank charges	17 901	19 413
Exchange losses	-	133 864
Legal fees	104 947	78 851
Insurance	225 990	185 908
	<u>1 127 631</u>	<u>1 078 864</u>
Depreciation	123 224	62 794
Total expenses	<u>25 686 501</u>	<u>26 379 069</u>
Excess of income / (deficit) over expenditure for year	<u>1 901 141</u>	<u>(1 749 940)</u>
Accumulated surplus brought forward	527 993	2 277 933
Accumulated surplus carried forward	<u>2 429 134</u>	<u>527 993</u>

The accompanying notes form an integral part of these financial statements.

FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

BALANCE SHEET AS AT 31 DECEMBER 2010

(all amounts in US dollars)

	2010	2009
<u>ASSETS</u>		
Current assets		
Cash on hand	9 395	4 037
Bank Current accounts	2 825 195	764 352
Short-term Deposits	19 955 000	13 000 000
Accounts receivable	941 859	1 505 898
Prepayments	2 870 444	2 716 923
	<hr/>	<hr/>
	26 601 893	17 991 210
Fixed assets		
Office furniture & fittings	39 040	52 596
Computers & printers	146 863	168 407
Electrical installations	8 675	11 153
Fax machine & telephones	38 171	1 626
	<hr/>	<hr/>
	232 749	233 781
Patents	15 414	23 121
Rental Guarantee Deposit	203 721	164 344
	<hr/>	<hr/>
Total assets	<hr/> <hr/>	<hr/> <hr/>
	27 053 777	18 412 456
 <u>LIABILITIES AND CAPITAL</u>		
Current liabilities		
Accounts payable	1 650 749	2 194 014
Accrued expenses	1 056 028	476 453
Contributions received in advance	21 502 915	15 173 566
Unrealized exchange gains	374 521	-
	<hr/>	<hr/>
	24 584 213	17 844 033
Capital and reserves		
Capital	40 430	40 430
General reserve	2 429 134	527 993
	<hr/>	<hr/>
Total liabilities and capital	<hr/> <hr/>	<hr/> <hr/>
	27 053 777	18 412 456

The accompanying notes form an integral part of these financial statements.

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FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

CASH FLOW STATEMENT FOR THE YEAR ENDED 31 DECEMBER 2010

(all amounts in US dollars)

	2010	2009
Excess of income over expenditure for the year	1 901 141	(1 749 940)
Add back non-cash charge - depreciation	123 224	62 794
	<u>2 024 365</u>	<u>(1 687 146)</u>
Cash flows - operating activities		
Increase/(decrease) in contributions in advance	6 329 349	(5 750 510)
Increase/(decrease) in unrealized exchange gains	374 521	-
Increase/(decrease) in accounts payable	(543 205)	(241 890)
Increase/(decrease) in accrued expenses	579 575	178 783
(Increase)/decrease in accounts receivable	564 039	(810 422)
(Increase)/decrease in prepayments	(153 521)	163 164
	<u>7 150 698</u>	<u>(6 460 865)</u>
Cash flows - investing activities		
Additional office furniture & fittings	(4 314)	(25 743)
Additional computers & printers	(71 380)	(148 477)
New electrical installations	-	(12 392)
Additional faxes and telephones	(38 811)	(1 626)
(Increase)/decrease in rental guarantee account	(39 377)	(66 379)
	<u>(153 862)</u>	<u>(274 617)</u>
Net increase in cash and cash equivalents for year	<u>\$ 9 021 201</u>	<u>(8 422 628)</u>
Cash and cash equivalents at start of year		
Cash on hand	4 037	2 945
Current accounts & short-term deposits	13 764 352	22 188 072
	<u>13 768 389</u>	<u>22 191 017</u>
Cash and cash equivalents at end of year		
Cash on hand	9 385	4 037
Current accounts & short-term deposits	22 780 195	13 764 352
	<u>22 789 580</u>	<u>13 768 389</u>
Net increase in cash and cash equivalents for year	<u>\$ 9 021 201</u>	<u>(8 422 628)</u>

The accompanying notes form an integral part of these financial statements.

FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

NOTES TO THE FINANCIAL STATEMENTS FOR THE YEAR ENDED 31 DECEMBER 2010

1. General

The Foundation for Innovative New Diagnostics (FIND) is an independent non-profit Foundation created under Article 80 of the Swiss Civil Code, and is registered in the Geneva Register of Commerce under statutes dated 28 September 2007.

FIND's mission is to support and promote the health of people in developing countries through the development and introduction of new but affordable diagnostics for infectious diseases.

FIND is exempt from federal and cantonal income and capital taxes.

On 9 December 2010 FIND and the Swiss Federal Council signed an agreement granting FIND certain privileges and immunities under the revised Host State Act which came into force on 1 January 2008. In accordance with this agreement FIND has been granted exemption from all federal, cantonal and communal taxes, from Value-Added Tax, and from regulations governing the employment of foreign nationals in Switzerland. This agreement came into effect on 1 January 2011.

2. Significant accounting policies

2.1 **Basis of presentation** The financial statements are prepared under the historical cost convention.

2.2 **Fixed assets** Fixed assets are recorded at cost and are depreciated under the straight-line method at 20% annually for office furniture and fittings, electrical installations and fax machine and telephones, and 33.3% annually for computers and printers.

2.3 **Patents** The Patents were purchased as part of an agreement completed with a project partner early in 2004, and are subject to amortization under the straight-line method over their remaining useful life (two years).

2.4 **Foreign currency** Accounting records are maintained in US dollars. Income and expenditures in other currencies are recorded at accounting rates approximating actual rates in effect at the time of the transaction. Year-end balances for assets and liabilities in other currencies are translated into US dollars at rates of exchange prevailing at balance sheet date. At 31 December 2010 the rate of exchange used for the Swiss franc, the main foreign currency for 2010, was USD/CHF = 0.93 (2009 - 1.03). Realized exchange gains as well as realized and unrealized exchange losses are included in the determination of net income. Unrealized exchange gains are deferred.

2.5 **Recognition of revenue** Contributions received are recorded according to the grant period agreed with donors, with amounts received relating to periods extending beyond balance sheet date recorded as contributions received in advance. Donor agreements in effect at 31 December 2010 provide for a total of USD 34.4 million to be paid to FIND between January 2011 and September 2015.

FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

NOTES TO THE FINANCIAL STATEMENTS

(continued)

2.6 **Accounts payable and accrued expenses** With the exception of emoluments payable to staff, which are accounted for when paid, accounts payable and accrued expenses represent expenditures chargeable in the 2010 financial year, for which invoices were not received for payment before year-end. Settlements are charged to the accruals in the following financial period.

2.7 **Rental guarantee deposit** The guarantee relates to the rental of the FIND office premises and is recoverable in accordance with the rental contract upon vacation of the premises.

2.8 **Projects** Payments to project partners during 2010 under contracts signed up to 31 December 2010 totalled USD 9,277,027 (2009 – USD 11,582,189). Commitments at 31 December 2010 for future payments under those contracts total USD 8,492,660 (2009 – USD 6,699,917).

3. Fixed assets and intellectual property

3.1 Fixed assets as at 31 December 2010 were as follows:

	<u>2010</u>	<u>2009</u>
Cost price		
Office furniture & fittings	116,822	112,508
Computers & printers	400,538	329,177
Electrical installations	12,392	12,392
Fax machine & telephones	41,965	3,155
	-----	-----
	571,717	457,232
Less: Accumulated depreciation	338,968	223,451
Net book value	USD 232,749	233,781
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Fire insurance cover as at 31 December 2010 was USD 107,530 (2009 – USD 128,155).

3.2 Intellectual property as at 31 December 2010 was as follows:

	<u>2010</u>	<u>2009</u>
Patents -- cost price	53,949	53,949
	-----	-----
	53,949	53,949
Less: Accumulated depreciation	38,535	30,828
Net book value	USD 15,414	23,121
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FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

Geneva, Switzerland

NOTES TO THE FINANCIAL STATEMENTS

(continued)

4. Contributions received

During 2010 contributions were received from donors as follows (*non-dollar amounts converted to USD at exchange rates on date of receipt*):

	<u>2010</u>	<u>2009</u>
Becton Dickinson and Co	125,000	50,000
Bill & Melinda Gates Foundation	17,460,125	11,553,558
DNDI	55,556	-
European Union	44,362	344,650
Government of Ireland	-	-
Government of Netherlands	3,006,387	-
Government of United Kingdom	3,189,889	-
Government of United States	178,828	-
JSI Research & Training	-	21,500
UBS	-	149,850
UNITAID	9,377,581	6,064,300
WHO	485,748	556,173
	<u>USD 33,923,476</u>	<u>18,740,031</u>

5. Pension Fund liabilities

No amounts were due to the pension fund at 31 December 2010 (*2009 – nil*).

6. Rent commitments

At 31 December 2010 FIND had future rent commitments totalling USD 2,629,290 up to 30 June 2014 (*2009 – USD 3,052,311 up to 30 June 2014*).

7. Funds

The Endowment Capital of CHF 50,000 is fully subscribed and equates to USD 40,430 at the rate of exchange on the date of payment.

8. Events subsequent to 31 December 2010

There were no events occurring subsequent to 31 December 2010 which could have a material impact on the understanding of these financial statements.

FIND

Board of Directors and Teams

Board of Directors

Dr. Gerard H. Moeller
Chairman of the Board



Dr. Jan Gheuens
Member



Dr. Bernard Mach
Member



Dr. Mphu Ramatlapeng
Member



Dr. Giorgio Roscigno
Member



FIND Team

Chief Executive Officer

Giorgio Roscigno

FIND Geneva

Eric Adam, *Senior Implementation Officer*
Audrey Albertini, *Scientific Officer Malaria*
Heather Alexander*, *Health Scientist*
Alexandra Amann, *Project Support Officer EXPAND-TB*
Krisztina Bagamery, *Communications Officer TB*
Sylvain Biéler, *Project Manager*
Catharina Boehme, *Senior Medical Officer*
Nora Champouillon, *Logistics Officer*
Louisa Chaubert, *Accounting Manager*
Diana Choa, *Personal Assistant to the CEO*
Herbert J. Clemens, *Chief Financial Officer*
Christine Crettenand, *Accounting Assistant*
Christiane Elfman, *Receptionist / Administrative Assistant*
Knut Feldmann, *Senior Project Manager EXPAND-TB*
Francoise Fichet, *Receptionist / Administrative Assistant*
Rossana Gambin, *Human Resources Officer*
Iveth J. González, *Scientific Officer Malaria*
Beatrice Gordis, *Communications Officer HAT & Malaria*
Julian Gordon*, *Medical Diagnostic Technologies & IP*
Christen Gray, *Clinical Trials Data Manager*
Cristina Gutierrez, *Senior Project Manager, Laboratory Support*
Sandy Haldimann, *Scientific Team Administrator, EXPAND-TB*
Linda Hinni, *Accounting Assistant*
Sandra Incardona, *Technical Officer Malaria*
Judith Kirorei-Ita, *Scientific Team Administrator TB*
Peter Koller*, *Quality Manager*
Francelle Kwankam, *Communications Officer TB*
Evan Lee, *Senior Policy Officer*
Solomon Haile Mariam*, *Advocacy Officer HAT*
Gerd Michel, *Senior Technology Officer*
Pamela Nabeta, *Medical Officer*
Joseph Ndung'u, *Head of HAT Programme*
Richard O'Brien, *Head of Product Evaluation and Demonstration*
Madhukar Pai *, *Consultant for Latent TB Infection*
C.N. Paramasivan, *Head of TB Laboratory Support*

Mark Perkins, *Chief Scientific Officer*
Catherine Pilet, *Accounting Assistant*
Bärbel Porstmann, *Senior Operating Officer*
Sharon Saacks, *Document Controller & Project Manager TB*
Hojoon Sohn*, *Health Economist*
Ákos L. Somoskövi, *Senior Project Manager, Laboratory Support*
Lakshmi Sundaram, *Advocacy Officer*
Ranald Sutherland *, *Technology and Business Development*
Tatiana Titova*, *Web consultant*
Eloise Valli, *Technical Officer Clinical Team*
Alessandra Varga*, *Events and Image Development*
Julie Vercruyssen, *Scientific Team Administrator Malaria*
Thomas Verges, *Logistics Officer, EXPAND-TB*
Hanna Yirga, *Scientific Team Administrator HAT*
Diego Zallocco*, *TB Projects*

FIND India

V.V.H. Balasangameshwara*, *Senior Programme Manager and Technology Officer*
Jacques Debayle, *Liaison Office Manager*
Anil Samuel James*, *Office Support*
Uday Gurung*, *Data Entry Operator*
R. Narayanaswamy*, *Deputy Drugs Controller (I) (RETD.)*
Shailaja Paramathma, *Senior Administrative Assistant*
Neeraj Raizada*, *Medical Officer*
Arman Singh*, *Data Entry Operator*

FIND Uganda

Heidi Albert*, *Senior Scientist, Head of Laboratory Research*
Caroline Asiimwe, *Malaria Diagnostics Implementation Project*
Heidi Hopkins, *Medical Officer*
George Lukyamuzi, *Scientific Officer*
Zoe Nakuya*, *Lead Advisor*
Jean Nsekera, *Office Manager*
Barnabas Nyesiga, *Assistant Scientific Officer*
Ajay Kumar Thirumala, *Programme Manager & Technology Officer*

* *Consultant*

Publications

Tuberculosis programme

TB antigen discovery

- Submitted manuscript: Shui *et al.* (2011) Mycolic acids as diagnostic markers for TB infection and drug efficacy; submitted to PNAS.

TB antibody detection

- Kunnath-Velayudhan S, Salamon H, Wang H-Y, Davidow AL, Molina DM, Huynh VT, Cirillo DM, Michel G, Talbot EA, Perkins MD, Felgner PL, Liang X, Gennaro ML. Dynamic antibody responses to the *Mycobacterium tuberculosis* proteome, *PNAS*, 2010, vol. 107, no. 33, 14703–14708
- Oral presentation: CHI European POC Congress, Hannover, Germany; NUS workshop on “Workflows towards generating anti-lipid antibodies”, Singapore; Genomics and Proteomics Conference, Santorini, Greece.

Lateral Flow Immunoassay sensitivity increase

- Kunnath-Velayudhan S, Salamon H, Wang H-Y, Davidow AL, Molina DM, Huynh VT, Cirillo DM, Michel G, Talbot EA, Perkins MD, Felgner PL, Liang X, Gennaro ML. Dynamic antibody responses to the *Mycobacterium tuberculosis* proteome, *PNAS*, 2010, vol. 107, no. 33, 14703–14708

Automated TB DNA Detection

Published manuscripts

- Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, *et al.* Rapid molecular detection of tuberculosis and rifampin resistance. *The New England Journal of Medicine*, 2010; Sep;363(11):1005-15.
- Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, *et al.* Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology* 2010. Jan;48(1):229-37.
- Small PM, Pai M. Tuberculosis diagnosis--time for a game change. *The New England Journal of Medicine* 2010. Sep 9;363(11):1070-1.
- Van Rie A, Page-Shipp L, Scott L, Sanne I, Stevens W. Xpert® MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? *Expert Review of Molecular Diagnostics* 2010. Oct;10(7):937-46.

Manuscripts currently undergoing review

- Andrea Rachow, Alimuddin Zumla, Norbert Heinrich, Gabriel Rojas-Ponce, Bariki Mtafya, Klaus Reither, Elias N. Ntinginya, Justin O’Grady, Jim Huggett, Keertan Dheda, Catharina Boehme, Mark Perkins, Elmar Saathoff and Michael Hoelscher. Rapid and Accurate Detection of *Mycobacterium Tuberculosis* in Sputum Samples by Cepheid Xpert MTB/RIF Assay - A Clinical Validation Study. Accepted for fast-track review by *Lancet*.
- Viral Vadwai, Catharina Boehme, Pamela Nabeta, Anjali Shetty, David Alland, Camilla Rodrigues. Xpert MTB/RIF, a new pillar in the diagnosis of extrapulmonary tuberculosis? Under review at *Lancet Infectious Diseases*.
- Bowles, Edmee; Freyee, Benthe; van Ingen, Jakko; Mulder, Bert; Boeree, Martin; van Soolingen, Dick. The GeneXpert, a novel automated PCR-based tool for the diagnosis of tuberculosis. Revision accepted as technical note by *The International Journal of Tuberculosis and Lung Disease*.

Manuscripts under preparation

- Use of Xpert MTB/RIF for the diagnosis of tuberculosis and multi-drug resistance at point-of-treatment: Feasibility and impact of decentralized testing
- Use of Xpert MTB/RIF for the diagnosis of tuberculosis and multi-drug resistance at point-of-treatment: A cost-effectiveness analysis
- Xpert MTB/RIF: Positioning where the need is greatest: The South African perspective
- Diagnosis of pulmonary tuberculosis in HIV-infected and uninfected hospitalized children using Xpert MTB/RIF: a prospective study

Published posters

- S. Naidoo (Lancet Laboratories, South Africa) Evaluation of GeneXpert MTB/RIF Assay on pulmonary and extrapulmonary samples in a high throughput routine laboratory. ECCMID, Vienna, April, 2010
- S. Naidoo (Lancet Laboratories, South Africa) Evaluation of Xpert MTB/RIF Assay on pulmonary samples in a high throughput routine laboratory. Pathvine Congress, Cape Town, August, 2010
- P. Ioannidis, D. Papaventsis, S. Nikolaou, S. Karabela, M. Panagi, A. Raftopoulou,
- E. Konstantinidou, I. Marinou, S. Kanavaki (National Reference Laboratory, Greece)
- Evaluation of GeneXpert MTB/RIF Assay for MTB Detection and Rifampicin resistance in Athens, Greece. 31st Annual ESM Congress, Bled, Slovenia, July 2010
- Nataša Fajfar, Urška Bidovec-Stojkovič, Manca Žolnir-Dovč (University Clinic of Respiratory and Allergic Diseases Golnik, Slovenia) Evaluation of GeneXpert MTB/RIF assay for detection of *Mycobacterium tuberculosis* and rifampicin resistance in a routine laboratory setting in Slovenia. 31st Annual ESM Congress, Bled, Slovenia, July 2010
- M.T. Tórtola, L. Nieto, M.G. Codina, N. Martín-Casabona (Microbiologia, Hospital Universitari Vall d'Hebron, Barcelona) Detection of *M. tuberculosis* and rifampicin-resistance using a commercial PCR real time technique in respiratory and extrapulmonary samples
- B. Malbruny, G. Le Marrec, K. Courageux, R. Leclercq, V. Cattoir (Service de Microbiologie, CHU Côte de Nacre, Caen, France). Rapid and Efficient Detection of *Mycobacterium tuberculosis* by the Cepheid Xpert MTB/RIF Assay, ICAAC, Boston, September 2010
- T. Bodmer and A. Ströhle (University of Berne, Switzerland) Diagnosing pulmonary tuberculosis in a low prevalence setting: the Xpert® MTB/RIF test. ESM, 2010
- S. Filippo, Mitchelmore I, Pillai P, Mulla R (Luton and Dunstable Hospital NHS Foundation Trust, UK), Rapid diagnosis of *Mycobacterium tuberculosis* using Cepheid Xpert MTB/RIF PCR. HPA conference, 2010

HAT programme

HAT LAMP

- Ndung'u J.M., Bieler S. and Roscigno G. (2010). "Piggy-backing" on diagnostic platforms brings hope to neglected diseases: the case of sleeping sickness. *PLoS Negl Trop Dis* 4(5): e715. doi:10.1371/journal.pntd.0000715.

HAT staging markers

- Ndung'u J.M., Bieler S. and Roscigno G. (2010). "Piggy-backing" on diagnostic platforms brings hope to neglected diseases: the case of sleeping sickness. *PLoS Negl Trop Dis* 4(5): e715. doi:10.1371/journal.pntd.0000715.
- Tiberti N., Hainard A., Lejon V., Robin X., Ngoyi D.M., Turck N., Matovu E., Enyaru J., Ndung'u J.M., Scherl A., Dayon L., Sanchez J.C. (2010). Discovery and verification of osteopontin and beta-2-microglobulin as promising markers for staging human African trypanosomiasis. *Molecular and Cellular Proteomics* 9:2783–2795.
- Hainard, A., Tiberti, N., Robin, X., Ngoyi, D.M., Matovu, E., Enyaru J.C.K., Müller, M., Turck, N., Ndung'u, J.M., Lejon, V., and Sanchez, J.C. (2010). Matrix metalloproteinase-9 and intercellular adhesion molecule 1 are powerful staging markers for human African trypanosomiasis. *Tropical Medicine & International Health* 16:119–126.

LED Fluorescence Microscopy

- Publication of animal study results: manuscript submitted
- Ndung'u JM, Bieler S, Roscigno G (2010) "Piggy-backing" on diagnostic platforms brings hope to neglected diseases: the case of sleeping sickness. *PLoS Negl Trop Dis*. 25; 4(5):e715.

HAT Antibody detection

- Ndung'u JM. Addressing the diagnostic challenges of diseases of poverty. *Stakeholders' consultation meeting of the WHO/TDR Disease Reference Group on Chagas disease, human African trypanosomiasis and Leishmaniasis. Rio de Janeiro, Brazil, 30th March 2010*
- Ndung'u JM. Rapid tests for HAT, impact of early diagnosis at community level. FIND Symposium at the Global Health Congress, Washington DC, 14th June 2010.
- Ndung'u JM. Diagnosis of African trypanosomiasis: challenges and opportunities. 18th IPR International Biomedical Conference, Nairobi, Kenya - 6th to 8th July 2010.
- Ndung'u JM. New diagnostics for neglected protozoan diseases. European Commission Conference on Neglected Protozoan Diseases, Institute Pasteur, Paris 24th September 2010.
- Ndung'u JM. Challenges and opportunities in the diagnosis of African trypanosomiasis. Annual Scientific Conference on African Trypanosomiasis. Nairobi, Kenya 4th – 5th Oct. 2010.

Malaria programme

RDT evaluations

- Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: Round 1 (2008). WHO/FIND/TDR/CDC, 2008. http://www.wpro.who.int/sites/rdt/who_rdt_evaluation/call_for_testing.htm.
- Malaria Rapid Diagnostic Test Performance. Results of WHO product testing of malaria RDTs: Round 2 (2009). WHO/TDR/FIND/CDC. http://www.wpro.who.int/sites/rdt/who_rdt_evaluation/call_for_testing_round2.htm.
- Baker J, Ho MF, Pelecanos A, Gatton M, Chen N, Abdullah S, Albertini A, Arie F, Barnwell J, Bell D *et al*: Global sequence variation in the histidine-rich proteins 2 and 3 of *Plasmodium falciparum*: implications for the performance of malaria rapid diagnostic tests. *Malar J*, 9:129.
- Baker J, McCarthy J, Gatton M, Kyle DE, Belizario V, Luchavez J, Bell D, Cheng Q: Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. *J Infect Dis* 2005, 192(5):870-877.
- Bell D, Peeling RW: Evaluation of rapid diagnostic tests: malaria. *Nat Rev Microbiol* 2006, 4(9 Suppl):S34-38.
- Bell D, Perkins MD: Making malaria testing relevant: beyond test purchase. *Trans R Soc Trop Med Hyg* 2008, 102(11):1064-1066.
- Bell D, Wongsrichanalai C, Barnwell JW: Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nat Rev Microbiol* 2006, 4(9):682-695.
- Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, Incardona S, Perkins M, Bell D, McCarthy J *et al*: A large proportion of *P. falciparum* isolates in the Amazon region of Peru lack pfrp2 and pfrp3: implications for malaria rapid diagnostic tests. *PLoS One*, 5(1):e8091.
- Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, McCarthy J: Effect of sequence variation in *Plasmodium falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: Implications for rapid diagnostic tests for malaria. *J Clin Microbiol* 2006, 44(8):2773-2778.
- Lee N, Baker J, Bell D, McCarthy J, Cheng Q: Assessing the genetic diversity of the aldolase genes of *Plasmodium falciparum* and *Plasmodium vivax* and its potential effect on performance of aldolase-detecting rapid diagnostic tests. *J Clin Microbiol* 2006, 44(12):4547-4549.
- Perkins MD, Bell DR: Working without a blindfold: the critical role of diagnostics in malaria control. *Malar J* 2008, 7 Suppl 1:S5.
- Talman AM, Duval L, Legrand E, Hubert V, Yen S, Bell D, Le Bras J, Arie F, Houze S: Evaluation of the intra- and inter-specific genetic variability of *Plasmodium lactate dehydrogenase*. *Malar J* 2007, 6(1):140.

Detection of low-density malaria infections: LAMP for malaria

- Polley SD, Mori Y, Watson J, Perkins MD, González IJ, Notomi T, Chiodini PL, Sutherland CJ. Mitochondrial DNA targets increase sensitivity of malaria detection using loop-mediated isothermal amplification, *J Clin Microbiol.* 2010 Aug; 48(8):2866-71

Non-malarial febrile illnesses (NMFI)

- Jorgensen P, Nambanya S, Gopinath D, Hongvanthong B, Luangphengsouk K, Bell D, Phompida S, Phetsouvanh R.: High heterogeneity in *Plasmodium falciparum* risk illustrates the need for detailed mapping to guide resource allocation: a new malaria risk map of the Lao People's Democratic Republic. *Malaria Journal* 2010, 9:59doi:10.1186/1475-2875-9-59

Improving roll-out and use of RDTs

- Lon Chanthap, Frédéric Arieu, Duong Socheat, Reiko Tsuyuoka and David Bell: Low-technology cooling box for storage of malaria RDTs and other medical supplies in remote areas. *Malaria Journal* 2010, 9:31 doi:10.1186/1475-2875-9-31

Published posters

- How can community malaria diagnosis be made accurate and safe? Improving diagnostic accuracy of malaria RDTs at the community level through the use of training materials (Collaborative Project of FIND, WHO, TDR, URC, MALARIA CONSORTIUM, ZAMBIA NMCC, RITM)

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