

New Laboratory Diagnostic Tools for Tuberculosis Control



RETOOLING
TASK FORCE



World Health
Organization

Stop TB Partnership

WHO Library Cataloguing-in-Publication Data :

New laboratory diagnostic tools for tuberculosis control.

1.Tuberculosis - diagnosis. 2.Tuberculosis - prevention and control 3.Laboratory techniques and procedures - trends. 4.Reagent kits, Diagnostic - utilization. 6.Product surveillance, Postmarketing. 7.Diffusion of innovation 8.Decision making. I.World Health Organization. II.Stop TB Partnership. Task Force on Retooling.

ISBN 978 92 4 159748 7 (NLM classification: WM 200)

© World Health Organization 2008

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; e-mail: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

This publication contains the collective views of the Retooling Task Force of the Stop TB Partnership and does not necessarily represent the decisions or the policies of the World Health Organization.

Writing and Design: Timothy Ryan, SOLVE. Bangkok, Thailand.

Printed in France.

Preface

The Global Plan to Stop TB anticipates the availability of new diagnostics, drugs and vaccines by 2015.

The New Diagnostics Working Group (NDWG) is an essential component of the Stop TB Partnership and contributes in a major way in coordinating and facilitating the development, evaluation and implementation of new and modified diagnostics in a scientifically acceptable and timely manner by linking all stakeholders involved in the diagnostic development and evaluation pathway.

Recognizing considerable delays between the availability of new tools and routine use at country level, the Stop TB Partnership's Coordinating Board established the Retooling Task Force (RTF) to support the new tools and implementation Working Groups in preparing national programs for the introduction of new technologies. The RTF includes members of the product development, programme implementation, donor and scientific communities. It serves as a bridge and communicator between Stop TB Partnership Working Groups.

New diagnostic tools (technologies and approaches) for TB are being developed and evaluated at an unprecedented rate. The World Health Organization has already endorsed 3 new diagnostic tools, and they are currently being rolled out in countries.

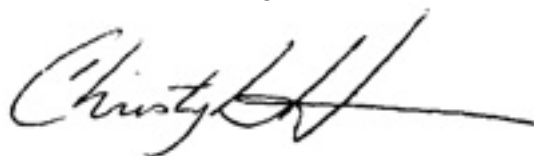
Moreover, the diagnostics pipeline developed by partners in the NDWG is now well populated with candidate new diagnostic tools.

The NDWG of the Stop TB Partnership is working hard to produce a scientific blueprint for TB diagnostics development that will allow an objective, staged, inclusive pipeline to be developed and that will help outline the appropriate value chain for the development of new diagnostics to be submitted for technical review and endorsement to the WHO and to inform WHO diagnostic policy development. This is something that may take some time but should permit the objective description of a diagnostics pipeline with realistic estimates of the time before tools become available for widespread use.

The RTF and NDWG recognize that there is a lack of easily-digestible information available to NTP staff for programmatic planning, or to funding and technical agencies that may wish to support the development, evaluation or implementation of new TB diagnostic tools.

With this in mind the RTF, with the full collaboration of the NDWG and the Global Laboratory Initiative (GLI) and inputs from the New Vaccines and New Drugs Working Groups, has produced this brochure to act as an interim document until the blueprint can be developed and the pipeline based on this can be created.

Christy Hanson
Chair of the Retooling Task Force



Giorgio Roscigno
Chair of the New Diagnostics Working Group





Introduction

Research in new diagnostics, drugs and vaccines for tuberculosis (TB) is happening at an unprecedented rate. The Global Plan to Stop TB 2006-2015 anticipated the development of numerous technologies and provided a framework to make them accessible to high TB burden settings. The Stop TB Partnership established a Task Force on Retooling to stimulate the adoption, introduction and implementation of these tools.

Thanks to the work of the Foundation for Innovative New Diagnostics (FIND) and other organizations, several new diagnostics tools are already available and more will become available in the coming years. These tools are desperately needed. The World Health Organization (WHO) reports that although treatment success rates are improving every year, case detection rates are increasing more slowly. In Africa, less than 50% of estimated cases are being detected in DOTS programmes. Compounded by new challenges, such as drug resistance and TB/HIV co-infection, the lack of effective, quality controlled diagnostic tools and laboratory systems endangers the gains that have been made in TB control.

This document describes nineteen (19) new or improved diagnostic tools of the many that have been or are being developed. WHO has a process for evaluating and endorsing new tools for TB control programmes. Three (3) of the tools described in this document have already been endorsed by WHO and are being implemented by countries, while the others are still under development or in piloting phase and are expected to be ready for review for appropriateness for scaled-up use in the coming years. The purpose of this document is not to recommend specific tools, but rather to provide summary information about them so that all who play a part in TB control, especially in National TB Programmes, can understand the tools better and make well-informed decisions when retooling.

Challenges faced in TB diagnosis

Sputum smear microscopy remains the cornerstone of TB diagnosis in developing countries. The method depends upon the quality and bacterial load of the sputum specimen and the training and motivation of laboratory technicians. Although highly specific in most countries, smear microscopy is insensitive – it detects roughly 50% of all the active cases of TB. Sensitivity can be as low as 20% in children and HIV-infected people. Furthermore, smear microscopy cannot detect resistance to drugs.

Smear microscopy also places a burden on the patient. A person with suspected TB typically has to visit the clinic at least twice before a diagnosis can be made, and then has to return again for the results. This process can be expensive in terms of transportation costs and lost wages from time off work. Some people never complete the testing process, or do not return for their results.

Culture of TB bacteria on liquid or solid media is a more sensitive method for TB diagnosis than smear microscopy and it permits testing for drug resistance. TB culture has limitations, however: it requires biosafety facilities that are expensive to build and maintain and specially trained laboratory technicians to perform the procedure. Some national TB programmes in developing countries have no functioning TB culture facility at all. In others, TB culture is performed only at national reference laboratories or in hospital laboratories in large cities. Few developing countries have capacity for good-quality drug-susceptibility testing (DST) for first-line drugs and even fewer have the capacity to test for second-line drug resistance.

Even where capacity exists, diagnosing TB with culture can take weeks because of the slow growth rate of TB bacilli. In most countries TB culture is reserved for retreatment cases. Specimens are often sent to distant laboratories. This can delay processing of specimens and lead to inaccurate results. Furthermore, test results must travel long distances back to reach the clinic and the patient.

Who is suffering from the lack of effective TB diagnostic tools?

TB is an airborne infectious disease that is preventable and curable. Left untreated, each person with active TB will infect on average between 10 and 15 people every year. The WHO estimated that 1.7 million people died from TB in 2006. Although one in three people in the world is infected with TB bacteria, not everyone who is TB-infected becomes ill with tuberculosis.

HIV/AIDS and TB are so closely connected that they are often referred to as co-epidemics or dual epidemics. HIV affects the immune system and increases the likelihood of people becoming infected with TB and developing TB disease. HIV promotes both the progression of latent TB infection to active disease and relapse of the disease in previously treated patients. TB is the leading cause of death in people infected with HIV, particularly in low-income countries. Of the 1.7 million deaths from TB in 2006, 200,000 were among people infected with HIV, the virus that causes AIDS. Many HIV-infected TB patients suffer from extrapulmonary TB. Extrapulmonary TB is particularly hard to diagnose. Even when there are clinical symptoms or x-ray findings suggestive of TB, they can be hard to distinguish from other conditions.

Drug resistance is a major public health problem that threatens TB control. Drug resistance is caused by improper use of TB drugs in chemotherapy. Drug-resistant TB can be passed from person to person. Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) are two forms of TB that are resistant to the most effective first-line and second-line TB drugs (respectively).

Diagnosis of childhood tuberculosis is challenging. The disease frequently bears little resemblance to the pulmonary presentation usually seen in adults. Furthermore, children often have difficulty producing sputum and conventional tests like sputum smear microscopy are therefore not useful to diagnose TB in children.

New TB drugs and vaccines

In 2000, there were virtually no prospective new drugs for TB. Today, members of the Working Group on New TB Drugs have built a pipeline of drug development projects, with several in advanced stages. Moxifloxacin [Bayer, TB Alliance, UCL, British MRC] and gatifloxacin [OFLOTUB Consortium] may help shorten TB therapy to 3 to 4 months and if successful, could be registered within the next 5 years. Other anti-TB drug candidates (PA-824 [TB Alliance], OPC 67683 [Otsuka Pharmaceuticals], TMC 207 [Tibotec], LL-3853 [Lupin Limited] and SQ-109 [Sequella, Inc.]) are also active against multidrug-resistant strains of TB. All of the new drugs should be suitable for areas where HIV/AIDS is common.

A new vaccine for TB is also needed. Today's TB vaccine, Bacille Calmette-Guérin (BCG), provides some protection against severe forms of TB in children but is unreliable against pulmonary TB, which accounts for most of the worldwide disease burden. In recent years, members of the Working Group on New TB Vaccines, including non-profit organizations such as Aeras Global TB Vaccine Foundation (www.aeras.org) and the Tuberculosis Vaccine Initiative (www.tbvi.eu), and other researchers from the public, private and academic sectors have developed multiple new TB vaccine candidates. Some of these candidates would replace BCG, while others would improve or "boost" BCG, thereby extending the vaccine's protective effects.

TB vaccines under development could work in several ways, including by preventing infection, disease, or reactivation of latent TB infection, or by improving the response to chemotherapy. Several new vaccine candidates are in various stages of clinical trials, with more in preclinical development.

TB experts hope that at least one new vaccine will be ready for global distribution by 2015. A list of TB vaccines under development is available at www.stoptb.org/retooling.

Selecting and adopting new diagnostic tools

The Stop TB Partnership's Retooling Task Force has published valuable materials for countries seeking to implement new tools, including a "Framework for their Adoption, Introduction and Implementation," guidance on "Engaging Stakeholders for Retooling TB Control" and a checklist for the introduction of liquid culture. The information in these publications will be helpful as you prepare to select and adopt new tools for your programme. You can find the publications on the Retooling Task Force website (<http://www.stoptb.org/retooling>) or ask for them by email (stoptbretooling@who.int).

What should you consider when assessing technologies?

The above publications describe many factors to keep in mind when reviewing potential new tools for your programme, including:

- The evidence base. This includes evidence not only of the accuracy of a new tool under the conditions of controlled clinical trials but also its effectiveness under actual field conditions. Some tools becoming available look promising but their value in TB control activities is yet to be demonstrated on a wide scale in developing countries, or among the TB population they target (such as tools for DST in countries with a high prevalence of MDR-TB). Define the diagnostic needs and priorities of your programme (higher general case-detection, enhanced case-detection in HIV positive people, detection of MDR-TB, and so on) and ensure there is evidence that a tool meets those needs. Note that the actual impact of new diagnostic tools on TB control is unknown at present.
- Your programmatic readiness. Implementation of tools often requires changes in national policy and treatment guidelines, meaning that decision-makers must be well informed about the prospective advantages of the tools and be ready to adopt and set policy that supports their use. Furthermore, none of the new tools will – alone – address all of your needs. Programme managers and the heads of laboratory networks must think through the potential roles of different tools and how they can be used (or combined) in different country settings for best effect, and develop a plan for the required laboratory capacity strengthening. Piloting new tools under programme conditions, with assistance from donors and partners if necessary, can help build the programme capacity and evidence you need before scaling up nationwide.
- Turnaround time for results. Although several new tools provide results faster than existing diagnostic methods, their practical role within your larger diagnostic network must be carefully considered to ensure they achieve the aims you expect. A fast tool that cannot deliver reliable, timely results to the point of care because of transport and other issues has limited additional value.
- Costs of the products required and their maintenance. Many tools bring with them high initial costs for equipment and ongoing costs for maintenance and for consumable products. The costs-versus-benefits of each tool should be carefully considered. Slightly reduced (but acceptable) benefits may be achievable at highly reduced costs. Forecasted needs and secure finances are essential to finalizing tool selection. Procurement systems and teams must be in place and properly informed to ensure continuity of the supply needed for sustainable, high-quality TB diagnostic services.
- Regulatory and quality control measures. Evidence review is a fundamental step required by many countries before a new product is authorized to enter countries and be integrated into the health care system. In many countries, the product registration process includes submission of a dossier containing information on efficacy, safety and other properties. Furthermore, tools bring with them ongoing quality assurance, logistical requirements and costs, which place demands on national quality control programmes. Both internal quality controls and external quality assurance are critical, as a good test done badly may be worse than no test at all. These factors should be considered and arrangements made with national regulatory systems and quality control programmes before beginning implementation. It is recognized that in many countries, national regulatory mechanisms for diagnostics do not exist or are weak.
- Safety considerations. Many of the tools becoming available require sophisticated, expensive laboratory biosafety infrastructure that must be established before you can use the tool. Proper infrastructure must be established and staff trained in necessary safety procedures before you can begin using the tool.
- Staff capacity and training capacity. Staff must understand the advantages of a new tool and how to use it properly. For some tools, sensitization and training of staff in one or two laboratories will be relatively simple. For other tools (even very simple ones), training activities will need to be carefully coordinated with supply management, essential infrastructure changes and the development of laboratory-specific operating procedures, particularly where tools are being introduced in phases in the country.

Important new WHO policies

Given current evidence and consensus, WHO has established the following new policies:

1. The definition of a new sputum smear positive pulmonary TB case has been revised, and is now based on the presence of at least one acid fast bacillus (AFB) in at least one sputum sample in countries with a well functioning quality assurance system.
2. The minimum number of sputum specimens to be examined in the investigation of suspected TB by smear microscopy has been reduced from three to two in settings with a well-functioning external quality assurance scheme, and where workload is high and human resources limited.
3. The use of liquid culture and rapid species identification to address the needs for culture and DST is recommended, based on a country-specific plan for laboratory capacity strengthening. The use of liquid culture and rapid species identification should be implemented in a phased approach.
4. The use of line probe assays for rapid detection of MDR-TB is recommended within the context of country plans for appropriate management of MDR-TB patients, including the development of country-specific screening algorithms and timely access to quality-assured second-line anti-tuberculosis drugs.

Find out more

www.who.int/tb/dots/laboratory/policy

Who can help?

The ultimate responsibility for how you retool your diagnostic services lies with your programme and Ministry of Health, however, several resources exist to advise you during planning and to provide finances or technical assistance to help you realize those plans. You may already have contacts with partners that can assist you. The Stop TB Partnership Retooling Task Force may also be a help to you. If you require additional support, however, you can begin by contacting:

Summary of new diagnostic technologies (2008-2009)

Many new diagnostic tools are under development/evaluation (i.e. in the diagnostics pipeline). It is expected that the evidence for several of these will be sufficient to merit review by the WHO research-into-policy process within the next few years. Some of these diagnostic tools are likely to be endorsed by WHO. This document does not aim at being exhaustive but at illustrating the pipeline by describing a selection of some of the most promising and/or well-known tools. There are published reviews in scientific literature that provide a more extensive listing of all available diagnostic tests for TB (see page 16).

Tools for screening for latent TB

The Tuberculin Skin Test (TST) is one of the most common tests to determine if someone has been exposed to and become infected with the TB bacteria. A positive skin test is indicated by a skin reaction at the point of the injection. However, vaccination with the BCG vaccine can also lead to a reaction at the TST site (as can repeated TST testing), which limits the test's usefulness in vaccinated children or people repeatedly tested because of high risks of exposure (such as healthcare workers). One new tool developed to supplement or replace the TST are described in this document: [Interferon gamma release assays \(page 12\)](#).

Tools for improving smear microscopy

Smear microscopy is not a very sensitive technique and requires that the patient comes at least twice to the clinic – but it is also relatively fast, inexpensive, and specific for TB in high incidence areas. Thus, in the absence of better alternatives, it is a useful tool in the basic laboratories common in developing countries. Many researchers have focused on new tools and methods to improve the sensitivity and/or efficiency of microscopy, three of which are described in this document: [Front-loaded smear microscopy \(page 12\)](#), [LED fluorescence microscopy \(page 13\)](#) and [bleach microscopy \(page 15\)](#).

Tools for improving culture and DST services

WHO has recommended liquid culture and rapid species identification to address the needs for culture and DST. Liquid culture systems reduce the delays in obtaining positive cultures. The rapid growth in liquid culture results in reduced delays for DSTs also, compared with conventional solid media. Liquid systems are more sensitive for detection of mycobacteria and may increase the case yield by 10% over solid media. With increased sensitivity and reduced delays, liquid systems may contribute significantly to improved patient management. It should be noted, however, that just because a tool relies on a liquid culture medium, does not necessarily mean it is endorsed by WHO. The Expert Meeting on liquid culture convened by WHO only reviewed evidence on the use of commercial broth-based culture systems, and recommendations only pertain to these systems. This document describes four new tools for improving culture diagnosis and DST: [Liquid culture: Manual liquid culture technique and commercial broth-based culture systems \(page 9\)](#), [Solid culture: Nitrate reduction assay and Thin layer agar culture \(page 14\)](#), and [Colorimetric redox indicators \(page 11\)](#).

Tools for rapidly identifying the species of culture isolates

Speciation is necessary to discriminate between *Mycobacterium tuberculosis* complex and other species of mycobacteria which may grow in cultures. Traditional biochemical methods of speciation can take weeks. With commercially-available broth culture systems, the organisms need to be removed from the culture tubes and tested. Researchers are working to develop and/or scale up tools for rapidly speciating the results of culture, one of which is described in this document: [Strip speciation \(page 10\)](#).

Tools for molecular testing

Molecular techniques that could reduce turnaround times in the laboratory for diagnosing TB and detection of drug resistance are being developed and evaluated in many countries. WHO has already approved tools for detection of drug resistance-associated genetic mutations directly from the sputum of smear-positive patients or from isolates grown in culture. An automated technology, as well as two molecular line probe assays focused on rapid detection of rifampicin resistance alone or in combination with isoniazid resistance, are described in this document: [Automated detection and MDR screening \(page 11\)](#) and [Molecular line probe assay \(page 9\)](#).

Case study: Lesotho

Since late 2006, global and national partners had already been working to improve Lesotho's TB diagnostic capabilities, including strengthening of the National Reference Laboratory (NRL). WHO recommends implementing the new technologies first in the country's national reference laboratory, so that it can provide experience during scale-up to the rest of the laboratory system.

Thanks to the close collaboration among multiple stakeholders, Lesotho established a state-of-the-art mycobacteriology laboratory and rapidly introduced new TB diagnostic technologies needed to address the challenges posed by MDR- and XDR-TB.

Partners in Health (PIH), with funding from the Open Society Institute, had established a partnership with the Ministry of Health and Social Welfare (MOH&SW) to launch a treatment programme for MDR-TB.

In addition, the Foundation for Innovative New Diagnostics (FIND) had signed a memorandum of understanding with the government in April 2007 to help prepare the laboratory system in Lesotho to introduce new diagnostic technologies. The MOH&SW coordinated several levels of global and national support to rehabilitate the NRL and institute laboratory staff training programmes. It installed new equipment bought through the Global Fund, developed standard operating procedures, streamlined workflow, and recruited additional technicians to tackle the new workload.

WHO provided technical guidance and appointed a country-based WHO medical officer to support laboratory strengthening. PIH provided logistics and financial assistance. FIND provided an on-site technical expert for renovation and provided a liquid culture system and rapid strip test to detect TB in cultures. In October 2008, a new molecular laboratory was finished and Lesotho started doing line probe assays as well.

Summary of technologies			Estimated costs			
Technology	Description	Product	Training ¹	Infrastructure ²	Equip. ³	Consumables
WHO-endorsed tools (2006-2008)						
Liquid culture	Commercial broth-based culture systems detect TB bacteria (manual and automated systems are available); can be configured for DST.	BacT/ALERT 3D; MGIT	Extensive (3 weeks)	■ ■ ■	High	High
Molecular line probe assay	Strip test simultaneously detects TB bacteria and genetic mutations that indicate isoniazid and/or rifampicin resistance.	GenoType® MTBDR and MTBDRplus; INNO-LiPA Rif.TB	Moderate (3 days)	■ ■ to ■ ■ ■	High	High
Strip speciation	Strip speciation test detects a TB-specific antigen from positive liquid or solid cultures to confirm the presence of TB bacteria in culture samples.	Capilia TB Rapid Diagnostic Test	Minimal (1 day)	■ ■ ■	Low	Medium
Tools in late-stage development/evaluation						
Automated detection and MDR screening	Device allows automated sample processing, DNA amplification and detection of M. tuberculosis and screening for rifampicin resistance.	Cepheid GeneXpert device and Xpert MTB cartridge	Minimal	■	High	High
Colorimetric redox indicators	Technique detects isoniazid and rifampicin resistance in culture samples after incubation with redox dyes.	Non-commercial method (Resazurin)	Extensive	■ ■ ■	Low	Medium
Front-loaded smear microscopy	Based on 2 or 3 specimens but aims to examine specimens on the day that patient presents to the health service (thus identifying 95% of TB cases).	n/a	Minimal	■	Low	Low
Interferon gamma release assay	Blood test detects specific cellular immune responses indicating TB infection.	QuantIFERON®-TB Gold In Tube; T-SPOT.TB®	Moderate	■	Low	High
LED fluorescence microscopy	Robust fluorescence microscopy (FM) systems based on light-emitting diodes (LEDs) that could allow the advantages of FM at levels of the health system where conventional FM would be impractical.	Fraen	Moderate	■	Medium	Low
		LW Scientific	Moderate	■	Medium	Low
		Zeiss	Moderate	■	Medium	Low
Microscopic Observation Drug Susceptibility (MODS)	Manual liquid culture technique uses basic laboratory equipment (incl. an inverted light microscope) and microscopy skills to detect TB bacteria.	Non-commercial method	Extensive	■ ■ to ■ ■ ■	Medium	Medium
New solid culture methods	Solid culture technique measures nitrate reduction to indicate isoniazid and rifampicin resistance.	Non-commercial method (Nitrate reductase assay)	Moderate	■ ■ to ■ ■ ■	Low	Medium
	Solid culture technique simultaneously detects TB bacteria and indicate isoniazid and rifampicin resistance.	Non-commercial method (Thin layer agar culture)	Extensive	■ ■ to ■ ■ ■	Low	Medium
Tools in early phase of development						
Tool		Level of health system	Tool		Level of health system	
Breathalyser screening test		Community or point-of-care	Sodium hypochlorite (bleach) microscopy		Peripheral laboratory	
First-generation loop-mediated isothermal amplification technology platform (LAMP)		Peripheral laboratory	Sputum filtration		Peripheral laboratory	
Lipoarabinomannan (LAM) detection in urine		Peripheral laboratory	TB Patch Test		Health post	
Phage-based tests		Reference laboratory	Vital fluorescent staining of sputum smears		Peripheral laboratory	
² Key	Description					
■	Basic laboratory ⁴ ; no specialized biosafety equipment.					
■ ■	Biosafety level 2. Specialized biosafety equipment required, such as biosafety cabinet.					
■ ■ ■	Biosafety level 3. Biosafety cabinet and other primary safety equipment required. Controlled ventilation system that maintains a directional airflow into the laboratory required.					
8	¹ Estimates assume that technicians are already trained in existing TB diagnostic techniques (such as smear microscopy and culturing) and the necessary laboratory safety precautions. ³ Product prices may vary depending on geographical location and terms of supply. Ranges are indicative only: Low (minimum-2000 US\$); Medium (2001-7000 US\$); high (7001+ US\$). ⁴ Detailed information available in the WHO Laboratory Biosafety Manual: http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/ .					

WHO-endorsed tools (2006-2008)

Liquid culture: Commercial broth-based culture systems

New tools can detect fluorescence in a liquid culture medium, enriched with oxygen, to indicate the presence of bacteria. As bacteria grows in the culture, the oxygen is utilized, causing it to be fluorescent when placed under UV light. Methods for testing for drug susceptibility follow the same principle but use two culture samples: one with a drug added and one without the drug (a growth control). If the test drug is active against the TB bacteria, it will inhibit growth and suppress fluorescence. In the manual system, a technician visually identifies fluorescence using a hand-held UV lamp. Automated systems have the capacity for up to 960 cultures at a time.

Potential advantages: Such systems have been thoroughly evaluated in clinical settings and approved for use in industrialised countries for years. Liquid culture systems provide results significantly faster than solid culture techniques: on average diagnosis can be performed in 7-14 days (up to 42 days for a negative result). DST takes on average 7-14 days (range 4-14 days) after the initial culture. Automated systems can benefit laboratories with a high workload and provide standardized reading of samples. Such systems have a sensitivity and specificity of nearly 100% (slightly less for manual systems). Studies have shown that both automated and manual systems perform well in detection of isoniazid and rifampicin susceptibility, but are not as effective for ethambutol and streptomycin.

Considerations: The machine (required for the automated system) and the growth indicator tubes (required for both manual and automated systems) are costly, although a “Developing country” price has been negotiated for MGIT by FIND. Manual systems typically require additional manipulation and use of a hand-held UV lamp. Liquid media are more prone to contamination than solid media, leading to invalid tests unless carefully controlled. Automated machines must be maintained, requiring on-site technical support from manufacturers or their agents.

Requirements: As with all TB culture systems, BSL-2 facilities are required for processing specimens and inoculating cultures. BSL-3 is required if tubes need to be opened and the cultured organisms manipulated (for speciation or further testing). Since speciation of organisms grown in commercial broth-based culture systems requires tubes to be opened and cultures to be manipulated, BSL-3 facilities are required. Laboratory technicians require training in BSL-3 safety precautions and training in the use of these methods and tools. A stable source of electricity is required for automated systems. Laboratories performing rapid culture in liquid medium require sophisticated technology (such as the BACTEC™ MGIT™ 960 System for the automated Mycobacterium Growth Indicator Tube test) or a hand-held UV lamp (for manual systems) as well as several consumable products for which a supply chain must be established, such as BBL MGIT Indicator Tubes, centrifuge tubes, sodium hydroxide, sodium citrate solution, N-acetyl-L-cysteine powder, phosphate buffer, vortex mixer, incubator, pipettes and pipette tips, sodium sulfite solution, mycobacterial agar or egg-based medium, tissue homogenizer or sterile swab, Normal saline, ATCC reference strains, microscope and materials for staining slides, and blood agar plates. Some methods of rapid culture in liquid medium require proprietary products and countries may find it difficult to find local/national suppliers, so establishing long-term support agreements with international suppliers is critical.

Product information: BacT/ALERT 3D (industry.biomerieux.com); BACTEC™ MGIT™ 960 System, BBL MGIT Indicator Tubes (www.bd.com/ds); hand-held UV lamp (www.ambion.com)

Current known developers: Becton, Dickinson and Company (BD, www.bd.com) in collaboration with FIND (www.finddiagnostics.org); BioMerieux (www.biomerieux.com)



Molecular line probe assay

Strip test detects TB DNA and genetic mutations associated with drug resistance from smear-positive sputum specimens or culture isolates after DNA extraction and PCR amplification.

Potential advantages: Studies have shown such assays perform well when used directly on smear-positive sputum specimens (with sensitivity exceeding 97% and specificity exceeding 98%), confirming their value in rapid screening of patients suspected of MDR-TB. While molecular methods are not yet sufficiently developed to fully replace conventional methods for culture and DST, implementation of such assays may reduce the demand for sophisticated and expensive conventional laboratory infrastructure, especially in high MDR-TB burden settings. There is the potential for savings in overall TB diagnostic cost if such assays are judiciously introduced in screening algorithms for detection of MDR-TB.

Considerations: While specificity is excellent for isoniazid resistance, sensitivity estimates are modest and highly variable. Geographical variation in prevalence of mutations associated with rifampicin - and in particular with isoniazid resistance - may result in varying performance of line probe assays in different epidemiological settings. Introduction of line probe assays should preferably be preceded by an assessment of sensitivity and specificity of these assays in a representative collection of MDR- and non-MDR isolates at country or at regional level. Line probe assays are as complex to perform as conventional culture and DST methods and require skilled and well-trained laboratory personnel, as well as adequate laboratory space and design to reduce the risk of false-positive results. Line probe assays do not eliminate the need for conventional culture and DST capability, as culture remains necessary for definitive diagnosis of TB in smear-negative patients, while conventional DST is required to diagnose XDR-TB. Problems with contamination may be experienced, especially when newly-trained staff perform the assay.

Requirements: Processing of smear-positive specimens for direct testing should be performed in a BSL-2 level laboratory, whereas performing line probe assays on positive cultures requires BSL-3 laboratory infrastructure and equipment, including a class I or class II microbiological safety cabinet equipped with uninterrupted power supply (UPS), autoclave, screw-topped tubes and bottles, pipetting aids, disposable transfer loops and personal protective equipment such as gloves, gown/lab coat, respirator and eye protection. Laboratory technicians require training in either BSL-2 or BSL-3 safety precautions (depending on the procedure) and in how to perform the line probe assay. Reagents used in line probe assays must be refrigerated or frozen, while amplification and hybridization procedures must be conducted under closely monitored temperature conditions. Line probe assays require specific equipment for molecular procedures, such as a thermal cycler, shaking platform and water bath, heating block, sonicator, micro centrifuge and tubes, fridge, freezer, micropipettes and pipette tips, and PCR tubes. UPS is required during PCR amplification and use of the automated hybridization systems to avoid interruption of the procedure and subsequent loss of results. The laboratory must be designed to address the risk of amplicon contamination. Certain equipment such as incubators and automated hybridization systems are product-specific. Technical support from test distributors is required to address hardware problems and to assist with troubleshooting related to unusual results and contamination. Establishing a long-term support agreement with an international supplier is critical.

Product information: GenoType® MTBDR and MTBDRplus (www.hain-lifescience.com); INNO-LiPA Rif.TB (www.innogenetics.com)

Current known developers: GenoType® MTBDR and MTBDRplus (Hain Lifescience GmbH in collaboration with FIND); INNO-LiPA Rif.TB (Innogenetics NV)



Strip speciation

Strip speciation test can detect a TB-specific antigen (MPB64) from positive liquid or solid cultures to confirm the presence of organisms belonging to *M. tuberculosis* complex. Speciation is necessary to differentiate *M. tuberculosis* complex and other mycobacteria grown in cultures.

Potential advantages: Studies have demonstrated that the rapid speciation tests compare favourably with other established phenotypic or genotypic methods. This test provides results within 15 minutes and is highly sensitive and specific (98.6% and 97.9%, respectively). Other than the equipment needed to culture TB, no additional equipment or consumables are needed to perform the test and can detect TB even when mixed with nontuberculous mycobacteria.

Considerations: The results of *M. bovis* and *M. bovis* BCG cultures may vary since some BCG strains are known to lack MPB64 antigen production. Although limited data exists on the product's performance and cost-effectiveness further studies are required to validate its readiness for large-scale implementation in countries. The test detects organisms belonging to *M. tuberculosis* complex but does not specifically identify *M. tuberculosis* strains.

Requirements: Rapid speciation tests require BSL-3 laboratory infrastructure and equipment, including a class I or class II microbiological safety cabinet. Laboratory technicians require training in BSL-3 safety precautions and training in the use of such tests. Establishing a long-term support agreement with an international supplier is critical to enable uninterrupted supply. In addition, a well-established laboratory and consumables required to perform liquid or solid culture (see above).

Product information: Capilia TB Rapid Diagnostic Test (www.capilia.com/en/TBInsert.html)

Current known developers: Tauns Laboratories, Inc. (<http://www.tauns.co.jp>) in collaboration with FIND



Case study: United Republic of Tanzania

The Tanzanian National TB and Leprosy Programme (NTLP) has made progress in recent years, successfully treating 81% of its cases and expanding its TB/HIV coordinated activities. Nonetheless, it faces challenges. Two of its greatest ambitions are to improve detection of new, smear-positive TB cases and detection of drug-resistance. New diagnostics were identified by Tanzania as one way to help achieve these goals. For years, the NTLP worked closely with national diagnostics experts to evaluate the new diagnostic tools becoming available. With careful consideration and considerable political will, retooling is expected to meet the challenges of the NTLP.

In 2007, the NTLP began evaluating LED fluorescence microscopy as a more appropriate tool for improving case detection at the periphery. Standard light microscopes were converted to LED using an adaptor. Conventional fluorescence microscopy, not LED-based, is on average 10% more sensitive than traditional Ziehl-Neelsen microscopy but also more prone to false positive results. Because it builds upon existing techniques, it does not require costly infrastructure or extensive training. The positive response to the LED trials has prompted the NTLP to begin rapidly scaling-up its availability at all district hospitals and high-volume sites. The adoption and implementation of such new tools requires large programmatic changes, unfortunately making it impossible to implement immediately at peripheral laboratories, where the need (and potential impact in high-volume laboratories) is likely to be greatest.

Tanzania has also begun introducing faster, more sensitive liquid culture and DST at national and regional reference laboratories. The availability of these tests is expected to improve the diagnosis and management of TB patients. Increasing detection of drug resistant cases poses different challenges at the periphery. As in most countries, samples from patients failing treatment are sent to a central reference laboratory. Transporting these samples can be a challenge – and specimens may be delayed for long periods or even lost. Long delays and other adverse conditions of transport can result in the specimens being unculturable (because of contamination) or falsely considered negative. Delayed detection of resistance can have tragic results.

In 2009, the NTLP will assess the feasibility of the Hain GenoType® MTBDRplus rapid test for detecting drug resistance. The assessment will be conducted in the same limited number of laboratories conducting culture and DST but specimens may not be so sensitive to adverse transport conditions. Results should be available quicker. Studies have shown that line probe assays (endorsed by WHO in 2008) adequately detect rifampicin resistance when used directly on smear-positive patients, thus confirming their potential value for screening suspected MDR-TB patients.

Tools in late-stage development/evaluation

Automated detection and MDR screening

Amplification and detection of *M. tuberculosis* DNA is one of the fastest and most sensitive ways to detect tuberculosis, and also may allow the detection of genetic mutations associated with drug resistance. Unfortunately, conventional DNA amplification methods are cumbersome, require specialized training, and can only be performed on material that has been subjected to processing and DNA extraction. This has limited their use to reference laboratories. The Xpert MTB device is a fully automated system that allows a relatively untrained operator to perform sample processing, DNA amplification, and detection of *M. tuberculosis* and screening for rifampin resistance in less than 2 hours and only minutes of hands-on time.

Potential advantages: Results can be available while patient waits in clinic. The only manual step, adding sample treatment reagent to the specimen cup before loading the cartridge, kills over 99.9% of TB bacilli in the specimen. The test detects TB in essentially all smear-positive samples and the majority of smear-negative samples. The presence of non-tuberculous mycobacteria does not confound testing. The cartridges are stable at room temperature. The same device may in the future be used for HIV viral load detection.

Considerations: The device that automates the procedure is a computer-driven, sophisticated piece of equipment that will require reliable energy supply, security and maintenance. Test costs will be much higher than the cost of tests routinely done at lower level laboratories, where high performance detection and drug resistance screening are not currently performed.

Requirements: Laboratories performing the Xpert MTB assay will require BSL-1 laboratory infrastructure and equipment. Technicians require training in BSL-1 safety precautions and the use of the GeneXpert device. A reliable source of electricity is needed as well as a room that can be secured outside working hours. All buffers and reagents are included in the cartridge. A plastic Pasteur pipette is used to transfer sputum to the Xpert MTB cartridge. Cepheid, Inc., and its distributors will be the only source of consumables and maintenance, so a long term supply and service agreement will be necessary.

Product information: Cepheid GeneXpert device and Xpert MTB cartridge (www.cepheid.com)

Current known developers: Cepheid, Inc. (www.cepheid.com) in collaboration with FIND



Colorimetric redox indicators

Colorimetric methods are based on the reduction of an indicator solution added to a liquid culture medium after TB organisms have been exposed to different antibiotics. Isoniazid and rifampicin resistance is detected by a change in colour of the indicator.

Potential advantages: There is limited evidence that colorimetric methods are highly sensitive (about 95%) and specific for the detection of MDR-TB and faster than conventional solid or liquid culture DST methods (first results between 7 and 14 days after culturing). Colorimetric methods do not require sophisticated equipment and are therefore less expensive to perform than some other liquid culture techniques for DST.

Considerations: Colorimetric methods cost approximately the same as solid culture techniques for DST. Additional studies and cost-effectiveness analyses are required to validate this technique and determine if appropriate for large-scale implementation in countries with a high prevalence of MDR-TB.

Requirements: As with all TB culture systems, BSL-2 facilities are required for processing specimens and inoculating cultures. BSL-3 facilities are required if tubes need to be opened and the cultured organisms manipulated (for speciation or further testing). Since the colorimetric test requires that the microtitre plates be opened and indicator solution pipetted into the wells, BSL-3 facilities are required. Laboratory technicians require training in BSL-3 safety procedures and in the colorimetric DST methodology. Laboratories performing colorimetric DST require the equipment (centrifuge, balance, Bunsen burner, freezer, incubator, refrigerator, vortex mixer and water distillator) and consumables (antiseptics and dispensing syringes) required to perform liquid culture (see above). Furthermore, the test requires equipment (microtitre plates) and consumable products for which a supply chain must be established, including Middlebrook 7H9 broth and a redox indicator (MTT solution or resazurin).

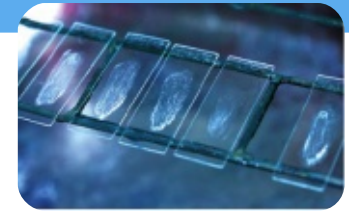
Product information: Middlebrook 7H9 broth (www.bd.com); MTT reagent (www.millipore.com); resazurin powder (uk.vwr.com); microtitre plates (www.bdbiosciences.com)

Current known developers: Academic laboratories



Front-loaded smear microscopy

Microscopy services currently require patients to make repeat visits to the healthcare facility. This is associated with considerable patient costs and patient drop-out during diagnosis. The new WHO definition of a smear positive case does not require confirmatory smears. This allows patients to be diagnosed on a single smear. Two smears have been shown to diagnose 95-98% of patients. In front-loaded microscopy the first two specimens are collected and examined on the same day a patient presents. Patients with negative smears can be asked to return with a morning specimen the next day, depending on whether routine services are based on two or three specimens being examined.



Potential advantages: The costs for visits incurred by patients, particularly by poor patients, are often prohibitive. This new method intends to reduce these costs and the considerable proportion of patients dropping out of the diagnostic process. TB patients can be offered treatment on the day they present.

Considerations: In addition to this method, programmes can seek ways to provide better follow-up in order to reduce patient drop-out. Data on the impact and effectiveness of this method is limited; studies to validate the method are currently in progress. To make best use of this method, clinics may have to reorganize their clinical and laboratory services so that people have both their smears examined on the same day – making it possible to put them onto treatment rapidly.

Requirements: Does not require any equipment or biosafety measures beyond that already required for direct smear microscopy. Some supervised training of existing smear microscopy laboratory technicians is required to change from a two-day to one-day sampling. Laboratories performing front-loaded microscopy require the equipment and consumables needed for traditional smear microscopy. National guidelines for TB control, forms and registers may need revision to account for changed protocols.

Current known developers: UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR, www.who.int/tdr)

Interferon gamma release assay

Interferon gamma release assays detect cellular immune responses to proteins that are specific for latent TB infection. If a patient is infected with TB, their immune cells will release IFN-gamma in response to stimulation by TB antigens. The results of interferon gamma release assays are based on the amount of IFN-gamma that is released in response to these antigens.



Potential advantages: Very high specificity and much less likely than the tuberculin skin tests (TST) to be confounded by exposure to environmental mycobacteria or by prior BCG vaccination. Does not boost responses that will be measured by subsequent tests, as happens with TST. Interferon gamma release assays do not require a second clinical contact to evaluate the test result, thus potentially reducing costs to the patient. Results can be available within 24 hours. Preliminary studies indicate that at least one test (the T-SPOT.TB® test) may have better overall sensitivity than the TST and better sensitivity for HIV-infected people and patients with extrapulmonary TB than the TST. Interferon gamma release assays are frequently packaged in easy-to-use kits.

Considerations: Although studies suggest assays are more closely associated with recent exposure risks than TST, they may have lower sensitivity than the TST for past infection. Assays do not discriminate between active and latent TB infection. Performance in immunosuppressed populations and children has not been extensively evaluated but studies suggest assays may be more sensitive than the TST. The test is moderately complex and requires standard ELISA equipment that may only be appropriate for use at district hospitals in many developing countries. Blood samples have to be incubated within 16 hours of being collected, which may require the use of portable incubators or establishment of systems enabling transportation to properly equipped laboratories for testing. Requires that blood be drawn from the patient with a needle, which can lead to other infections. The application of such tests in disease-endemic, developing countries where half the population is likely to be infected with M. tuberculosis is the subject of extensive investigation.

Requirements: Laboratory technicians require supervised training in the use of these tests. Depending on the kits chosen, laboratories using these tests may require some or all of the following: PBMC separation equipment and PBMC counting equipment (such as an inverted light microscope or an automated cell counter), an ELISPOT plate reader, a centrifuge, an incubator, water bath, microplate shaker, microplate washer and a microplate reader fitted with reference filters. Furthermore, the test requires several consumable products for which a supply chain must be established, including needles, centrifuge- and microcentrifuge tubes, PBS, cell culture media (RPMI 1640 or GIBCO AIM-VTM is recommended), Trypan Blue stain, variable volume and multichannel pipettes and pipette tips and 24-well culture plates. Interferon gamma release assay kits are typically proprietary products and countries may find it difficult to find local/national suppliers, so establishing a long-term support agreement with an international supplier is critical.

Product information: QuantiFERON®-TB Gold In Tube (QFT™) (www.cellestis.com); T-SPOT.TB® (www.oxfordimmunotec.com)

Current known developers: Cellestis, Inc. (www.cellestis.com); Oxford Immunotec Ltd. (www.oxfordimmunotec.com)

LED fluorescence microscopy

The use of fluorescence microscopy (FM) could potentially increase the sensitivity of smear microscopy and improve the efficiency of facilities. These microscopes allow a much larger area of the smear to be seen, resulting in more rapid examination of the specimen (up to four times faster) and making it easier to count bacilli.

Potential advantages: Light-emitting diodes (LEDs) are more sustainable and user-friendly than the quartz-halogen lamps or high-pressure mercury vapour lamps typically used in FM: powered correctly, they have an extremely long life expectancy (10,000 hours versus 200 for a conventional mercury lamp). Unlike the light emitted by mercury vapour lamps, LEDs do not produce ultra-violet (UV) light (a cause of concern to many users). LEDs significantly decrease the instrument's power consumption, allowing long-lasting battery operation. LED technology allows FM at a much lower cost than conventional FM.



Considerations: In some countries, it may be difficult to find the LED lamps required. Also, the Royal Blue colour LED lamp which works best for FM was only very recently developed and may not be widely available. Some issues with the stability of reagents under field conditions, and the stability of stained smears for blinded rechecking, have been reported. These need further investigation. Proper training is critical; it must be carefully considered and carried out properly. The sensitivity, specificity, cost-effectiveness and cost-benefit of this approach have not yet been adequately established. International guidance on quality assurance for FM does not currently exist.

Requirements: Apart from an LED microscope (or an adaptor to convert an existing microscope to LED FM) and stains for FM, this technology does not require equipment or biosafety measures beyond that already required for smear microscopy. Training of existing smear microscopy laboratory technicians is required to perform FM. LED FM may be powered by electricity or batteries. There are various manufacturers of LED fluorescence microscopes or adaptors converting standard light microscopes to LED fluorescence microscopes. These are proprietary products and countries may find it difficult to find local/national suppliers, so establishing a long-term support agreement with an international supplier is critical. Some LED equipment (the Fraen and the Lumin) can be used as add-ons to existing microscopes, while others (the iLED) are complete microscope kits.

Product information: Fraen AFTER® LED add-on kit or ready-to-use instrument (www.fraensrl.com/flmicro.html); LWS Lumin™ and QBC Paralens™ fluorescence microscopy system; Primo Star iLED microscope (www.zeiss.com); auramine stain (www.sigmaaldrich.com)

Current known developers: Carl Zeiss, Inc. (www.zeiss.com) in collaboration with FIND; Fraen Corporation (www.fraensrl.com); LW Scientific (lwscientific.com) and QBC Diagnostics (www.qbcdiagnostics.com); multi-centre evaluations being conducted by TDR.

Microscopic observation drug susceptibility (MODS)

Microscopic observation drug susceptibility (MODS) is a manual liquid culture technique that uses basic laboratory equipment (including an inverted light microscope) and microscopy skills to detect TB bacteria. Microscopic colonies (micro-colonies) of *M. tuberculosis* are observed in the culture media using an inverted microscope, through the bottom of a sealed plastic container. The characteristic pattern of growth of *M. tuberculosis* complex allows ready identification. Concurrent culture of sputum in drug-free and drug-containing media facilitates direct rifampicin and isoniazid DST.

Potential advantages: For detection of TB in sputum, MODS has been demonstrated in multi-center studies to be more sensitive and faster than conventional solid culture and automated liquid culture (median time to culture-positivity of the same samples cultured in MODS, automated MBacT and LJ were 7, 13 and 26 days respectively). Because MODS utilizes direct DST, median MDR detection time is also 7 days. MODS is relatively inexpensive compared to commercial broth-based liquid culturing systems.

Considerations: Like all liquid and solid culture techniques, MODS is more challenging than smear microscopy. Laboratory technicians may have difficulty distinguishing between the micro-colonies of TB and some nontuberculous mycobacteria (NTM). This could potentially have impact where NTM prevalence is high. MODS is delicate and requires experienced personnel. As with all TB culture systems, biosafety of laboratory workers must be taken into consideration. Further studies are required to adequately validate this technique.

Requirements: As with all TB culture systems, BSL-2 facilities are required for processing specimens and inoculating cultures. BSL-3 facilities are required if tubes need to be opened and the cultured organisms manipulated, such as for speciation or further testing. Laboratory technicians require training in BSL-2 or BSL-3 safety precautions as appropriate and in the use of MODS. Laboratories using MODS require functioning biosafety cabinets, a fridge/freezer for storing media, a vortex and centrifuge for sputum decontamination, an incubator and an inverted light microscope (such as the Radical RTC-6). Furthermore, MODS relies on a proprietary liquid medium called Middlebrook 7H9. A cold supply chain for OADC and PANTA and agreement with suppliers for proper support for equipment must be established.

Product information: Middlebrook 7H9, OADC and PANTA (www.bd.com) and Radical RTC-6 (www.radicalindia.com/)

Current known developers: Academic laboratories; PATH (www.path.org); Tulip Diagnostics (www.tulipgroup.com). Detailed technical information available at www.modsperu.org.

New solid culture methods: Nitrate reduction assay (NRA), e.g. Griess method

Solid culture technique measures nitrate reduction to indicate resistance to isoniazid and rifampicin. This technique is based on the property of TB to reduce nitrate to nitrite, which is revealed as a color change of the culture media.

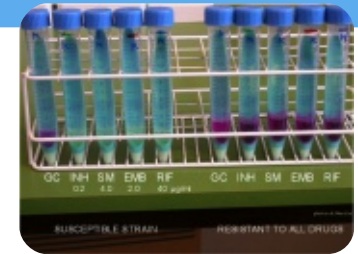
Potential advantages: The nitrate reductase assay (NRA) is less expensive to perform than liquid culture techniques for DST. Furthermore, since the NRA method makes use of the recognition of nitrate reduction as a sign of growth, results are acquired earlier than by eye examination of colonies in solid culture. Studies have shown that the specificity and sensitivity of NRA were comparable to traditional solid culture methods for DST of isoniazid and rifampicin. It uses solid media, which may be safer than liquid media. NRA does not need sophisticated equipment, is not complex to perform and could therefore be appropriate for laboratories with limited resources.

Considerations: The culture is killed by the mix reagent used to develop the assay, requiring that multiple cultures be prepared if comparative testing will be performed. Only fresh cultures must be used (<14 days). Additional studies and cost-effectiveness analyses are required to validate this technique and determine if it is appropriate for large-scale implementation in countries with a high prevalence of MDR-TB.

Requirements: As with all TB culture systems, BSL-2 facilities are required for processing specimens and inoculating cultures. BSL-3 facilities are required if tubes need to be opened and the cultured organisms manipulated, such as for speciation or further testing. Since speciation of *M. tuberculosis* growing in NRA cultures is done by visualization of a colour change in the tube (without opening them) BSL-2 facilities may be sufficient. Laboratory technicians require training in appropriate safety precautions and in how to perform NRAs. Laboratories performing NRA require the equipment and consumables required to perform solid culture (see above). Furthermore, the test itself requires several consumable products for which a supply chain must be established, including sodium nitrate and either hydrochloric acid, sulfanilamide and N-naphthylethylene-diamine (if the test will be performed with liquid reagents) or sulfanilic acid, N-(1-naphthyl)-ethylenediamine dihydrochloride and tartaric acid (if the test will be performed with a crystalline reagent).

Product information: sulfanilamide (www.merck.com); N-(1-naphthyl)-ethylenediamine dihydrochloride (www.sigmaaldrich.com); hypochloric acid (www.sigmaaldrich.com)

Current known developers: Academic laboratories



New solid culture methods: Thin layer agar culture (TLA)

The technique uses a standard microscope to simultaneously detect TB bacteria and indicate isoniazid and rifampicin resistance. Plates with a thin layer of agar medium are incubated and examined microscopically on alternate days for the first two weeks and less frequently thereafter.

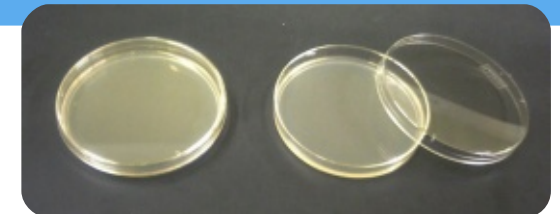
Potential advantages: Inexpensive compared to other culture techniques. Test can be done with a standard light microscope. At least one study has demonstrated that this technique provides results faster (in about 11 days) than traditional solid culture using Löwenstein-Jensen medium (26.5 days). Sensitivity obtained with thin layer agar is slightly better than with Löwenstein-Jensen. TB bacteria can be detected in as little as 7 days, with results for DST between 10-15 days. Uses solid media, which may be safer than liquid media. Simpler to manage large numbers of samples than for manual liquid culture.

Considerations: The contamination rate is slightly higher than when using Löwenstein-Jensen medium. Although reportedly faster than traditional solid culture techniques, it is not as fast or as sensitive as liquid culture. Further studies are required to adequately validate this technique.

Requirements: As with all TB culture systems, BSL-2 facilities are required for processing specimens and inoculating cultures. BSL-3 facilities are required if tubes need to be opened and the cultured organisms manipulated, such as for speciation or further testing. Since speciation of *M. tuberculosis* growing in TLA cultures is done without opening the Petri dishes, BSL-2 facilities may be sufficient. If Petri dishes cannot be adequately sealed, however, then BSL-3 facilities are required. Laboratory technicians require training in the appropriate safety precautions and in the use of thin layer agar cultures. Laboratories performing the thin layer agar technique require the equipment and consumables to perform solid culture (see above), as well as additional consumable products for which a supply chain must be established: OADC, PANTA, Middlebrook agar-based medium and Petri dishes.

Product information: OADC, PANTA, Middlebrook 7H10 or 7H11 agar (www.bd.com)

Current known developers: Academic laboratories



Case study: Peru

According to the most recent WHO Global Tuberculosis Control Report, in 2006 there were 34,311 TB cases and 893 confirmed MDR-TB cases in Peru. One of the strategies of the Peruvian National TB Control Programme to control MDR-TB includes decentralization of first-line DST to district laboratories to screen patients at risk of MDR-TB. As part of this strategy the National Reference Laboratory first validated the direct Griess method, a colorimetric method using nitrate reductase reaction to indicate the growth of TB on solid medium. From 2005 this method was then progressively decentralized to district laboratories. Two district laboratories served 228 health establishments with a population of more than 5 million people. From January 2005 to July 2008 these district laboratories performed a total of 14,907 DSTs. With adequate quality assurance and training, this method performed well as a rapid test to screen for MDR-TB at district level. The method proved to be inexpensive, simple and robust. The median time from sputum collection to DST result was 31 days as compared to 99 days for conventional DST in a pilot district laboratory. Decentralization of first-line DST to district laboratories decreased the bottleneck of the central level and improved access to early diagnosis and treatment.

Tools in an early phase of development

Researchers continue to develop and evaluate diagnostic tools beyond those described in this document. There is a wide array of products in the pipeline with different expected dates of availability – and a new tool may be superseded quickly by a newer tool, where appropriate evidence exists to support it. It is important that programmes continue to monitor the progress of research and carefully consider the potential of future tools when planning to invest in existing tools. Below is a short summary of several other promising new diagnostic tools that still require further development and/or evaluation, but evidence for some could be ready for review by WHO in the next few years.

Breathalyser screening test

A test for respiratory forms of the disease that could be used in the community to screen high risk groups. Patients cough into a disposable device which is placed in the instrument for detection of volatile organic compounds. The instrument is fully portable and runs off rechargeable AA batteries. The test is performed and readout obtained in under 10 minutes. Limited performance data is available and further evaluation studies are required. **Developers:** Rapid Biosensor Systems Ltd (www.rapidbiosensor.com). **Level of health system:** community or point-of-care.

First-generation loop-mediated isothermal amplification (LAMP) assay

Molecular amplification methods are proven technologies for the detection of TB but have not been widely used in remote settings because of the cost and complexity. LAMP is a simple DNA amplification method that does not require a thermocycler or detection system and reportedly allows visual detection of amplification, possibly allowing it to be used at lower levels of the health system. **Developers:** Eiken Chemical Co. Ltd. (www.eiken.co.jp) and FIND. **Level of health system:** Peripheral laboratory.

Lipoarabinomannan (LAM) detection in urine

M. tuberculosis LAM has been shown to be excreted in the urine of TB patients. Urine is an easier specimen to collect than sputum, and may be less variable in quality and safer to handle. There are several versions of this assay in development, including in-tube ELISA and dipstick methods. Urinary antigen detection may be of particular value in diagnosing TB in HIV-coinfected patients. It may prove valuable for rapid and simple diagnosis of TB in particular in developing countries at peripheral levels. **Developers:** Inverness Medical Innovations, inc. (www.invernessmedical.com). **Level of health system:** Peripheral laboratory.

Phage-based tests

A method for detecting rifampicin resistance directly from sputum smear positive samples or indirectly from culture. The test has a manual format and results are read by eye. Results are reportedly available within 2 days and utilize basic microbiological equipment and skills available in most laboratories. The performance of the FASTPlaque-Response has been evaluated in a TDR sponsored Phase III clinical trial in Peru. Phase IV demonstration evaluations are necessary to assess use of the diagnostic in the public sector of low-income countries. **Developers:** Fast-Plaque™ Response Test, by BIOTEC Laboratories, Ltd. (www.biotec.com); academic laboratories. **Level of health system:** Reference laboratory.

Sodium hypochlorite (bleach) microscopy

The digestion of sputum with household bleach prior to sputum smear preparation and microscopy has been reported to be an effective, simple method to improve the yield of smear microscopy even in high HIV-prevalence settings. Progress on the development of a bleach microscopy method has been complicated by the wide heterogeneity, and lack of standardization, in methods described. A standardized bleach method, the Mathare Sodium Hypochlorite (MaSH) method, has recently been developed and evaluated in MSF-sponsored studies in Mathare, Nairobi. The addition of a standardized sodium hypochlorite solution to sputum followed by overnight sedimentation resulted in a 15% increase in TB cases detected. The MaSH Method is now being evaluated by TDR under operational conditions in large multi-country studies. **Developers:** TDR. **Level of health system:** Peripheral laboratory.

Sputum filtration

In this method, sputum is liquefied and passed through a filter, which is then stained or cultured by standard techniques. Filtration considerably concentrates mycobacteria, increasing sensitivity. Another advantage of using concentrating sputum is the reduction in time spent on sputum examination. Some studies have been published on this method, but further validation studies are required. **Developers:** Academic laboratories. **Level of health system:** Peripheral laboratory.

TB Patch Test

The TB Patch Test is a point-of-care diagnostic used to diagnose active TB. The Patch delivers MPT64, a protein specific to the organisms that cause TB. In those with active, infectious TB, a localized immune response consisting of erythema and/or vesiculation appears 3–4 days after application to the skin. The patch reportedly avoids responding to BCG vaccination, to other mycobacteria or to a previous TB infection that has been cured with drugs. Enrollment in a Peru clinical trial complete; data analysis ongoing. Additional trials in the Philippines began in 2007. **Developers:** Sequella (www.sequella.com). **Level of health system:** Health post.

Vital fluorescent staining of sputum smears

Unlike most fluorescent stains, fluorescent vital dye only stains living, culturable organisms. While a positive finding provides a basis for initiating antibiotic treatment, the sensitivity of the direct smear is highly variable. Note that use of this dye requires a fluorescent microscope. This technique might be most appropriate for use with patients not responding to therapy. **Developers:** Academic laboratories. **Level of health system:** Peripheral laboratory.

Select documents and resources for further reading

The following are a selection of documents that may be valuable to you as you prepare to retool your diagnostics programme and consider new diagnostic tools.

- Evidence-based tuberculosis diagnosis. M Pai, A Ramsay, R O'Brien. PLoS Med. 2008 Jul 22;5(7):e156.
- Using a bus service for transporting sputum specimens to the Central Reference Laboratory: effect on the routine TB culture service in Malawi. AD Harries, J Michongwe, TE Nyirenda, JR Kemp, SB Squire, AR Ramsay, P Godfrey-Faussett, FM Salaniponi. International Journal of Tuberculosis and Lung Diseases. 2004. 8(2): 204-210
- Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient? M Yagui, MT Perales, L Asencios, L Vergara, C Suarez, G Yale, C Salazar, M Saavedra, S Shin, O Ferrousier, P Cegielski. Int J Tuberc Lung Dis. 2006 Aug;10(8):838-43.
- Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. KR Steingart, A Ramsay, M Pai. Expert Reviews in Anti-infective Therapy, 2007. Jun;5(3):327-31).
- Reducing the number of sputa examined, and thresholds for positivity: an opportunity to optimize smear microscopy. M Bonnet, A Ramsay, F Varaine, W Githui, L Gagnidze, P Guerin. Accepted by International Journal of TB and Lung Diseases. 2007. Sep;11(9):953-8.
- The bleach microscopy method and case detection for tuberculosis control. A Ramsay, SB Squire, K Siddiqi, J Cunningham, MD Perkins. Int J Tuberc Lung Dis. 2006 Mar;10(3):256-8.
- Bleach Sedimentation: An opportunity to optimize smear microscopy for tuberculosis in settings of high prevalence of HIV. M Bonnet, A Ramsay, W Githui, L Gagnidze, F Varaine, PJ Guerin. Clinical Infectious Diseases, 2008. June. 46(11): 710-6)
- Commercial serological tests for tuberculosis - do they work? K Steingart, A Ramsay, M Pai. Future Microbiology. July 2007. Aug;2:355-9.
- Laboratory-based evaluation of 19 commercially-available rapid diagnostic tests for tuberculosis. WHO/TDR. 2008. World Health Organization, Geneva.
- New Diagnostics for Latent and Active Tuberculosis: State of the Art and Future Prospects. M Pai, R O'Brien. Semin Respir Crit Care Med. 2008. 29: 560-568.
- Facing the crisis: Improving the diagnosis of tuberculosis in the HIV era. MD Perkins, J Cunningham. 2007. Journal of Infectious Diseases. 196 (Supp 1): s15-s27.
- Diagnostics for Tuberculosis: Global Demand and Market Potential. 2007. WHO/TDR.
- Operational feasibility of using loop-mediated isothermal amplification (LAMP) for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. CC Boehme, P Nabeta, G Henostroza, R Raqib, Z Rahim, M Gerhardt, E Sanga, M Hoelscher, T Notomi, T Hase, MD Perkins. J Clin Microbiol. 2007 Jun;45(6):1936-40. Epub 2007 Mar 28.
- Stop TB Partnership Retooling Task Force. New Technologies for TB Control: A Framework for their Adoption, Introduction and Implementation. Geneva, World Health Organization, 2007 (WHO/HTM/STB/2007.40).
- Stop TB Partnership Retooling Task Force. Engaging Stakeholders For Retooling TB Control. Geneva, World Health Organization, 2008.
- Stop TB Partnership Retooling Task Force. Liquid Culture Checklist. Geneva, World Health Organization. 2008.

Abbreviations/acronyms used in this document

Abbreviation	Means
BCG	Bacille Calmette-Guérin – current vaccine for TB
BSL	Biosafety level
CDC	United States Center for Disease Control and Prevention
DST	Drug susceptibility testing
FIND	Foundation for Innovative New Diagnostics
FM	Fluorescence microscopy
LED	Light-emitting diode
MDR-TB	Multidrug-resistant TB
MGIT	Mycobacterium Growth Indicator Tube
MODS	Microscopic Observation Drug Susceptibility
NTLP	National TB and Leprosy Programme
NTM	Nontuberculosis mycobacteria
NRA	Nitrate reductase assay
NTP	National TB Programme
TB	Tuberculosis
TDR	UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TST	Tuberculin skin test
UPS	Uninterrupted power supply
UV	Ultra-violet
WHO	World Health Organization
XDR-TB	Extensively drug-resistant TB

Acknowledgements

The following individuals were involved in the development of this framework, and their contribution is gratefully acknowledged.

Stop TB Partnership Task Force on Retooling

Mohamed Aziz, Rachel Bauquerez (Secretariat), Delia Boccia, Jean-Francois de Lavison, Saidi Egwaga, Ulrich Joseph Fruth, Michel Gasana, Christy Hanson (Co-chair), Barbara Laughon, David Lee, Davide Manissero, Robert Matiru, Vinand Nantulya (Co-chair), PR Narayanan, Ikushi Onozaki, Philip Onyebujoh, Andrew Ramsay, Guy Stallworthy, Javid Syed, Francis Varaine, Rosalind Vianzon, Karin Weyer, Jennifer Woolley.

Working Group on New TB Diagnostics

Giorgio Roscigno (Chair), Andrew Ramsay (Secretariat), core group members and sub-group chairs.

Other reviewers and contributors

Michael Brennan (Aeras), Leopold Joseph Blanc (WHO), Marcos Espinal (Stop TB Partnership), Ruth McNerney (London School of Hygiene & Tropical Medicine), David Moore (Imperial College), Madhukar Pai (McGill University), Mark Perkins (FIND), Mario Raviglione (WHO), Véronique Vincent (WHO), Diana Weil (WHO).

Additional support

The development of this document was made possible thanks to the generous contribution of the Stop TB Partnership.

Photos courtesy of

Becton, Dickinson and Company, Carl Zeiss, Inc., Cellestis, Inc., Hain Lifescience, Inc., Hangzhou Genesis Biodetection and Biocontrol, Ltd., Dr. Anandi Martin of the Institute of Tropical Medicine Antwerp, and TDR.

About the Stop TB Partnership's Retooling Task Force

Previous experience with the introduction of new tools for the control of other communicable diseases has shown that there can be a significant delay between the availability of new tools at global level and their eventual adoption and implementation at country level. Recognizing this important delay between formulation and implementation of policy, the Stop TB Partnership (<http://www.stoptb.org>) has established a Task Force on Retooling. Its task is to develop a framework for catalyzing policy-makers and practitioners at global and national levels towards accelerated introduction of new tools into national TB programmes and national immunization programmes. One of its aims is to stimulate discussion and planning for optimal, timely and appropriate introduction, adoption and implementation of new tools as they become available.

The main activities of the Retooling Task Force are to:

- Consolidate and share information from the working groups on drugs, diagnostics and vaccines on current product pipelines and timelines/milestones.
- Create opportunities for consultative dialogue with stakeholders from high TB burden countries, including ministries of health, NGOs, affected communities, etc.
- Facilitate the mobilization of financial and human resources for country-level introduction and deployment.
- Consolidate relevant lessons learnt from other disease areas to inform TB-specific processes for adoption, introduction and implementation.
- Facilitate operational research on introduction of new tools.
- Generate evidence to support the adoption of new tools.
- Fast-track the incorporation of new tools into WHO and national policies and guidelines.
- Enhance communication among all working groups around the theme of retooling.

More information about the Retooling Task Force is available at <http://www.stoptb.org/retooling/> or by email to stoptbretooling@who.int.

Partner	Contact
Aeras Global TB Vaccine Foundation	http://www.aeras.org/
Mérieux-Alliance	http://www.biomerieux.com/
Foundation for Innovative New Diagnostics (FIND)	http://www.finddiagnostics.org/
Global Alliance for TB Drug Development	http://new.tballiance.org/
Médecins Sans Frontières (MSF)	http://www.msf.org/
National Institute of Allergy and Infectious Diseases (NIAID)	http://www3.niaid.nih.gov/
Research Institute of Tuberculosis (RIT)	http://www.jata.or.jp/eindex.htm
South African Medical Research Council	http://www.mrc.ac.za/
Stop TB Partnership	http://www.stoptb.org/
Stop TB Partnership Global Drug Facility	http://www.stoptb.org/gdf
Stop TB Partnership Global Laboratory Initiative	http://www.who.int/tb/dots/laboratory/gli
Treatment Action Group (TAG)	http://www.aidsinfonyc.org/tag/
United States Agency for International Development (USAID)	http://www.usaid.gov/
WHO Department of Technical Cooperation for Essential Drugs and Traditional Medicine	http://www.who.int/medicines/about/tcm/en/
WHO Special Programme for Research and Training in Tropical Diseases (TDR)	http://www.who.int/tdr/
WHO Stop TB Department	http://www.who.int/tb/en/

Stop TB Partnership and World Health Organization. New Laboratory Diagnostic Tools for TB Control. Geneva, World Health Organization, 2008.

www.stoptb.org

ISBN 978 92 4 159748 7

