

TB DIAGNOSTICS PIPELINE

**New TB Diagnostics Working Group
November 2007**

Health services have been divided into three levels based on consideration of the intended level of the health system where the new tools are to be used: reference laboratory, peripheral laboratory and health post.

A. **Reference laboratory** is defined as a regional, tertiary or national level laboratory with mycobacterial culture capability.

B. **Peripheral laboratory** is defined as a laboratory at health centre or higher level where sputum smear microscopy is conducted, and where auxiliary equipment such as biosafety cabinets, centrifuges and incubators is unlikely to be present.

C. **Health post** is defined as a primary health care facility with no on-site access to microscopy or other laboratory testing.

The selected technologies described below as examples are grouped according to their intended level of implementation. These examples were selected as being illustrative of a process which involves submission to STAG-TB for consideration.

In general, the highest impact on case-finding will accrue from implementation at the lowest level of health services.

Some of the diagnostic tools expected to be introduced into control programmes will be incremental improvements on existing technologies, while others will be radically new. The speed and extent of adoption of new technologies will depend on the balance between the benefits they bring and the degree of disruption their implementation causes. For instance, a simplified microscopy method may see greater adoption than a novel alternative that necessitates changes in the way testing or case notification are carried out. On the other hand, a new method that rapidly identifies all smear-positive and many smear-negative cases might, if suitably robust and specific, see widespread use and substantially replace microscopy.

Disclaimer: The TB portfolio are not intended to be fully comprehensive but it rather represents those products in development that have been brought to the attention of the working groups. Please note that no standard evaluation criteria have been applied for the development of this TB portfolio. The list will be revised continuously by the Stop TB working groups. Updated versions will be published annually through the Retooling and working groups' websites.



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A. REFERENCE LABORATORY

1. Liquid culture system for case detection and drug susceptibility testing (DST)

Type of product
Liquid culture system
Product
Mycobacterium growth indicator tube (MGIT-TB) and MGIT-DST
Developers
Foundation for Innovative New Diagnostics (FIND) and Becton, Dickinson and Company (BD)
Rationale
It is recognized that direct smear microscopy has limited sensitivity, especially in some patient groups such as children and individuals coinfecting with HIV. Culture is substantially more sensitive than microscopy. Liquid culture systems are more rapid and more sensitive than solid culture.
Product profile
The MGIT-TB culture system allows for the rapid growth and detection of <i>M. tuberculosis</i> . The average time to detection is 10–14 days as opposed to 3–4 weeks with traditional solid egg or agar-based culture. MGIT can also be used to diagnose multidrug-resistant TB (MGIT-DST). FIND, together with BD, is evaluating MGIT-DST to determine the feasibility and impact of its wider use in disease-endemic settings.
Stage of development
Both of these products are already in use in wealthier countries and in the private sector of some developing countries. Currently, MGIT-TB culture and MGIT-DST are being evaluated in demonstration projects to assess their effectiveness, efficiency and impact in the field situation in national control programmes of low-income countries with high TB prevalence.
Considerations
Limitations: tests intended for use where culture and biosafety facilities exist or can be built. Additional care is required to minimize contamination of culture.
Earlier date for availability for adoption in public sector
Beginning of 2008

Under "Considerations": State "Manual and automated systems available".]

2. Alternative liquid culture system for case detection and drug susceptibility testing.

Type of product
Liquid culture system
Product
MODS (Microscopic-Observation Drug-Susceptibility)
Developers
Academic laboratories
Rationale
Sputum smear microscopy is insensitive. Culture is considerably more sensitive. Liquid culture is quicker and more sensitive than solid culture.
Product profile
Microwell observation of growth, reduced biohazard, rapid diagnosis and simultaneous DST, colorimetric indicators being developed to reduce workload. Modification for colorimetric readout under evaluation. Inexpensive 'in house' technique costing <\$2 per test but inverted light microscope required.
Stage of development
Validation papers published. Requires evaluation in demonstration projects.
Considerations
Good step towards simplifying culture. Reduced biohazard issues by double-sealing cultures after sputum processing. Microscopic examination allows concurrent TB detection and DST in 1-3 weeks.
Earlier date for availability for adoption in public sector
2008

3. Rapid solid culture system for case detection and drug susceptibility testing.

Type of product
Rapid solid culture system for case detection and drug susceptibility testing
Product
TLA (Thin Layer Agar) Culture
Developers
Academic labs
Rationale
Inexpensive 'in house' technique costing <\$2 per test and uses standard microscope.
Product profile
<ul style="list-style-type: none"> ▪ Thin layer agar for rapid diagnosis and simultaneous identification and DST (H,R) directly on sputum smear positive samples. May be performed in microwell format (equivalent to MODS on solid media) or in traditional Petri dish format. ▪ Modification for colorimetric readout and DST for FQ under evaluation.
Stage of development
Validation papers published. Requires evaluation in demonstration projects. Validation of colorimetric method
Considerations
Possibly easier to perform than MODS but solid media likely to be slightly less sensitive and rapid. Reduced biohazard issues by double-sealing cultures after sputum processing. Microscopic examination allows concurrent TB detection and DST in 1-3 weeks.
Earlier date for availability for adoption in public sector
2008

4. Rapid solid culture system for case detection and drug susceptibility testing.

Type of product
Rapid method for DST in solid medium
Product
NRA (Nitrate reductase Assay)
Developers
Academic labs
Rationale
Conventional DST method takes weeks to give results. NRA is a rapid method that can be applied directly to sputum smear-positive sample for first-line drugs.
Product profile
Color reaction to detect growth on LJ slants, can be used direct from sputum, quicker than the conventional LJ method, simple and robust
Stage of development
Validation paper published. NRA well evaluated in clinical isolates. Meta-analysis on going. Require demonstration project in sputum smear positive samples.
Considerations
Cheap and quick; throughput limited.
Earlier date for availability for adoption in public sector
2008

5. Colorimetric DST profiling

Type of product
Colorimetric DST profiling
Product
Resazurin, MTT as redox-indicators
Developers
Academic labs
Rationale
Liquid culture is quicker and more sensitive than solid culture. DST results can be obtained in 8 days for first and second-line drugs.
Product profile
Inexpensive “in house” technique. Starting from culture, rapid DST profiling available using overnight incubation with redox dye.
Stage of development
Well validated, meta-analysis
Considerations
Needs initial primary culture, but is cheap and quick and can handle high throughput easily.
Earlier date for availability for adoption in public sector
2008



5b. Slide drug susceptibility testing (for district level)

Type of product
Rapid method for DST in solid medium
Product
Slide DST (slide drug susceptibility testing)
Developers
Academic labs
Rationale
Inexpensive "in house" technique. Detection of microcolonies growth on culture slide view under a standard microscope
Product profile
Rapid detection of resistance to rifampicin, kanamycin and gatifloxacin directly on sputum smear positive samples
Stage of development
Under early evaluation
Considerations
Microscope examination allow rapid detection of DST
Earlier date for availability for adoption in public sector
2010

6. Speciation test

Type of products
Test for confirming <i>M. tuberculosis</i> grown in culture
Product
Capilia TB
Developers
FIND and Tauns Co. Ltd
Rationale
Not all AFB grown in culture are <i>M. tuberculosis</i> . Existing tests for confirming identification of <i>M. tuberculosis</i> are time consuming and complex. The Capilia TB test is a simple and fast lateral flow technology that allows the confirmation of <i>M. tuberculosis</i> in cultures in 15 minutes.
Product profile
The Capilia test is a lateral flow (strip test) detecting a TB-specific antigen.
Stage of development
The test is in the demonstration phase to assess effectiveness in real-life disease control programmes.
Considerations
Limitations: tests intended for use where culture facilities exist or can be built.
Earliest date for availability for adoption in the public sector
Late 2008

7. Phage-based DST

Type of product
Phage-based test
Product
FASTPlaque-Response test
Developers
FIND and Biotec Laboratories
Rationale
Conventional detection of multidrug resistance requires isolation of <i>M. tuberculosis</i> in culture prior to DST. This leads to a long turnaround time of weeks to months. The FASTPlaque-Response assay is applied directly to sputum smear-positive samples, with rifampicin-susceptibility results obtained in just two days.
Product profile
The FASTPlaque assay is bacteriophage-based and allows detection of rifampicin resistance directly from smear-positive sputum or indirectly from culture. Rifampicin resistance in most settings serves as a marker for multidrug resistance.
Stage of development
The test is in the demonstration phase in selected countries where its efficiency and effectiveness are to be assessed.
Considerations
Limitations: tests intended for use where culture and biosafety facilities exist or can be built.
Earliest date for availability for adoption in the public sector
End of 2008

8. Manual nucleic acid amplification DST

Type of product
Molecular technique
Product
PCR-based assay
Developers
FIND and HAIN Lifescience GmbH
Rationale
The rapid detection of multidrug resistance could facilitate early initiation of correct treatment or appropriate measures to prevent transmission. The manual-based nucleic acid amplification DST detects rifampicin and isoniazid resistance in one day.
Product profile
PCR-based line-probe assay
Stage of development
The test is entering the evaluation phase.
Considerations
Limitations: tests intended for use where culture, biosafety and polymerase chain reaction (PCR) capabilities exist or can be built. Requirement for specialized equipment (Thermocycler) and training. The sensitivity of the assay for isoniazid resistance is only 60–70%.
Earliest date for availability for adoption in public sector
2008

9. Automated nucleic acid amplification test DST

Type of product
Molecular technique
Product
PCR-based
Developers
FIND and Cepheid
Rationale
Molecular amplification is a proven technology for the detection of <i>M. tuberculosis</i> . Current test methods, however, are too complex for routine widespread implementation in developing countries. Sample processing and DNA extraction adds significantly to this complexity. An assay that automates all of these steps could make nucleic acid amplification testing (NAAT) much simpler to implement. Molecular detection of rifampicin resistance could speed targeted treatment and other measures for controlling MDR-TB.
Product profile
FIND and Cepheid are working to develop an automated method to integrate sputum processing, DNA extraction as well as amplification and detection of TB DNA and rifampicin-resistance encoding mutations.
Stage of development
Under development
Considerations
Limitations: requirement for specialized equipment and limited training; security; electricity.
Earliest date for availability for adoption in the public sector
2010



10. Urinary nucleic acid amplification

Type of product
Molecular technology
Product
PCR-based test for <i>M. tuberculosis</i> DNA in urine
Developers
FIND, University College London, Spaxen
Rationale
Fragments of <i>M. tuberculosis</i> DNA have been shown to be excreted in urine. Urine is less variable and easier to collect than sputum and may be safer to handle. These characteristics may make this method applicable in less complex settings if paired with an appropriately simple amplification method.
Product profile
This is a method, not a product, and will need to be applied to an existing molecular amplification platform.
Stage of development
This method is under development.
Considerations
Limitations: tests intended for use where PCR capabilities exist or can be built. Requirement for specialized equipment (Thermocycler) and training.
Earliest date for availability for adoption in the public sector
2011

B. PERIPHERAL LABORATORY

1. Same-day sputum smear microscopy

Type of product
Optimizing sputum microscopy
Product
Same-day two-smear strategy
Developers
TDR
Rationale
The current sputum smear microscopy strategy requires direct examination of three sputum specimens obtained on 2–3 different days. This strategy delays the diagnosis and leads to patient drop-out during the diagnostic process.
Product profile
The same-day approach requires a patient to produce two separate sputum samples to curtail patient drop-out, thereby increasing case detection.
Stage of development
Under evaluation in operational settings; moving towards demonstration.
Considerations
More cases require links with treatment centre/drugs. Possible cost saving/consumables. Reorganization of the laboratory workflow to maximize benefit.
Earliest date for availability for adoption in the public sector
End of 2008

2. Low-cost fluorescence microscopy

Type of product
Sputum microscopy
Product
Fiber-optic-based fluorescence microscopy system
Developers
TDR
Rationale
Fluorescence microscopy increases the sensitivity of direct smear microscopy. The expense of the conventional fluorescence microscopy system precludes its widespread use at peripheral level. This system offers a cheaper alternative. Potential exists for further optimization of smear microscopy by combining low-cost fluorescence microscopy with bleach digestion and/or same-day approaches.
Product profile
A simple objective with light filters that can be fitted to most standard makes of microscopes and is connected by a fiber-optic cable to a halogen light source.
Stage of development
The product is already available and in use by some organizations in the field. Evaluation in operational settings and a move to demonstration projects are planned for 2007.
Considerations
Requires training. As yet, no guidelines for external quality assessment (EQA). Fluorescence microscopy may represent a broad platform, with advantages in the diagnosis of other diseases. Considerable time will be saved in microscopic examination of smears.
Earliest date for availability for adoption in the public sector
End of 2008

3. Bleach digestion of sputum

Type of product
Sputum smear microscopy
Product
Bleach
Developers
TDR
Rationale
Digestion of sputum with bleach (sodium hypochlorite) prior to the preparation of smears has been shown to increase the sensitivity of smear microscopy.
Product profile
Bleach digestion of sputum. Various methods of bleach digestion have been described involving different digestion times and supplementary processes.
Stage of development
Developing a standardized bleach digestion method to be put into demonstration projects in 2008.
Considerations
The expected reduction in biohazard resulting from bleach digestion will be evaluated prior to demonstration projects.
Earliest date for availability for adoption in the public sector
End of 2009

4. LED fluorescence microscopy

Type of product
Fluorescent microscope
Product
LED microscope
Developers
FIND and commercial partner
Rationale
Existing conventional fluorescence systems increase the sensitivity of direct smear microscopy. The LED microscope lamp is inexpensive in comparison to mercury vapour or the halogen lamp used in the regular fluorescent microscope and may have a lifespan of more than 50 000 hours.
Product profile
Simple binocular microscope incorporating LED light source.
Stage of development
Microscope in development phase.
Considerations
Training; as yet no EQA system for fluorescence microscopy; may represent a broad platform, with advantages in the diagnosis of other diseases; considerable time-savings in microscopic examination.
Earliest date for availability for adoption in the public sector
2009

5. First-generation isothermal nucleic acid amplification

Type of product
Molecular technique
Product
First-generation LAMP-based assay (loop-mediated isothermal amplification technology platform).
Developers
FIND and EIKEN Chemical Co. Ltd
Rationale
A simple DNA amplification method that does not require an expensive thermocycler or detection system and that allows visual detection of amplification could allow sensitive molecular methods to be used at lower levels of the health system.
Product profile
Preliminary data suggest high sensitivity and specificity. Modifications of the assay may be suitable for implementation at microscopy level. It is envisaged that the method may be applied to sputum, urine and blood specimens.
Stage of development
This product is in the development phase.
Considerations
Cross-disease platform; training required; somewhat more complex than microscopy, but with potential for further simplification and implementation at peripheral laboratory.
Earliest date for availability for adoption in the public sector
2010

6. Filter concentration of sputum

Type of product
Sputum concentration
Product
Sputum filtration
Developers
Academic Labs
Rationale
A method for concentrating sputum that does not require centrifugation or long sedimentation times.
Product profile
Sputum liquefied and passed through filter which is then stained or cultured by standard techniques. Filtration considerably concentrates mycobacteria, increasing sensitivity.
Stage of development
Some results published, optimization and validation studies needed
Considerations
Effective but throughput limited in published formats.
Earliest date for availability for adoption in the public sector
2009

7. Early detection of treatment failure

Type of product
Vital staining of smears
Product
Fluorescent vital dye for modified sputum smear microscopy
Developers
Academic labs
Rationale
Current smear microscopy methods cannot distinguish between live and dead bacilli. This is a limitation when using smears to monitor response to treatment. Vital stains (ie only stain live bacilli) may be used to overcome this.
Product profile
Simple rapid and inexpensive procedure equivalent to traditional auramine microscopy but stains only living, culturable, not dead mycobacteria. Performed before and after 10-14 days therapy identifies patients who are not responding to therapy and may have MDRTB.
Stage of development
Under early evaluation.
Considerations
Identifies probable MDRTB for early identification of treatment failure.
Earliest date for availability for adoption in the public sector
2010



C. HEALTH POST

No tests are currently available for use at the health post level of the health system. Bringing diagnostics to this level would be a tremendous achievement, with great implications for the ability of a control programme to increase case detection. In general, the technology best suited for this level is lateral flow or other immunochromatographic strip test.

1. Urinary antigen detection

Type of product
Antigen detection test
Product
LAM (mycobacterial lipoarabinomannin) antigen detection in urine.
Developers
FIND and partners
Rationale
<i>M. tuberculosis</i> 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.
Product profile
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. There is potential for further development and simplification, resulting in a lateral flow test.
Stage of development
This test is under development.
Considerations
Lateral flow test – implementation could significantly increase case-finding through improved access to testing. The ELISA format has potential to increase case-finding if combined with smear microscopy and/or culture in settings of high HIV prevalence. Possible improvements in the diagnosis of paediatric and extrapulmonary TB.
Earliest date for availability for adoption in the public sector
2010



2. Antibody detection tests

Type of products
Antibody detection tests
Product
Development pending identification of suitable antigens
Developers
FIND and partners
Rationale
TB patients often have detectable antibodies to a variety of <i>M. tuberculosis</i> antigens. Currently, the commercially-available serological tests have been shown to perform poorly. A limited number of potential diagnostic antigens have been evaluated. FIND is systematically interrogating the proteome of <i>M. tuberculosis</i> for potential diagnostic antigens.
Product profile
The likely final product would be an immunochromatographic lateral-flow or flow-through test.
Stage of development
Antigen identification stage.
WHO guidance on adoption and recommendations
A performance dossier will be presented to STAG at its meeting in May 2011.
Considerations
Antibody detection may not perform well in patients with HIV-mediated immunosuppression.
Earliest date for availability for adoption in the public sector
2011