



**NON-COMMERCIAL CULTURE AND DRUG-SUSCEPTIBILITY
TESTING METHODS FOR SCREENING OF PATIENTS AT RISK
OF MULTI-DRUG RESISTANT TUBERCULOSIS**

- POLICY STATEMENT -

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EXECUTIVE SUMMARY

Commercial liquid culture systems and molecular line probe assays have been endorsed by the World Health Organization (WHO) as gold standards for rapid detection of multidrug-resistant tuberculosis (MDR-TB); however, due to technical complexity, cost, and the need for sophisticated laboratory infrastructure, uptake of these technologies has been limited in many resource-constrained settings.

Several non-commercial culture and drug susceptibility testing (DST) methods have been developed over recent years, aimed at settings with limited access to sophisticated laboratory infrastructure and technical expertise. Several methods have shown initial promise as rapid, inexpensive methods. Those most advanced include microscopic observation of drug susceptibility (MODS), colorimetric redox indicator (CRI) methods, thin-layer agar (TLA) methods, the nitrate reductase assay (NRA), and mycobacteriophage-based assays.

- **MODS:** A microcolony direct method in liquid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
- **TLA:** A microcolony direct method on solid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
- **CRI methods:** Indirect testing methods based on the reduction of a coloured indicator added to liquid culture medium in a microtitre plate after in vitro exposure of *M. tuberculosis* strains to anti-TB drugs;
- **NRA:** A direct and/or indirect method based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a coloured reaction;
- **Phage-based assays:** Assays which uses bacteriophages to infect and detect the presence of viable *M. tuberculosis* in clinical specimens and culture isolates.

In 2009, the strength of the evidence for these noncommercial methods was assessed by WHO following standards appropriate for evaluating both the accuracy and patient/public health impact of new TB diagnostics. Results showed that current evidence is insufficient to recommend the use of TLA or phage based assays. CRI methods, MODS and NRA were judged to have sufficient evidence to consider their use under clearly-defined programmatic and operational conditions, in reference laboratories and under strict laboratory protocols, and as an interim solution while capacity for genotypic and/or automated liquid culture and DST are being developed.

Under these conditions, MODS and NRA are recommended for direct testing of sputum specimens. Together with CRI methods, MODS and NRA are also recommended for indirect DST of *Mycobacterium tuberculosis* isolates grown from conventional culture. Time to detection of MDR-TB may not necessarily be faster in indirect testing and none of these methods are able to detect extensively drug-resistant TB. Conventional culture and DST capacity is therefore still required in all settings.

POLICY STATEMENT

NON-COMMERCIAL CULTURE AND DRUG-SUSCEPTIBILITY TESTING METHODS FOR RAPID SCREENING OF PATIENTS AT RISK OF MULTI-DRUG RESISTANT TUBERCULOSIS

1. Background

Early detection of drug resistance in tuberculosis (TB) allows the use of appropriate treatment regimens for patients, which has an important impact for improved TB control. Spread of drug resistant strains of *Mycobacterium tuberculosis* and the management of patients diagnosed with drug resistant disease is one of the most formidable obstacles faced by national tuberculosis control programmes, compounded by a lack of appropriate diagnostic tools and vastly inadequate laboratory capacity.

The development of rapid methods for drug susceptibility testing (DST) is crucial due to increasing rates of multidrug-resistant tuberculosis (MDR-TB) worldwide and the emergence of extensively drug-resistant tuberculosis (XDR-TB), with very high reported HIV-associated mortality. Conventional culture and DST methods require prolonged periods to confirm mycobacterial growth and detect drug resistance, during which time patients may be inappropriately treated, drug resistant strains may continue to spread, and amplification of resistance may occur. Rapid diagnosis of TB and drug resistance will therefore have obvious patient- as well as public health benefits, including better prognosis, increased survival, prevention of acquisition of further drug resistance, and reduced spread of drug resistant strains to vulnerable populations.

No single test currently satisfies all the demands of 'quick', 'cheap', and 'easy'. Commercially available liquid culture systems and molecular line probe assays for rapid detection of MDR-TB have been endorsed by WHO; however, due to their complexity and cost, as well as the need for sophisticated laboratory infrastructure, uptake has been limited in many resource-constrained settings.

Several non-commercial culture and DST methods have been developed at the same time, aimed at use in laboratories that lack access to more sophisticated infrastructure and techniques. Among these methods, microscopic observation of drug susceptibility (MODS), colorimetric redox indicator (CRI) methods, thin-layer agar (TLA) methods, the nitrate reductase assay (NRA), and mycobacteriophage-based assays have shown initial promise as rapid, inexpensive methods. These were recently assessed by WHO and results are summarized below.

- MODS: A microcolony direct method in liquid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;
- TLA: A microcolony direct method on solid culture, based on inoculation of specimens to drug-free and drug-containing media followed by microscopic examination of early growth;

- **CRI methods:** Indirect testing methods based on the reduction of a coloured indicator added to liquid culture medium in a microtitre plate after in vitro exposure of *M. tuberculosis* strains to anti-TB drugs;
- **NRA:** A direct and/or indirect method based on the ability of *M. tuberculosis* to reduce nitrate, which is detected by a coloured reaction;
- **Phage-based assays:** Assays which uses bacteriophages to infect and detect the presence of viable *M. tuberculosis* in clinical specimens and culture isolates.

CRI methods, MODS and NRA were subsequently judged to have sufficient evidence to consider their use under clearly-defined conditions outlined below. These recommendations should also be read in conjunction with the *WHO Policy Framework for Implementing New Diagnostics, 2010*, available at (www.who.int/tb/dots/laboratory/policy)

2. Evidence base for policy formulation

2.1 Process

In September 2009, WHO assessed the evidence base for the above-mentioned non-commercial culture and DST methods through a systematic, structured process: The first step constituted a systematic review and meta-analysis of available data (published and unpublished) using standard methods appropriate for diagnostic accuracy studies. The second step involved the convening of an Expert Group to a) evaluate the strength of the evidence base; b) recommend operational and logistical considerations for these methods within national TB control programmes; and c) identify gaps to be addressed in future research.

In accordance with current WHO standards for evidence assessment in the formulation of policy recommendations, the GRADE system (<http://www.gradeworkinggroup.org>), was used to assess the findings of the Expert Group. The GRADE approach provides a systematic, structured framework for evaluating both the accuracy and the patient/public health impact of new interventions.

The Expert Group findings and the final GRADE evaluation are available at (www.who.int/tb/dots/laboratory/policy) and were presented to the WHO Strategic and Technical Advisory Group for Tuberculosis (STAG-TB) in November 2009. STAG-TB acknowledged the evidence base on selected non-commercial culture and DST methods and advised WHO to proceed with policy guidance under clearly defined premises and conditions (http://www.who.int/tb/advisory_bodies/stag/en/index.html).

2.2 Premise

- Current gold standards for culture and DST (conventional solid and automated liquid culture and DST systems, as well as molecular line probe assays) need to be phased in and scale up as a matter of urgency and priority;

- Genotypic (molecular) methods have considerable advantages for scaling up programmatic management and surveillance of drug-resistant TB, particularly related to speed of diagnosis, standardized testing, potential for high through-put, and biosafety;
- Rapid DST methods done directly on sputum specimens have the most important patient and public-health benefit. Rapid DST is essential to identify patients at risk of MDR-TB and should be a first priority in screening strategies;
- Rifampicin resistance is a reliable proxy for MDR-TB. Once MDR-TB has been confirmed, additional first- and second-line DST should be obtained based on current WHO recommendations and available laboratory capacity;
- Non-commercial methods are less expensive, enable laboratories to be independent of single-test commercial providers, and may represent an incentive to commercial providers to lower prices; on the other hand, non-commercial methods are prone to errors related to lack of standardization and due to local variations in methodology;
- The evidence base for selected non-commercial culture and DST methods has been reviewed and the performance of these methods found to be acceptable under stringent laboratory protocols in reference/national laboratories in selected settings;
- Technologies/methods for culture and DST are not mutually exclusive. Molecular line probe assays and the selected non-commercial culture and DST methods are suitable for direct application on smear-positive specimens only. Conventional culture is still required for smear-negative specimens, while conventional DST is needed to detect XDR-TB.

2.3 *Summary of results*

2.3.1 *Colorimetric redox indicator (CRI) methods*

CRI methods are indirect tests, done on *M. tuberculosis* isolates grown from conventional culture. Time to diagnosis of MDR is therefore not faster than conventional phenotypic DST using liquid culture or genotypic testing using line probe assays.

Accuracy data showed that CRI methods are highly sensitive (pooled estimate 98%; 95CI 96% - 99%) and specific (pooled estimate 99%; 95CI 99% - 100%) for the detection of rifampicin resistance as well as isoniazid resistance (pooled sensitivity 97%; 95CI 96% - 98%; pooled specificity 98%; 95CI 97% - 99%).

Compared to the conventional indirect proportion DST method on LJ medium, CRI methods require additional staff skills, similar equipment, but additional consumables that may be difficult to obtain; compared to the conventional indirect proportion method

in liquid culture medium, CRI methods require similar staff skills, less equipment, and consumables that may be readily available.

CRI methods have been standardized and testing protocols are available on <http://www.tbevidence.org>. CRI methods require manipulation of concentrated suspensions of mycobacteria, creating a high risk for aerosol creation. CRI methods should therefore be performed under laboratory biosafety level 3 conditions.

CRI methods are suitable for use at reference laboratory level; scale-up/decentralisation to lower level laboratories is not recommended.

2.2.2 *Microscopically observed drug susceptibility (MODS)*

MODS can be done as a direct or indirect test, by observing micro-colony growth and typical cord-formation of *M. tuberculosis* in sealed microtitre plates containing liquid culture medium, through an inverted microscope.

Accuracy data on combined (direct and indirect) use showed that MODS is highly sensitive (pooled estimate 98%; 95CI 95% - 99%) and specific (pooled estimate 99%; 95CI 96% - 100%) for the detection of rifampicin resistance and slightly less so for isoniazid (pooled sensitivity 91%; 95CI 87% - 95%). High sensitivity and specificity are retained in direct MODS testing.

Initial concerns about the ability to microscopically differentiate *M. tuberculosis* from non-tuberculous mycobacteria were addressed by a revised MODS platform that includes a microtitre well containing p-nitrobenzoic acid (PNB). *M. tuberculosis* fails to grow in PNB. Absence of growth combined with cord-formation in non-PNB containing wells are therefore indicative of *M. tuberculosis* (similar to current WHO recommendations for PNB use in conventional solid culture and DST methods). Having adding a PNB-containing well to the microtitre plate also obviated the need to re-open the plate and consequently reduced the biosafety risk.

Compared to the conventional indirect proportion DST method on LJ medium, MODS requires additional staff skills, an additional inverted microscope, and additional consumables that may be more difficult to obtain; compared to the conventional indirect proportion method in liquid culture medium, MODS requires additional staff skills, less equipment, and consumables that may be readily available.

MODS has been standardized, with testing protocols and online support available through a dedicated website. The revised MODS platform is considered to have a biosafety risk similar to that of conventional culture on solid medium and therefore requires biosafety level 2 precautions.

MODS is suitable for use at reference laboratory level; scale-up/decentralisation to lower level laboratories is not recommended.

2.3.3 Nitrate reductase assay (NRA)

NRA can be used as a direct test on smear-positive sputum specimens or as an indirect test on *M. tuberculosis* isolates grown from conventional solid culture. Indirect testing using NRA is therefore not faster than conventional phenotypic DST using solid media.

Accuracy data on combined (direct and indirect) use showed that NRA is highly sensitive (pooled estimate 97%; 95CI 95% - 98%) and specific (pooled estimate 100%; 95CI 99% - 100%) for the detection of rifampicin resistance as well as for isoniazid resistance (pooled sensitivity 97%; 95CI 95% - 98%; pooled specificity 99%; 95CI 99% - 100%).

Sub-analysis of diagnostic accuracy of NRA on direct testing alone did not differ significantly although sensitivity values for individual studies showed greater variation (range 85% to 100%) and data were more limited.

Reagents for NRA are non-proprietary and relatively inexpensive. Compared to the conventional indirect proportion DST method on LJ medium, NRA requires similar staff skills, similar equipment, and no additional consumables; compared to the conventional indirect proportion method in liquid culture medium, NRA requires fewer staff skills, equipment and consumables.

Procedures for NRA have been standardized and testing protocols are available on <http://www.tbevidence.org>. NRA uses solid culture media and biosafety requirements are therefore similar as for conventional solid culture (biosafety level 2); however, adding NRA reagent require tubes to be regularly opened, which poses a significant risk of aerosol generation and therefore should be done inside an appropriate biological safety cabinet.

NRA is suitable for use at reference laboratory level; scale-up/decentralisation to lower level laboratories should not be considered until such laboratories have demonstrated proficiency in performing solid culture.

3. Policy recommendations

The GRADE process confirmed that there is sufficient generalisable evidence to recommend the use of selected non-commercial culture and DST methods as an interim solution in resource-constrained settings, under clearly defined programmatic and operational conditions, while capacity for genotypic and/or automated liquid culture and DST are being developed.

With due consideration of the issues raised under section 2.2 above, WHO recommends the selective use of one or more of the following non-commercial culture and DST methods, in reference laboratories, and under strict laboratory protocols:

- **CRI methods**, as indirect tests on *M. tuberculosis* isolates from patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB would not be faster (but less expensive) than conventional DST methods using commercial liquid culture or molecular line probe assays;
- **MODS**, as direct or indirect tests, for rapid screening of patients suspected of having MDR-TB;
- **NRA**, as direct or indirect tests, for screening of patients suspected of having MDR-TB, and acknowledging that time to detection of MDR-TB in indirect application would not be faster than conventional DST methods using solid culture.

To ensure testing standards and consistency, WHO will:

- Review existing documents on technical procedures, standard operating procedures, and biosafety requirements for each method;
- Develop and disseminate procedures for internal quality control and external quality assurance for each method.

4. Target audience

This policy statement should be used to guide implementation of LED microscopy for TB diagnosis within national TB control programmes, and is intended to be used by National TB Control Programme Managers and Laboratory Directors, in coordination with external laboratory consultants, donor agencies, technical advisors, laboratory technicians, laboratory equipment procurement officers, warehouse managers, other service providers, other relevant government officials, and implementing partners involved in country-level TB laboratory strengthening. Individuals responsible for programme planning, budgeting, resource mobilization, and training activities for TB diagnostic services may also benefit from using this document.