EXPERT REVIEWS

Selected culture and drug-susceptibility testing methods for drug-resistant *Mycobacterium tuberculosis* screening in resource-constrained settings

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Vicente Ausina Ruiz*[1–4], Gema Fernández-Rivas[1,3] and Cristina Vilaplana Messeguer[2,4]

[1]*Servicio de Microbiología, Hospital Universitario Germans Trias i Pujol, Badalona, Spain*
[2]*Instituto de Investigación Biomédica Germans Trias i Pujol, Badalona, Spain*
[3]*Universidad Autónoma de Barcelona, Barcelona, Spain*
[4]*CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain*
**Author for correspondence: vausina.germanstrias@gencat.cat*

Evaluation of: Adikaram CP, Perera J, Wijesundera SS. The manual Mycobacteria Growth Indicator Tube and the Nitrate Reductase Assay for the rapid detection of rifampicin resistance of *M. tuberculosis* in low resource settings. *BMC Infect. Dis.* 12, 326 (2012).

The control of drug-resistant tuberculosis (TB) requires an accurate and a rapid diagnostic for the detection of critical patterns of drug resistance. Rifampicin resistance is a good predictor of the presence of multidrug-resistant TB. Traditionally, *in vitro* susceptibility methods for *Mycobacterium tuberculosis* are time consuming and laborious. More rapid methods are available but are very expensive for routine use in low-resource settings. According to the article under evaluation, the Manual Mycobacteria Growth Indicator Tube and Nitrate Reductase Assay, in liquid medium, agreed well with the agar proportion method for the rapid detection of rifampicin resistance in low-resource settings. The results of both susceptibility tests will be available in less than 2 weeks and the cost per test is low. Major efforts are needed to improve the diagnostic and treatment success rate among patients with drug-resistant TB. Since 2007, the WHO has promoted new diagnostic tests such as Xpert *Mycobacterium tuberculosis*/rifampicin; point-of-care tests are nowadays under development.

Keywords: determination of drug sensitivity • drug-resistant tuberculosis • line-probe assays • Manual Mycobacteria Growth Indicator Tube • Nitrate Reductase Assay in liquid medium • Xpert *Mycobacterium tuberculosis*/rifampicin

This article evaluates a recently published article by Adikaram *et al.*, which suggests that Manual Mycobacteria Growth Indicator Tube (MGIT) and the Nitrate Reductase Assay (NRA) in liquid medium agreed well with the agar proportion method (APM) – the gold standard for susceptibility testing of *Mycobacterium tuberculosis* – for the rapid detection of rifampicin resistance in low-resource settings [1].

Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to isoniazid and rifampicin [2], and extensively drug-resistant TB (XDR-TB), defined as MDR-TB plus resistance to additional drugs – fluoroquinolones and at least one second-line injectable drug (e.g., amikacin, kanamycin and/or capreomycin) [3], are major threats to tuberculosis (TB) control.

It is estimated that among the notified pulmonary TB cases in 2011, 310,000 (range: 220,000–400,000) were MDR-TB cases [4]. India, China and the Russian Federation represent 60% of these cases. The high levels of MDR-TB are still worrisome in some parts of the world, notably in eastern Europe and central Asia [5]. In some of these countries, 9–32% of new cases are MDR-TB and more than 50% of previously treated cases are MDR-TB [3]. In 84 countries, XDR-TB has been reported; the average proportion of MDR-TB cases with XDR-TB is 9.0% [5].

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To treat MDR-TB and XDR-TB patients, the classic 6-month treatment with first-line anti-TB drugs is not effective anymore, so they must be treated with less efficacious, more toxic and much more costly drugs [1]. The duration of treatment is up to 2 years. Major efforts are needed to improve treatment success rates among patients with drug-resistant TB (DR-TB) [4,5]. In the next few years, a substantial and rapid expansion of diagnosis and treatment of DR-TB is required and clearly recognized in the Global Plan to Stop TB 2011–2015 [4]. Limited laboratory capacity and cost are the main reasons why only 5% of MDR-TB cases are diagnosed [6].

The widely available diagnostic tool for identifying TB is still microscopic examination of sputum in most high-burden countries [7], but this method lacks effectiveness. If drug-susceptibility testing (DST) is available, it is usually performed after treatment failure, delaying the diagnosis of drug-resistant cases and failing to interrupt transmission in many opportunities [2,5].

In the last 10 years, several assays have been developed for MDR screening [8]. Since 2007, the WHO endorsed the use of these rapid detection tests [5,9], and recommends the detection of rifampicin resistance in low-resource settings as a marker of MDR-TB [10].

Summary of methods & results

The main objective of the article published by Adikaram *et al.* [1] was to evaluate the suitability of the manual MGIT and the NRA for the rapid detection of rifampicin resistance in low-resource settings. Fourteen-day-old *M. tuberculosis* strains (n = 373) isolated onto solid media were used for DST by APM, NRA in liquid medium and the manual MGIT method. Rifampicin-free and rifampicin-incorporated (final concentrations: 1 µg/ml) media were inoculated with the recommended concentrations of mycobacterial suspensions and incubated at 37°C in 5% CO_2.

In the APM, the proportion of colonies in drug-containing medium was determined. In the NRA, the color change in the medium was compared with a standard color series after day 6 and day 12 of incubation. Growth in the MGIT was detected using the manual MGIT reader from day 2 onwards. The two evaluation methods were compared with the APM – the gold standard – to determine sensitivity and specificity; agreement between the methods was calculated using κ statistics. The *rpoB* gene mutations, of the rifampicin-resistant isolates, detected by any of the phenotypic methods, were identified by DNA sequencing.

When compared with the APM, the sensitivity of detection of rifampicin resistance was 85% for the NRA and 93% for the manual MGIT and the specificity was 99 and 100%, respectively. Both assays, NRA (κ = 0.86) and manual MGIT (κ = 0.94), were in excellent agreement with the APM. The mean turnaround time for the manual MGIT method and NRA were 8 and 10 days, respectively.

The NRA in liquid medium and the manual MGIT are useful alternatives to APM for rapid DST of *M. tuberculosis* in low-resource settings.

Expert commentary

The control of DR-TB requires rapid and accurate detection of drug resistance [2]. The need to identify cases of MDR-TB through detecting resistance to rifampicin is now well recognized [10]. Establishment of a more rapid DST method would positively impact the management of a patient harboring a drug-resistant strain [9].

Diagnosis of TB and DR-TB has been constrained by conventional technologies, but new, rapid tests now have the potential to change TB care [4]. WHO has recommended the use of approved tests and to avoid the use of poorly performing and/or costly tests [11].

NRA and MGIT use an indicator to detect growth in the liquid medium, eliminating the need for visualization of growth as colonies. Therefore, NRA in liquid medium and MGIT methods are an attractive alternative to conventional methods. In this study, a good agreement was observed between APM and NRA in liquid medium or manual MGIT in the detection of rifampicin resistance. Similar results for detection of rifampicin resistance by manual MGIT and NRA have been reported previously [12–15].

The consumable cost per test for NRA and manual MGIT is around US$3.00 and US$7.00, respectively. However, both assays' results of susceptibility testing will be available in less than 2 weeks. The manual MGIT reader is a reliable and suitable instrument for use in low-resource countries. The cost of a MGIT reader is a round US$3000. Alternatively, a simple UV lamp (365 nm) may be used to detect growth [16].

Introduction of these and other noncommercial methods recommended by WHO (colorimetric redox indicators, microscopic observation drug susceptibility, thin-layer agar, and so on) for low-resource settings will make the determination of rifampicin resistance faster and more cost-effective. All these methods are faster than the conventional methods (7–14 days) and use cheaper in-house supplies and equipment [7–9].

In 2008, WHO endorsed the use of rapid nucleic acid amplification tests such as line-probe assays based on detection of mutations associated with rifampicin resistance. INNO-LiPA Rif TB is one of these and it is very sensitive and specific for this purpose in culture isolates. The other, the GenoType MTBDR assay, has an excellent sensitivity and specificity even when used directly on clinical specimens [7,17]. Countries should take decisive steps to prohibit tests that are too expensive and poor-performing, and introduce technologies recommended by WHO [9].

Five-year view

The future development of new TB tests looks promising [11]. The automated molecular test for TB, Xpert *M. tuberculosis*/rifampicin, eluded most of the pitfalls of conventional nucleic acid amplification tests (safety, contamination and ease of use, among others). It does not need very highly trained personnel and published data suggest an excellent performance in smear-positive and smear-negative patients, and high reliability for determination of rifampicin resistance [18]. Thus, the high sensitivity and easy-to-use performance permit detection of *M. tuberculosis* from clinical specimens in less than 2 h. After approval by WHO in 2010, 67 low- and middle-income countries have purchased 1.1 million tests [5]. South Africa leads the purchase with 37% of tests. A price reduction of US$6.89 in August 2012 has accelerated its use [4,6].

Major efforts are needed to improve treatment success rates among patients with DR-TB. The development of a point-of-care

test for TB and MDR-TB continues, and other diagnostic tests are in process [11].

Development and validation of tools for rapid detection of drug resistance, including for XDR-TB and standardization of DST for second-line drugs, are research priorities [7]. Second-line DST continues to be a challenge, mutations are not well defined and standardization is a problem with phenotypic methods. Development of rapid molecular (genotyping) assays for MDR/XDR-TB will allow rapid identification of DR-TB [4,7,11].

For the first time in many years, a coordinated portfolio of 11 promising new anti-TB drugs or repurposed anti-TB drugs is under development and in clinical trials [5,19,20]. New guidelines could be used to treat both drug-sensitive TB and MDR-TB and to reduce treatment duration showing promising results in clinical trials [20].

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Key issues

- Major efforts are needed to improve diagnostic and treatment success rates among patients with drug-resistant tuberculosis (TB).
- Traditional methods of *in vitro* drugsusceptibility testing are time consuming and laborious. More rapid methods are available, but are very expensive for routine use in countries with high levels of TB endemicity.
- The RNA in liquid medium and the manual Mycobacteria Growth Indicator Tube assays are suitable alternatives to the agar proportion method and can be used to determine rifampicin resistance, especially in low-resource settings.
- Point-of-care test for TB and multidrug-resistant TB and other diagnostic tests are still under development.

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