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Review

Rapid culture-based methods for drug-resistance detection in *Mycobacterium tuberculosis*

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ABSTRACT

Tuberculosis still represents a major public health problem, especially in low-resource countries where the burden of the disease is more important. Multidrug-resistant and extensively drug drug-resistant tuberculosis constitute serious problems for the efficient control of the disease stressing the need to investigate resistance to first- and second-line drugs. Conventional methods for detecting drug-resistance in *Mycobacterium tuberculosis* are slow and cumbersome. The most commonly used proportion method on Löwenstein–Jensen medium or Middlebrook agar requires a minimum of 3–4 weeks to produce results. Several new approaches have been proposed in the last years for the rapid and timely detection of drug-resistance in tuberculosis. This review will address phenotypic culture-based methods for rapid drug susceptibility testing in *M. tuberculosis*.

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1. Introduction

Tuberculosis (TB) represents a major public health problem, especially in low-resource countries where the burden of the disease is more important. According to estimates of the World Health Organisation (WHO), two billion people, roughly one third of the

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world's population is latently infected with *Mycobacterium tuberculosis* the causative agent of the disease. In 2005 there were 8.9 million new cases of TB and 1.6 million deaths were attributed to the disease (World Health Organization, 2007a,b). This scenario is aggravated by the human immunodeficiency virus (HIV) pandemic with roughly one third of the 40 million people currently infected with HIV also co-infected with TB (Friedland et al., 2007). Multidrug-resistant (MDR)-TB patients, those harbouring strains of *M. tuberculosis* resistant to at least rifampicin (RIF) and isoniazid (INH), two essential drugs in the treatment of TB, constitute a serious problem for the efficient control of the disease.

The latest report on anti-tuberculosis drug-resistance in the world prepared by the WHO/IUATLD Global Project on anti-tuberculosis drug-resistance surveillance, shows that MDR-TB has reached levels of up to 14% amongst new patients and rates as high as 50% in previously-treated patients in some settings (World Health Organization, 2004). In addition, the recent description of XDR strains, standing for 'extensively drug drug-resistant' stresses the need to also investigate resistance to second-line drugs. XDR-TB is defined as resistance to at least INH and RIF, in addition to resistance to any fluoroquinolone and to at least one of the three injectable drugs used in the treatment of TB: capreomycin, kanamycin and amikacin (World Health Organization, 2006). Thus, early detection of drug-resistance to first- and second-line drugs in *M. tuberculosis* represents a major priority for TB control programmes.

Conventional methods for detecting drug-resistance in *M. tuberculosis* are slow and cumbersome. Laboratory diagnosis is further complicated by the fastidious growth of the tubercle bacillus. For TB diagnostics laboratories in high-burden countries, primary isolation is the first step before performing drug susceptibility testing (DST) of *M. tuberculosis*. The traditionally accepted methods include the proportion method, the absolute concentration method, the resistance ratio method and the radiometric BACTEC TB-460 system (Becton Dickinson, Sparks, MD) (Canetti et al., 1969; Kent and Kubica, 1985; Heifets and Cangelosi, 1999). The most commonly used proportion method on Löwenstein–Jensen (LJ) medium or Middlebrook agar requires a minimum of 3–4 weeks to produce results. The radiometric BACTEC TB-460 system, on the other hand, using an enriched liquid medium decreased the turnaround time (TAT), but the fact that it relies on radioactive materials represented an inconvenience for its wider application especially in low-resource countries.

Due to the TAT required by conventional DST methods several new approaches have been proposed in the last years for the rapid and early detection of drug drug-resistant *M. tuberculosis* (Palomino, 2005). Among these, both phenotypic and genotypic methods have been introduced. The latter look for genetic determinants of resistance rather than the resistance phenotype involving nucleic acid amplification to detect gene mutations known to be associated with drug-resistance, while phenotypic methods are based on detection of growth in the presence or absence of antibiotics (Garcia de Viedma, 2003; Palomino, 2006). The costs involved in setting up genotypic methods and their requirement for skilled personnel have precluded their wider implementation in clinical mycobacteriology laboratories, especially in low-resource countries where the TB situation and the problem of drug-resistance are of major concern. Phenotypic methods are receiving renewed attention since in general they are simple to perform, allow for several drugs being tested simultaneously, and in principle they are applicable to all drugs. This review will address phenotypic culture-based methods for the rapid detection of drug-resistance in *M. tuberculosis*.

2. Liquid culture-based methods

Several liquid culture-based methods for cultivation and DST of *M. tuberculosis* have been introduced during the last years. Most of them are commercially available and rely on the fact that faster growth is usually obtained in liquid medium. Some of them are simple manual methods while others rely on more sophisticated automated systems. Recent recommendations by the WHO encourage the use of liquid culture media for cultivation and DST of *M. tuberculosis* in low- and middle-income settings (World Health Organization, 2007b).

2.1. The Mycobacteria Growth Indicator Tube (MGIT)

The MGIT manual system (Becton Dickinson, Sparks, MD) was one of the first commercial liquid systems introduced for the rapid detection of mycobacterial growth. It is based on a fluorescence quenching-based oxygen sensor embedded at the bottom of the tube containing an enriched Middlebrook 7H9 broth. Consumption of the dissolved oxygen by the growing mycobacteria produces an orange fluorescence when illuminated with an UV lamp (Palomino, 2000). The MGIT manual system was subsequently applied for rapid DST of *M. tuberculosis*. For this purpose, a set of tubes composed of a growth control and drug-containing tubes are inoculated with the *M. tuberculosis* isolate and incubated at 37 °C. Growth is detected by comparing the fluorescence in the drug-containing tubes and growth control allowing determination of susceptibility or resistance. Several studies have evaluated the MGIT manual system for DST of *M. tuberculosis* against the first-line drugs INH, RIF, ethambutol (EMB) and streptomycin (SM) with good results (Reisner et al., 1995; Palaci et al., 1996; Palomino et al., 1999). These studies found good accuracy for detecting resistance, especially for INH and RIF. There are very few published studies evaluating the manual MGIT method for DST of a second-line drug (Bastian et al., 2001; Martin et al., 2005a,b). It has performed, however, very well for kanamycin and ofloxacin, showing that although not frequently used, it could represent a simple and reliable method for DST to second-line anti-tuberculosis drugs. Very few studies also exist evaluating the manual MGIT directly in sputum samples (Goloubeva et al., 2001; El-Sayed Zaki and Goda, 2007). These studies have applied manual MGIT on smear-positive sputum samples and results have been obtained on an average of 5–8 days with accuracy above 90% for detecting resistance to RIF and INH. The manual MGIT, thus, remains an interesting option for the rapid detection of drug-resistant *M. tuberculosis* with the possibility to be applied directly on sputum samples.

2.2. The BACTEC MGIT960 automated system

Soon after the MGIT manual system was developed and tested for diagnosis and DST of *M. tuberculosis* an automated system based on the same principle was proposed. The BACTEC MGIT960 system (Becton Dickinson, Sparks, MD) performs incubation and reading of the tubes continuously inside the machine using a predefined algorithm to interpret the fluorescent signal and giving the results as positive or negative (Tortoli et al., 1999). When performing DST, the BACTEC MGIT960 interprets the results as susceptible or resistant to the antibiotic under study. Results are available within 8 days (Ardito et al., 2001). Many studies have been performed for evaluating the BACTEC MGIT960 system for DST of *M. tuberculosis* (Tortoli et al., 2002; Johansen et al., 2004; Scarparo et al., 2004). A recent meta-analysis of the published studies found high accuracy and high predictive values associated with the use of BACTEC MGIT960 (Piersimoni et al., 2006). Also, a multicentre validation has been performed to test susceptibilities of *M. tuberculosis* to classical second-line drugs giving accurate results as compared to the BACTEC TB-460 radiometric method (Rusch-Gerdes et al., 2006). The BACTEC MGIT960 system is now practically replacing the radiometric BACTEC TB-460 as a rapid and reliable method for diagnosis and DST of *M. tuberculosis*. The investment required for obtaining the equipment and its large capacity for workload may limit, however, its adoption by small- and medium-sized diagnostic laboratories especially in high-burden low-resource countries. Efforts to address this issue are currently under development (FIND, 2008).

2.3. The BacT/Alert 3D system

Formerly known as the MB/BacT system (bioMerieux, Durham, N.C.) it is an automated commercial liquid culture-based system originally developed for blood cultures and later applied for diagnosis and DST of *M. tuberculosis*. The system uses a modified 7H9 Middlebrook broth and incorporates a colorimetric sensor at the bottom of the vial that measures changes in CO_2 production by the metabolizing mycobacteria. A change in colour from green to yellow indicates a positive reaction and each vial is continuously monitored inside the apparatus (Rohner et al., 1997). Several studies have evaluated the BacT/Alert 3D system for rapid DST of *M. tuberculosis* to first-line drugs including pyrazinamide (Ängeby et al., 2003; Bemer et al., 2004; Brunello and Fontana, 2000). Although using different critical concentrations and timing for reading of results the BacT/Alert 3D system has also shown good sensitivity and specificity; however, further evaluations would be desirable to fully assess the usefulness of this system for rapid DST of *M. tuberculosis*. Also, no studies have been published yet for DST to second-line drugs.

2.4. The Versa TREK system

It was formerly known as the ESP culture system II (Trek Diagnostic systems, West Lake, OH). Also based on an automated format originally developed for blood culture and later applied for detection and DST of *M. tuberculosis*, it uses an enriched 7H9 Middlebrook broth for detection of growth by measuring pressure changes inside the culture vial due to mycobacterial metabolism. Incubation and reading is accomplished inside the same apparatus (Woods et al., 1997). Fewer studies have evaluated the Versa TREK system for DST of *M. tuberculosis* against first-line drugs (Bergmann and Woods, 1998; Ruiz et al., 2000; LaBombardi, 2002). Moreover, one of these studies reported a high proportion of discordant results for INH (Ruiz et al., 2000). For this reason, further studies are needed to evaluate the real usefulness of this system for DST of *M. tuberculosis*.

2.5. The microscopic observation broth-drug susceptibility assay (MODS)

MODS is an ‘in-house’ method based on the observation of the characteristic cord formation of *M. tuberculosis* when growing in a liquid medium (Caviedes et al., 2000). The test was originally described for detection and DST of *M. tuberculosis* in sputum samples. For performance of DST, decontaminated sputum samples are inoculated into 7H9 liquid medium in 24-well plates with or without antibiotics and incubated at 37 °C in a CO_2 incubator. Reading of the plates is performed with an inverted microscope at 40× magnification to identify the typical cord formation of *M. tuberculosis*. Growth in the drug-containing wells and in the growth control is interpreted as resistance. MODS has been evaluated against first-line drugs although better results have been obtained for RIF and INH (Moore et al., 2004, 2006). Although fairly accurate to detect resistance, especially to RIF, the requirement of an inverted microscope to read the plates and biosafety concerns related to the use of liquid medium in 24-well plates may hamper, however, its application outside large reference laboratories with the necessary logistic facilities.

2.6. The colorimetric redox indicator methods

These rapid colorimetric tests rely on the use of a coloured oxidation–reduction indicator that is added to the culture medium after *M. tuberculosis* has grown in the presence or absence of antibiotics. Reduction of the redox indicator will produce a change of colour in the medium that is easily interpreted visually. If the bacteria grow in the presence of the antibiotic as compared to the growth control it is considered as resistant to this drug. These assays can be performed in microtiter plates or in culture tubes with results available in 7–8 days. The method allows easily determining the minimal inhibitory concentrations (MICs) of anti-tuberculosis drugs (Palomino et al., 2007). Various redox indicators have been proposed, with Alamar blue (Yajko et al., 1995; Franzblau et al., 1998), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Mshana et al., 1998; Foongladda et al., 2002) and resazurin (Palomino et al., 2002; Banfi et al., 2003) giving the most reliable and accurate results for DST of INH and RIF. The redox indicator resazurin has also been used to assess DST to second-line drugs (Martin et al., 2003). Resazurin has also been used in a modified colorimetric assay to determine resistance to pyrazinamide using nicotinamide (Martin et al., 2006). In a multicentre study performed in seven laboratories in middle- and low-income countries, redox indicator-based assays performed well with an overall accuracy of 97% for detecting MDR-TB (Martin et al., 2005a,b). A recent meta-analysis of colorimetric methods using redox indicators for DST of *M. tuberculosis* showed that there is evidence of high sensitivity and specificity of these methods (Martin et al., 2007).

2.7. Slide-culture technique

This is an older method recently modified and evaluated for rapid detection of MDR-TB in sputum samples (Hamid Salim et al., 2006). It uses Sula liquid medium with added antibiotics to control contamination. For each sample tested, ten sputum smears are made on one end of autoclaved slides, cut in half longitudinally, decontaminated with 5% aqueous sulphuric acid for 15 min, washed in water, and placed individually in sterile glass bottles containing 7 ml of medium. Three bottles without antibiotics as growth controls, and additional individual bottles containing medium plus INH at 0.2 and 1 μg/ml; RIF at 1 μg/ml; EMB at 2, 5, and 10 μg/ml, and SM at 2 μg/ml are also set up. After overnight refrigeration, all bottles are incubated at 37 °C for 10 days, and heated at 85 °C for 30 min before opening. Dried smears are heat-fixed, stained by Ziehl–Neelsen and examined at 100× magnification for counting acid-fast microcolonies. Any number of well-developed colonies in the drug-containing bottles with at least one colony per low-power field in the growth control is interpreted as resistance. When applied on fresh smear-positive sputum samples the slide-DST technique gave an accuracy of 96% for detecting resistance to RIF. For INH, however, the accuracy was only 90% and much lower for EMB and SM. Although simple in its principle and interpretation of results, this technique follows a cumbersome procedure and requires the preparation of a large number of slides for each sample making it less appropriate to analyze several samples at the same time. Furthermore, there are biosafety concerns, since the same study found that there were still live *M. tuberculosis* bacilli able to grow colonies on LJ medium even after the slides were heated at 85 °C for 30 min previous to microscopical examination.

3. Solid culture-based methods

New alternative methods for rapid DST of *M. tuberculosis* using solid culture media have also been recently proposed. The majority of these alternative procedures have been developed as ‘in-house’ methods with the purpose of shortening the TAT to obtain final results.

3.1. Mycobacteriophage-based methods

Two formats of phage-based methods have been proposed as rapid techniques for drug-resistance detection in *M. tuberculosis*, the phage-amplification method and the luciferase reporter method. Phage-amplification methods are based on the replication of the phage D29 inside viable mycobacteria while the luciferase reporter method relies on the production of light by an engineered phage containing the luciferase gene (Palomino, 2005).

3.2. The FastPlaque assay

This is a commercial system based on the method originally described by Wilson et al. (1997) for rapid DST of *M. tuberculosis* (Wilson et al., 1997). The *FASTPlaque-Response* (Biotec, Ipswich, UK) is a phage-based assay to detect resistance to RIF in smear-positive sputum samples. According to the recommended procedure, decontaminated sputum samples are incubated in the presence or absence of RIF and then with a suspension of the phages. After a period of time to allow infection of *M. tuberculosis*, the extracellular phages remaining are removed with a virucidal solution and the replicating phages are detected as plaques of lysis when plated with an indicator non-pathogenic mycobacterium such as *M. smegmatis*. Results are read visually within 2 days. The presence of viable *M. tuberculosis* after incubation with RIF is interpreted as resistance to this drug. Several studies have evaluated the *FASTPlaque* assay as well as modifications of the 'in-house' phage method with variable results (Albert et al., 2001, 2004; Galí et al., 2003; Simboli et al., 2005; McNerney et al., 2007). In a recent meta-analysis six evaluation studies were considered, all looking at resistance to RIF. A sensitivity of 97–100% and specificity of 84–100% was reported (Pai et al., 2005). However, very few studies have been done with other antibiotics.

3.3. Luciferase reporter phages

Tests based on luciferase reporter phages have been less evaluated. They rely on infection of *M. tuberculosis* with phages harbouring the luciferase gene. Viable mycobacteria will allow replication of the phages and production of light in the presence of cellular ATP and the substrate luciferin that is added to the medium (Riska et al., 1999). When incubated in the presence of RIF this production of light will be inhibited in susceptible isolates (Bardarov et al., 2003; Hazbón et al., 2003). Seven studies were identified in the meta-analysis mentioned above reporting sensitivity of 100% and specificity of 89–100% (Pai et al., 2005). Due to the methodology employed and the requirement for engineered phages, this technique has not gained wide application yet in diagnostic clinical laboratories, and seems more appropriate for research laboratories with the capacity and technical expertise required for its execution.

3.4. The E-test

Although not developed exclusively for *M. tuberculosis* the E-test is a commercial system allowing rapid DST to several antibiotics (Yeung et al., 1993). It relies on plastic strips that have an impregnated gradient of the antibiotic allowing reading of MICs directly at the zone of growth inhibition when applied on the surface of an agar plate. When testing *M. tuberculosis* an inoculum equivalent to 3.0 McFarland was recommended with results available within 5–10 days (Wanger and Mills, 1996). More recent evaluations have found a good correlation with the reference proportion method in agar when testing susceptibility to first-line drugs (Joloba et al., 2000; Hazbón et al., 2000). However, the requirement of a very high inoculum concentration may represent a disadvantage, especially when dealing with potentially highly drug-resistant strains such as MDR and XDR *M. tuberculosis*.

3.5. The nitrate reductase assay

The nitrate reductase assay (NRA) is another simple procedure recently proposed as a rapid method for DST of *M. tuberculosis*. It is based on the capacity of *M. tuberculosis* to reduce nitrate to nitrite which is then easily detected in a coloured reaction (Ängeby et al., 2002). The NRA, also known as the Griess reaction, has been used before in the panel of biochemical tests for the identification of *M. tuberculosis* (Kent and Kubica, 1985). The NRA has been evaluated in recent studies to detect resistance to first-line drugs and ofloxacin showing a good concordance with the reference proportion method (Martin et al., 2005a,b; Ängeby et al., 2002; Montoro et al., 2005; Lemus et al., 2006). An interesting possibility of the NRA is its direct application on sputum samples. This has been accomplished recently in three different studies performed in middle- and low-income settings with encouraging results for detecting resistance to RIF and INH (Musa et al., 2005; Solis et al., 2005; Affolabi et al., 2007).

3.6. The microcolony method

Also known as the thin-layer agar method, on which the MODS assay described above is based, it is performed on Middlebrook 7H11 agar. It was originally described for the rapid detection of mycobacterial growth and later applied as a rapid DST method (Welch et al., 1993; Mejia et al., 1999). When tested directly on sputum samples it detected multidrug-resistance within 13 days in smear-positive samples and in 38 days in smear-negative samples (Schaberg et al., 1995). Further studies in target populations are necessary to assess the feasibility of implementation and accuracy of this method for rapid detection of drug-resistance in *M. tuberculosis*.

3.7. The TK medium

This is a commercial system based on solid media containing dye indicators. The metabolic activity of the mycobacteria will produce a change in the original colour of the medium (red) that allows differentiating mycobacterial growth (yellow) from growth of contaminants (green) (Baylan et al., 2004). *TK INH*, *TK RIF*, *TK STR* and *TK EMB* are individual tubes of the TK medium containing first-line

Table 1
Main culture-based methods for rapid drug-resistance detection of *M. tuberculosis*

Method	Format	Antibiotic	TAT[a] (days)	Reference
MGIT manual	Liquid in tubes	RIF[b], INH, EMB, SM, KAN, OFLO	5–8[c]	9,18,25,37,48,49,57
MGIT automated	Liquid in tubes automated	RIF, INH, EMB, SM, PZA, AMK, CAP, ETH, PTH, OFLO, RIB, LIN	5–8	6,30,56,61,62,67
BacT/Alert 3D	Liquid in tubes automated	RIF, INH, EMB, SM, PZA	6–8	5,11,13,59
Versa TREK	Liquid in tubes automated	RIF, INH, EMB, SM,	4–8	12,33,60,71
MODS	Liquid in 24-well plates	RIF, INH, EMB, SM,	7–9[d]	15,43,44
Colorimetric	Liquid in 96-well plates	RIF, INH, EMB, SM, PZA, KAN, OFLO, CAP, ETH, PAS	4–8	7,20,21,35,37–39,52,55,77
Slide-culture	Liquid on microscopic slides	RIF, INH, EMB, SM	10	26
FASTPlaque	Solid in plates	RIF	2–3	2,3
In-house phage-based method	Solid in plates Liquid in microplate	RIF	2–3	23,40,64
Luciferase reporter phage	Liquid in membranes or microplate	RIF	2–3	8,47,58
E-test	Solid in plates	RIF, INH, EMB, SM, OFLO, CIP	5–10	27,31,68
Nitrate reductase	Solid in tubes	RIF, INH, EMB, SM, OFLO	7–10[e]	4,34,42
Microcolony (TLA)	Solid in plates	RIF, INH, EMB, SM, PZA	13	63
TK medium	Solid in tubes	RIF, INH, EMB, SM	7	10

[a] TAT, turnaround time.
[b] RIF, rifampicin; INH, isoniazid; EMB, ethambutol; SM, streptomycin; KAN, kanamycin; OFLO, ofloxacin; PZA, pyrazinamide: AMK, amikacin; CAP, capreomycin; ETH, ethionamide; PTH, prothionamide; RIB, rifabutin; LIN, linezolid; PAS, *para*-amino salicylic acid.
[c] 6–9 days when performed directly in sputum samples.
[d] Performed directly in sputum samples.
[e] 14–28 days when performed directly in sputum samples.

antibiotics and presented as a rapid kit to detect resistance to these drugs. Unfortunately there is only one published study evaluating this method and with a very small number of isolates (Baylan et al., 2004).

Table 1 shows and overview of the main rapid culture-based methods for rapid DST of *M. tuberculosis*.

4. Future perspectives

The search for alternative and faster methods for drug-resistance detection of *M. tuberculosis* is a continuing endeavour due to the long TAT of conventional methods and the difficulties of implementing newer molecular techniques on a routine basis, especially in low-resource countries where the burden of TB and drug-resistance is more demanding.

In this context, several newer culture-based methods have been proposed and thoroughly evaluated in several settings. Among the commercially available methods described above, the MGIT system, both in its manual and automated versions, have shown high sensitivity and specificity in detecting resistance to RIF and INH, the two most important drugs in the treatment of TB. DST to other first- and second-line drugs have also shown good accuracy, although additional studies are needed for second-line drugs to determine the optimal critical concentrations to be tested (Rusch-Gerdes et al., 2006). More studies are also needed to assess the performance of MGIT for DST of PZA and for performing DST directly on sputum samples. In spite of this, and taking into account recent recommendations of the WHO that encourage the use of liquid media for culture and DST of *M. tuberculosis* in low- and medium-income settings (World Health Organization, 2007b), the BACTEC MGIT960 system represents today the best alternative to replace the old radiometric BACTEC TB-460 for rapid DST of *M. tuberculosis*.

Another commercial system, the *Fast*Plaque, seems to be accurate in detecting resistance to RIF. Almost no studies have been performed with other drugs. One recent study evaluating the 'in-house' version of this method reported lower accuracy, with sensitivity of 80.4% and specificity of 80.8% for INH (Chauca et al., 2007). More studies of the *Fast*Plaque assay are warranted in different settings to assess its performance and feasibility of implementation on a routine basis in TB diagnostic laboratories.

Methods based on the early detection of growth such as MODS and the thin-layer agar that look for microcolonies of *M. tuberculosis* need further validations in different settings to confirm previous results of sensitivity and specificity to detect resistance to RIF and INH. Application of these methods to second-line drugs would also be desirable.

Colorimetric methods using redox indicators have demonstrated accuracy and reliability for rapid detection of MDR *M. tuberculosis* (Martin et al., 2007). Good results have also been obtained when testing for second-line drugs. Although the microtiter format most commonly used in this method seems more appropriate for reference laboratories with the necessary biosafety facilities, the possibility to implement a macromethod in screw-cap tubes using one critical concentration of the drug (Coban et al., 2006) makes it more feasible for implementation in small clinical diagnostic laboratories. Using one critical concentration of RIF or INH has shown to be enough to obtain 97% accuracy in a previous study with Alamar blue for detection of drug-resistance (Palomino and Portaels, 1999).

The NRA also appears as a simple method for DST of *M. tuberculosis*. One additional advantage of this method is that it uses the same culture medium and tube format commonly employed in TB diagnostic laboratories, facilitating in this way its implementation without the need for additional equipment or laboratory supplies. It seems also appropriate as a direct method to be used on sputum samples.

As we have described there are new options available for rapid DST of *M. tuberculosis* based on culture techniques. Several have proved already their reliability and accuracy in detecting drug-resistance as compared to the current methods considered as reference standard. Other methods need more evaluation to prove their accuracy and reproducibility. One important consideration to take into account should be the feasibility of implementation of the new method whether it is of a commercial nature or not, if it is to be used for drug-resistance surveillance or for rapid drug-resistance detection, and the capabilities of the laboratory to adapt their routine work to the new techniques.

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