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Evaluation of Mycobacteria Growth Indicator Tube for Direct and Indirect Drug Susceptibility Testing of *Mycobacterium tuberculosis* from Respiratory Specimens in a Siberian Prison Hospital

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The manual Mycobacteria Growth Indicator Tube (MGIT) method was evaluated for performing direct and indirect drug susceptibility testing (DST) of *Mycobacterium tuberculosis* for isoniazid and rifampin on 101 strongly smear-positive sputum specimens in a Siberian prison hospital. Using the indirect method of proportion (MOP) as the "gold standard," the accuracies of isoniazid and rifampin susceptibility testing by the direct MGIT system were 97.0 and 94.1%, respectively. The accuracy of the indirect MGIT system was 98.0% for both drugs. The turnaround times from specimen processing to reporting of the DST results ranged between 4 and 23 (mean, 9.2) days by the direct MGIT method, 9 and 30 (mean, 15.3) days by the indirect MGIT method, and 26 and 101 (mean, 59.6) days by the indirect MOP. MGIT appears to be a reliable, rapid, and convenient method for performing direct and indirect DSTs in low-resource settings, but further studies are required to refine the direct DST protocol. Cost is the only factor prohibiting widespread implementation of MGIT.

Multidrug-resistant tuberculosis (MDRTB), defined as resistance to at least isoniazid and rifampin, is complicating tuberculosis (TB) control efforts in several low- and middle-income countries (17). Effective treatment and prevention of MDRTB rely upon the prompt availability of drug susceptibility testing (DST) results (7, 23). Conventional mycobacteriological methods using solid media require more than 6 weeks on average to report identification and susceptibility results (10). Various commercial broth-based methods with sensitive growth-detection systems have been developed to improve this turnaround time (TAT), and multiple evaluations have demonstrated the performance of these methods to be essentially equivalent (1, 9, 15, 16, 25). Unfortunately, cost and the requirement for sophisticated equipment have prevented the use of these systems in the resource-poor settings where MDRTB is endemic and where these methods are most needed.

Unlike many of these new technologies, the manual Mycobacteria Growth Indicator Tube (MGIT) system does not require additional instrumentation. The MGIT method uses a fluorescence quenching-based oxygen sensor embedded in the base of a tube containing a modified Middlebrook 7H9 broth. The fluorescence that indicates the presence of mycobacterial growth can be detected by transillumination with a 365-nm UV light (e.g., a simple Wood's lamp). Previous studies from high-income countries have validated the system for performing indirect DST (2, 3, 19, 21, 22, 24, 27), but there are no published evaluations of direct DST by MGIT. In the present study we therefore evaluated the performance and practicability of MGIT for performing direct and indirect susceptibility tests for isoniazid and rifampin on strongly smear-positive sputum specimens collected in a prison hospital in Mariinsk, Siberia (12).

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MATERIALS AND METHODS

Setting and specimens. Médecins sans Frontières (MSF)–Belgium has supported the TB program in the penitentiary hospital in Mariinsk since December 1995 (12). This hospital houses about 1,150 TB patients, among whom the estimated overall prevalence of MDRTB is 22.6%. The prison laboratory is well established and has participated in a quality assurance programme with the World Health Organization (WHO) supranational reference laboratory (SRL) in Antwerp since 1997.

Smear-positive sputum specimens that had been collected for routine diagnosis or follow-up and that contained more than 10 acid-fast bacilli (AFB) in at least 20 high-power fields (i.e., grade 3+ by the WHO scale [26]) were selected for inclusion in the study, which was conducted between September 1999 and March 2000. In view of the high prevailing rates of drug resistance in the prison population, specimens were further selected in an attempt to ensure that the study cohort contained a reasonable mixture of drug-susceptible and -resistant strains to effectively evaluate the MGIT system. The final cohort of 101 specimens therefore came from 65 patients who had not been treated previously in the prison hospital, 10 patients on treatment, and 26 patients who had failed the WHO-recommended category II treatment regimen.

Sputum specimens were decontaminated and digested by using the standard *N*-acetyl-L-cysteine (NALC)–NaOH method (11, 16), which provided exposure to 2% NaOH for 15 min. After centrifugation, the pellets were resuspended in 4 ml of sterile phosphate-buffered saline.

DST. (i) Direct MGIT test. Three MGIT tubes were supplemented with 0.5 ml of OADC (oleic acid, bovine albumin, dextrose, and catalase), 0.1 ml of PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin), and 0.1 ml of test antibiotic; the third tube, being the growth control (GC), received no test antibiotic. The antibiotics were provided by and prepared as recommended by the manufacturer. The final concentrations in the test tubes were isoniazid at 0.1 μg/ml and rifampin at 1.0 μg/ml. Equal volumes (0.5 ml) of the

TABLE 1. Isoniazid and rifampin susceptibility results by direct MGIT, indirect MGIT, and the proportion methods

Drug and MOP classification[a]	MGIT results (no. of isolates)[b]			
	Indirect R		Indirect S	
	Direct R	Direct S	Direct R	Direct S
Isoniazid				
Resistant	75			
Susceptible	2		1	23
Rifampin				
Resistant	49	4		2
Susceptible				46

[a] Gold standard results obtained by MOP in the Mariinsk laboratory with any discordant results confirmed in the Antwerp reference laboratory.
[b] MGIT results obtained by direct and indirect methods. R, resistant; S, susceptible.

processed specimen were inoculated into the three tubes and then incubated in normal atmosphere at 37°C. To exclude bacterial contamination, an aliquot of the processed specimen was also inoculated onto a TSAII blood plate (BBL), incubated at 37°C, and examined after 48 h.

Starting on day 3 after inoculation, tubes were examined daily using a 365-nm UV transilluminator, and their fluorescence levels were compared with negative and positive control tubes; the negative control was an uninoculated tube, and the positive control was an MGIT tube containing 0.4% (wt/vol) sodium sulfite solution. An isolate was considered susceptible to the test drug if the drug-containing tube did not fluoresce within 2 days of the GC tube fluorescing. Conversely, an isolate was defined as resistant if the drug-containing tube fluoresced before or within 2 days of the GC tube.

(ii) Indirect MGIT test. The inoculum preparation from the positive GC tube of the direct MGIT test and the methodology for the indirect DST by MGIT have been described previously (3, 19, 22, 24). The TAT for the indirect MGIT DST was defined as the interval between inoculating the direct MGIT test and obtaining the indirect DST results (i.e., this TAT included the interval required to perform the primary isolation in the GC tube of the direct MGIT test).

(iii) Indirect proportion method. The routine culture and DST procedures of the prison laboratory were performed in parallel with the MGIT tests. These routine procedures involved primary isolation on egg-based media and indirect DST by the standard method of proportion (MOP) on Lowenstein-Jensen medium with the final concentrations of isoniazid and rifampin being 0.2 and 40 μg/ml, respectively (4). The time taken to obtain the primary isolates on solid media were included in the TAT calculations for the indirect MOP, and these TATs represent the normal workflow of the Mariinsk laboratory.

All strains producing discrepant DST results in the two MGIT tests or the MOP were referred to the WHO SRL in Antwerp, where the DST was repeated and verified by the conventional MOP (4).

Statistical analysis. The sensitivity (ability to detect true resistance), specificity (ability to detect true susceptibility), predictive value for resistance (PVR), predictive value for susceptibility (PVS), and accuracy (the rate of correct results) were calculated as previously described (13). The statistical analyses were performed using the Epi Info computer package (version 6.04b; Centers for Disease Control and Prevention, Atlanta, Ga.). P values of ≤ 0.05 were considered significant.

RESULTS

All 101 sputum specimens entered in the study grew *M. tuberculosis* isolates both in MGIT and on solid media. No specimens had to be excluded from the study because of bacterial contamination. Susceptibility testing by the indirect MOP in the Mariinsk and Antwerp laboratories found that 25 were susceptible to isoniazid and rifampin, 21 were isoniazid resistant rifampin susceptible, one was isoniazid susceptible rifampin resistant, and 54 were multidrug resistant (Table 1).

When compared with the above "gold standard" test, the direct MGIT system produced three false-resistant isoniazid results and six false-susceptible rifampin results, while the indirect MGIT system gave two false-resistant isoniazid and two false-susceptible rifampin results (Tables 1 and 2). The performance characteristics of the direct and indirect MGIT systems are listed in Table 3. When compared with each other, the direct and indirect MGIT DSTs showed only one discrepant isoniazid result and four discordant rifampin results (99.0 and 96.0% accuracies, respectively); the indirect test agreed with the MOP on all five occasions (Tables 1 and 2). Table 4 describes the TATs with the three methods. The direct MGIT system provided DST results 2 to 13 (mean, 6.1) days sooner than the indirect MGIT method ($P \ll 0.001$), which in turn produced results 9 to 91 (mean, 44.3) days earlier than the indirect MOP ($P \ll 0.001$).

The manufacturer instructs that indirect MGIT DSTs are invalid and should be repeated if the GC tube does not fluoresce by day 12. No such invalid indirect MGIT tests occurred in this study. A similar interval for invalidating direct MGIT DSTs was not applied in this pilot evaluation. A review of the data found that the GC tube became positive more than 12 days after inoculation for two (22.2%) of the nine specimens producing discordant isoniazid or rifampin results by the direct

TABLE 2. Drug susceptibility results by direct MGIT, indirect MGIT, and the MOP for specimens producing discordant results[a]

Result group and specimen no.	Isoniazid			Rifampin		
	Direct MGIT	Indirect MGIT	MOP[b]	Direct MGIT	Indirect MGIT	MOP[b]
Discordant isoniazid result						
68	R	R	S	S	S	S
245	R	S	S	S	S	S
5186	R	R	S	S	S	S
Discordant rifampin result						
3969	R	R	R	S	S	R
4244	R	R	R	S	S	R
4318	R	R	R	S	R	R
4883	R	R	R	S	R	R
5114	R	R	R	S	R	R
5391	R	R	R	S	R	R

[a] Drug susceptibility result: R, resistant; S, susceptible.
[b] Gold standard results obtained by MOP in the Mariinsk laboratory with any discordant results confirmed in the Antwerp reference laboratory.

TABLE 3. Accuracy and reliability of direct and indirect MGIT compared with the MOP[a]

Drug and MGIT method	% Sensitivity (95% CI)	% Specificity (95% CI)	% PVR (95% CI)	PVS (95% CI)	Accuracy (95% CI)
Isoniazid					
Direct	100 (95.2–100)	88.5 (69.8–97.6)	96.2 (89.2–99.2)	100 (85.2–100)	97.0 (91.6–99.4)
Indirect	100 (95.2–100)	92.3 (74.9–99.1)	97.4 (90.9–99.7)	100 (85.8–100)	98.0 (93.0–99.8)
Rifampin					
Direct	89.1 (77.8–95.9)	100 (92.3–100)	100 (92.7–100)	88.5 (76.6–95.6)	94.1 (87.5–97.8)
Indirect	96.4 (87.5–99.6)	100 (92.3–100)	100 (93.3–100)	95.8 (85.7–99.5)	98.0 (93.0–99.8)

[a] Gold standard results were obtained by MOP in the Mariinsk laboratory with any discordant results confirmed in the Antwerp reference laboratory. 95% CI, 95% confidence interval calculated using the exact binomial method.

MGIT method compared with only 7 (7.6%) of 92 concordant specimens ($P = 0.18$).

DISCUSSION

This is the first published evaluation of direct DST using the MGIT system. Similar trials of direct DST by radiometric BACTEC were performed when that system was introduced in the 1980s (14). Both systems share the advantages of being rapid and of testing the actual mycobacterial population causing the patient's disease instead of a selected subset that is (preferentially) cultivated in vitro during primary isolation. Fortunately, the direct MGIT DST system does not appear to have some of the disadvantages that have limited the widespread use of direct radiometric BACTEC DST (8). For example, unlike the direct MGIT DST system which used a different "critical proportion" to define resistance than the indirect radiometric BACTEC DST, the criteria for defining resistance in the indirect MGIT DST also appears appropriate for the direct MGIT DST. The manufacturer stipulates that indirect MGIT DST results are only valid if the GC tube becomes positive within 12 days of inoculation. The present study found that discordant results tended to occur more frequently with the direct MGIT method among specimens incubated beyond this time. However, this association did not reach statistical significance, with only nine discordant results. Further experience with the direct MGIT DST method is required to define an upper limit for the incubation time that optimizes test performance.

Contamination did not prove to be a problem in the direct MGIT DST despite the enriched Middlebrook medium that is used in the tubes. As in the previous direct radiometric BACTEC DST evaluations, PANTA antibiotic solution was added to limit contamination. The high concordance (i.e., 96 to 99.0%) between the direct and indirect MGIT methods suggests that the addition of PANTA has had little effect on the direct DST results. Unnecessary performance of DSTs on nontuberculous mycobacteria was not a problem in this Siberian prison population with a high prevalence of TB but, as with the direct radiometric BACTEC DST, would presumably be a problem in low-prevalence populations. Finally, this evaluation found that the direct MGIT system produced DST results for both isoniazid and rifampin 2 to 13 (mean, 6.1) days earlier than when using MGIT for primary isolation and then an indirect DST. Though statistically significant, the actual clinical benefit of this 6-day time-saving remains to be defined.

The present study does have some limitations. First, this initial evaluation of direct MGIT DST used only strongly smear-positive specimens to ensure that a significant quantity of acid-fast bacilli was present in each DST. Second, we only evaluated the direct MGIT system for obtaining isoniazid and rifampin susceptibility results. This approach was adopted because these two drugs are the key elements in short-course

TABLE 4. TAT for reporting drug susceptibility results

Drug and DST method	TAT (days)[a]			% Reported by day:				
	Mean	SD	Range	7	14	21	28	42
Direct MGIT[b]								
Isoniazid	8.5	3.4	3–23	52.5	95.1	99.0	100	
Rifampin	9.2	3.4	4–21	39.6	94.1	100		
Both drugs	9.2	3.4	4–23	38.6	94.1	99.0	100	
Indirect MGIT[c]								
Isoniazid	14.7	4.2	8–30	0	59.4	94.1	98.0	100
Rifampin	15.3	4.2	9–29	0	51.5	94.1	99.0	100
Both drugs	15.3	4.2	9–30	0	51.4	94.1	98.0	100
MOP[b,c,d]	59.6	21.2	26–101	0	0	0	1.0	17.8

[a] TAT calculated in days from the date of specimen processing to the date of the DST report (including the time required for primary isolation by MGIT for the indirect MGIT DST and on solid media for the MOP).

[b] Significant difference in TAT for obtaining isoniazid, rifampin, and both drug susceptibility results by direct MGIT compared with the MOP results ($P \ll 0.001$ for all three comparisons).

[c] Significant difference in TAT for obtaining isoniazid, rifampin, and both drug susceptibility results by indirect MGIT compared with the MOP results ($P \ll 0.001$ for all three comparisons).

[d] The cumulative percentages of DST results available by the proportion method after 56, 70, 84, and 112 days were 59.4, 68.3, 80.2, and 100%, respectively.

chemotherapy and provide the most robust DST results (13). Third, the study cohort contained only 26 isoniazid-susceptible specimens, so the estimated performance of the direct MGIT system for isoniazid susceptibility testing is inexact, with wide 95% confidence intervals (e.g., specificities of 69.8 to 97.6%; Table 3). Further studies will therefore be required to assess the performance and TAT of direct MGIT DST for weakly smear-positive specimens and for performing streptomycin and ethambutol susceptibility tests and to evaluate in more detail isoniazid susceptibility testing by the direct MGIT method.

Finally, the present study did not compare direct MGIT DST with direct DST on a solid medium because the Mariinsk laboratory did not routinely perform such tests. Direct agar dilution susceptibility testing is a recognized inexpensive alternative that can provide DST results within 3 to 4 weeks (8, 11, 14). However, direct agar DST can be confounded by bacterial contamination, under- or overgrowth in controls that invalidate about 15% of tests, and potential inactivation of the test drug during prolonged incubation. For example, Libonati et al. (14) found that direct agar DST provided reportable results in only 41% of smear-positive cases and 62% of culture-positive cases. Other low-technology techniques, such as the colorimetric Alamar Blue assay, microscopic observation of broth cultures, and direct DST on novel agar media (5, 6, 8, 20), have also been developed for rapid DST in low-income countries, but these "in-house" alternatives may not be as robust as MGIT and do require considerable laboratory expertise.

In contrast, the MGIT system was quickly and easily implemented in this low-resource prison TB laboratory. Only one modification to the laboratory's standard practices was required. In the training period before this study commenced, some growth failures in the MGIT system were attributed to pH variations in inocula processed by the Petroff method (the usual decontamination method used in the prison laboratory); use of the NALC-NaOH method as recommended by the manufacturer quickly resolved this problem, and no growth failures occurred during the study.

In summary, this study has demonstrated that the nonautomated MGIT system is a dependable, rapid method for performing direct DST. This evaluation has also confirmed that the excellent performance and rapid TAT reported for indirect MGIT DST in other studies (2, 3, 18, 19, 21, 22, 24, 27) can be reproduced in a low-resource setting. The MGIT system therefore represents appropriate technology for laboratories in these countries. Cost is the only prohibitive factor. The MGIT system and similar nonradiometric techniques are becoming the accepted gold standard methods for mycobacterial cultivation in high-income countries with low prevalences of TB (1, 9, 15, 16, 25). These techniques are even more necessary in areas with a high prevalence of MDRTB. International organizations, biomedical companies, and governments must develop arrangements that give low-income countries access to these new technologies if TB care is to be seen as globally equitable and the (MDR)TB epidemic controlled.

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