Validation of a Two Week 7H10 Agar Proportion Method for Mycobacterium tuberculosis complex Isoniazid and Rifampin Drug Susceptibility Testing

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INTRODUCTION

The Centers for Disease Control and Prevention recommend that first-line drug susceptibility test (DST) results be reported within 17 days from the identification of Mycobacterium tuberculosis complex (MTBC) from culture by the use of a rapid growth-based DST method (1). The Texas Department of State Health Services Laboratory, Austin Texas, routinely tests MTBC DST by 7H10 agar proportion (AP). AP DST is not considered a rapid DST method since results are not reported conventionally until three weeks (2). The objective of this study was to determine the accuracy of isoniazid (INH) and rifampin (RMP) AP results reported at two weeks. AP tests were examined at two and three weeks by high power (up to 100X) magnification. Compared to relatively low power examination by a dissecting microscope, high power magnification examination allowed the detection of microcolony growth significantly earlier than three weeks (3-6).

METHODS

The scope of this study included all AP tests performed from 10/26/2015 through 3/30/2016; no tests were excluded. The AP test consisted of two 7H10 quad plates. Plate 1 contained a drug-free growth control, INH (0.2 mcg/ml), RMP (1.0 mcg/ml), and ethambutol (5.0 mcg/ml). Plate 2 contained high concentration INH (1.0 mcg/ml), ethionamide (5.0 mcg/ml), ofloxacin (2.0 mcg/ml), and kanamycin (5.0 mcg/ml). Each drug concentration was color-coded with food color dye for identification purposes (Figure 1). For 0.2 and 1.0 mcg/ml INH and RMP, the AP test was resulted at two weeks as resistant, susceptible, inconclusive, or “wait for 3 week result”. For all drugs, the AP test was resulted at three weeks as resistant, susceptible, or inconclusive (7).

Figure 1. 7H10 Agar Proportion Plate Panel

RESULTS

The correlation of 0.2 mcg/ml INH, 1.0 mcg/ml INH, and RMP test results at two and three weeks post-inoculation is shown in Tables 1, 2, and 3, respectively. Typical MTBC growth is shown in Figure 2.

Table 1. INH 0.2 mcg/ml All Results

	Three Week 0.2 mcg/ml INH Result				
Two Week 0.2 mcg/ml INH Result	Inconclusive	Resistant	Susceptible	Total	% of Total
Inconclusive	20*			20	3.6%
Resistant		52		52	9.4%
Susceptible	1**		468	469	84.8%
Wait for 3 week result	3	7	2	12	2.2%
Total	24	59	470	553	100.0%

Table 2. INH 1.0 mcg/ml All Results

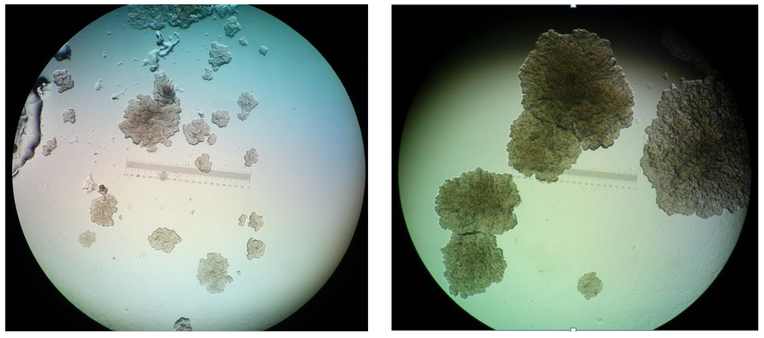
	Three Week 1.0 mcg/ml INH Result				
Two Week 1.0 mcg/ml INH Result	Inconclusive	Resistant	Susceptible	Total	% of Total
Inconclusive	21*			21	3.8%
Resistant		28		28	5.1%
Susceptible	2**		495	497	90.4%
Wait for 3 week result	1	3		4	0.7%
Total	24	31	495	550	100.0%

Table 3. RMP 1.0 mcg/ml All Results

	Three Week 1.0 mcg/ml RMP Result				
Two Week 1.0 mcg/ml RMP Result	Inconclusive	Resistant	Susceptible	Total	% of Total
Inconclusive	21*			21	3.8%
Resistant		20		20	3.6%
Susceptible			509	509	92.2%
Wait for 3 week result		2		2	0.4%
Total	21	22	509	552	100.0%

* Tests were inconclusive due to insufficient control growth or contamination
**Repeat test was susceptible at two and three weeks

Figure 2. Typical MTBC 7H10 Agar Proportion Growth at 50X Magnification



After Two Weeks Incubation | After Three Weeks Incubation

CONCLUSIONS

This study demonstrated that 0.2 and 1.0 mcg/ml INH and RMP AP results can be determined at two weeks with 100% sensitivity and specificity for at least 98.5% of the tests with conclusive results at three weeks. Only a small proportion (<=1.5%) of INH and RMP tests required a third week of incubation to yield definitive results. A limitation of this study is that agar proportion is performed at relatively few U.S. Public Health Laboratories; most labs use MGIT 960 or VersaTREK systems for rapid growth-based first-line DST. However, these broth systems are known to miss clinically significant rifampin and ethambutol resistance detected by solid medium proportion methods (8-12). Compared to broth-based DST, two week AP DST for INH and RMP may allow more accurate detection of drug resistance and susceptibility in a similar timeframe.

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