

CHAPTER IV

RESULTS

This study tried to adapt the MTT assay in drug susceptibility testing of *Mycobacterium tuberculosis* from direct clinical specimens for rapid report. Since the MTT assay could be tested after the culture process, then it was applied for using in direct clinical specimens. The incubation time of the result reading was observed at 7 days and 14 days. Firstly, the volume of clinical specimens was optimized using standard stain, H37RV and resistance strains in each drug (Rifampicin, Ethambutal, Isoniazid, Streptomycin). (Table 6)

Optimization of the inoculums size for the direct MTT assay.

The volume of clinical specimens was varied at 100, 200, 300, 400, 500 and 600 μ l for testing with the direct MTT method using the original MTT protocol. The test was done for five set in each volume. From Table 5, the volume at 500 μ l showed the best result for the direct MTT method violet color in control tube and yellow color in PBN tube. They also showed the same results with standard stain and resistant reference stains.

Table 6 The volume optimization for the direct MTT method

Volum e (μ l)	Color Result in each drug											
	Control	Test					Control	Standard strain (H37RV)				
		PN	RI	IN	EM	S		PN	RI	IN	EM	S
		B	F	H	B	M		B	F	H	B	M
100	Y	Y	Y	Y	Y	Y	V	Y	Y	Y	Y	Y
200	Y	Y	Y	Y	Y	Y						
300	Y	Y	Y	Y	Y	Y						
400	W V	Y	Y	Y	Y	Y						
500	V	Y	Y	Y	Y	Y						
600	V	Y	Y	Y	Y	Y						

Table 7 The volume optimization for the direct MTT method

Volum e (μ l)	Color Result in each drug											
	Control	Test					Control	Standard resistant strain				
		PN	RI	IN	EM	S		PN	RI	IN	EM	S
		B	F	H	B	M		B	F	H	B	M
*500	V	Y	V	Y	Y	V	V	Y	V	Y	Y	V
*600	V	Y	V	Y	Y	V	V	Y	V	Y	Y	V
**500	V	Y	Y	V	V	Y	V	Y	Y	V	V	Y
**600	V	Y	Y	V	V	Y	V	Y	Y	V	V	Y

Note: V= violet color ,Y= yellow color ,WV=weak violet,

PBN = para-nitrobezonic acid 100 μ g/ml, RIF = rifampicin 0.2 μ g/ml , INH= Isoniazid 0.04 μ g/ml, EMB=Ethambutal 1 μ g/ml, SM=Streptomycin 0.4 μ g/ml

* resistance strain to Rifampicin and Streptomycin

**resistance strain to Isoniazid and Ethambutal

Samples

The 150 specimens with AFB positive after decontamination and digestion and concentration were tested. The incubation time of the direct MTT assay was done 7 days and 14 days. The direct MTT method was tested by comparison to the indirect drug susceptibility testing using agar proportion method and MTT method.

Table 8 The result of AFB positive of sample.

Grading of the AFB strain	Number of specimens tested by MTT assay with incubation time at		
	7 days	14 days	Total
Scanty	1	0	1
1+	28	10	38
2+	63	27	90
3+	8	13	21
Total	100	50	150

Drug susceptibility results

1. directed MTT method

The direct MTT methods used clinical specimens which were divided in two periods for the test. In Period I (7 days), 95 samples showed susceptible results and 5 samples showed resistant results. When compared to the proportion, there was 75.79% sensitivity in susceptible result and 17.86 % in resistant results. In Period II (14 days), 43 samples showed susceptible results and 5 sample showed resistant results. In this study there were two samples of contamination. When compared to the proportion method, there was 100% sensitivity in both susceptible and resistant results. (Table 8)

Table 9 Drug Susceptibility results of Direct, Indirect MTT method and Proportion method for each period.

Methods (Amount)	Drug Susceptibility results in time Period			
	Period I :7 days		Period II : 14 days	
	Resistant	Susceptible	Resistant	Susceptible
*Direct MTT	5	95	5	43**
Sensitivity (%)	17.86	75.79	100	100
Indirect MTT	23	77	5	45
Sensitivity (%)	82.14	93.35	100	100
Proportion	28	72	5	45
Total sample	100		50	

Note: *The concentration of each drug not equal to the original MTT assay (1/5 time) (in Table 4)

**There were 2 tests of contamination.

2. Indirect MTT methods

The indirect MTT method used colony cultures for the test. This study showed similar drug susceptibility results both in Period I and Period II. In Period I, 77 samples showed susceptible results and 23 samples showed resistant results by Indirect MTT method. When compared to the proportion method there was 93.35% sensitivity in susceptible result and 82.14% in resistant results. In Period II (14 days of incubation), 5 samples showed resistant results and 45 sample showed susceptible results. The results showed 100% sensitivity in resistant and susceptible results when compared to the proportion method.

Comparison of drug susceptibility results in each drugs

The drug susceptibility results of direct MTT in drugs differed from indirect MTT and proportion method when the incubation time was 7 days (Period I). After

prolonging incubation to 14 days, they showed the same results as indirect MTT and proportion method in both susceptible and resistant groups. (Table 8)

In Period I (7 days incubation) of direct MTT, the sensitivity of drug susceptible results was 89.79%, 85.42%, 86.87% and 84.54% for isoniazid, rifampicin, ethambutal and streptomycin, respectively. The sensitivity of drug resistant results were 16.67%, 22.22%, 7.14% and 16.67% for isoniazid, rifampicin, ethambutal and streptomycin.

In Period II, the sensitivity of drug susceptible and drug resistant was 100% for all drugs and also equal to Indirect MTT and Proportion methods. (Table 9)

Table 10 Comparison of drug susceptibility results in each method.

Method	Direct MTT					In direct MTT					Proportion					
	RIF	INH	EMB	SM	RIF	INH	EMB	SM	RIF	INH	EMB	SM	RIF	INH	EMB	SM
Period	0.2	0.04	1	0.4	1.0	0.2	5.0	2.0	1.0	0.2	5.0	2.0	1.0	0.2	5.0	2.0
	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
Period I																
Resistance	2	4	1	3	8	13	12	12	12	18	14	18	12	18	14	18
	(16.67%)	(22.22%)	(7.14%)	(16.67%)	(66.67%)	(72.22%)	(85.71%)	(66.67%)	(85.71%)	(85.71%)	(66.67%)	(66.67%)	(66.67%)	(66.67%)	(66.67%)	(66.67%)
Susceptible	98	96	99	97	92	87	88	88	88	82	86	82	88	82	86	82
	(89.79%)	(85.42%)	(86.86%)	(84.54%)	(95.65%)	(94.25%)	(97.33%)	(93.18%)	(97.33%)	(94.25%)	(93.18%)	(93.18%)	(93.18%)	(93.18%)	(93.18%)	(93.18%)
Period II																
Resistance	3	4	4	1	3	4	4	1	3	4	4	1	3	4	4	1
	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)
Susceptible	47	46	46	49	47	46	46	49	47	46	46	49	47	46	46	49
	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)	(100%)

Note: Rifampicin (RIF), Isoniazid (INH), Ethambutol (EMB), and Streptomycin (SM).

The final concentration in each drug of direct MTT method was lower than direct MTT and proportion method (**decreased in to 1/5 time**). Then has an effect with the result in this test .The result showed low sensitivity all of resistance and susceptible in period I and need long incubation period for the test in period II. In case direct MTT used the same volume and concentration with the direct MTT and proportion method could be reliable to the result comparison. The direct MTT should be the new choice for the drug susceptibility test in general hospital.

The correlation of AFB grading between drug resistance results of proportion, direct and indirect MTT methods

The results of the sample with AFB grading was 3+ showed 40% sensitivity in Direct MTT and 100% in Indirect MTT in Period I (7 days). Whereas the incubation time was 14 days (period II), they showed similar results in indirect MTT and proportion methods both ABF grading 2+ and 3+. (Table 10)

Table 11 The correlation of AFB grading between drug resistance results of Proportion, Direct and Indirect MTT methods.

Drug resistance by	AFB results			
	AFB 3+	AFB 2+	AFB 1+	Scanty AFB
<i>Period I: 7 day</i>				
Proportion	5	15	8	0
Direct MTT	2	2	1	0
	(40%)	(13%)	(12.5%)	
Indirect MTT	5	14	4	0
	(100%)	(93.33%)	(50%)	
<i>Period II: 14 day</i>				
Proportion	4	1	0	0
Direct MTT	4	1	0	0
	(100%)	(100%)		
Indirect MTT	4	1	0	0
	(100%)	(100%)		