

## **REFERENCES**

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## **APPENDIX**

## APPENDIX A LIST OF SOLUTIONS AND BUFFERS

### 1) Decontamination solution by N-acetyl-L-cysteine (NALC)+NaOH method

#### 1.1) 2% NaOH

- NaOH	2 g
- Add distilled water to	100 mL

#### 1.2) N-acetyl-L-cysteine (NALC)+2% NaOH

- N-acetyl-L-cysteine (NALC)	0.25 g
- NaOH	100 mL

#### 1.3) 2.9% sodium citrate

- Sodium citrate	2.9 g
- Add distilled water to	100 mL

### 2) Phosphate buffer saline (PBS) pH 6.8

- NaH <sub>2</sub> PO <sub>4</sub> anhydrous	0.23 g
- Na <sub>2</sub> HPO <sub>4</sub> anhydrous	1.15 g
- NaCl	9 g
- Add distilled water to	1L

### 3) Solution for AFB stain (Ziehl-Neelsen method)

#### 3.1) Carbon fushin

- Basic fuchin	5 g
- Absolute ethanol	50 mL
- Phenol	25 g
- Add distilled water to	500 mL

#### 3.2) Acid alcohol

- HCl	3 mL
- Absolute ethanol	97 mL

#### 3.3) New methylene blue

- Saturated methylene blue in alcohol	300 mL
- 0.01% KOH	1 mL

**APPENDIX B Mycobacterium tuberculosis identification and drug susceptibility testing kit (TBI&S kit)**

<b>Name</b>	<b>Volume</b>	<b>Number</b>	<b>Composition</b>
1. M reagent(medium)	75 ml	3 bottles	medium
2. C reagent (control)	20 ml	1 bottles	culture medium M7H9 broth with 10% oleic acid albumin-dextrose
3. PNB reagent	20 ml	1 bottles	PNB medium
4. R reagent	20 ml	1 bottles	rifampicin 1 µg/ml medium
5. I1 reagent	20 ml	1 bottles	isoniazid 0.2 µg/ml medium
6. I2 reagent	20 ml	1 bottles	isoniazid 1 µg/ml medium
7. E1 reagent	20 ml	1 bottles	ethambutal 5 µg/ml medium
8. E 2 reagent	20 ml	1 bottles	ethambutal 10 µg/ml medium
9. S1 reagent	20 ml	1 bottles	streptomycin 2 µg/ml medium
10. S2 reagent	20 ml	1 bottles	streptomycin 10 µg/ml medium
11. MTT solution	3 ml	1 tube	MTT solution
12. L solution	100 ml	2 bottles	Lysis solution
13. 1 Mcfarland standard solution		1 tube	

The accessories support with the test set were following:

1. 20 zip lock plastic bags of 12 sterile polystyrene tubes with cap ,each bag contained of:
  - P1 labeled 14 ml – size tube containing glass beads for cells suspension preparation.
  - P2 labeled 14 ml – size tube for inoculums turbidity preparation.
  - P3 labeled 5 ml – size tube for storing prepared inoculums
  - 9 labeled 4.5 ml – size of C,PNB,R,I1,I2,E1,E2,S1,S2 for MTT – TBI&S testing
2. 20 zip lock plastic bag contained of 4 labeled 5 ml- size sterile plastic caped tubes of D1,D2,D3 and D4 containing 1.8 ml sterile distilled water for each sample testing for inoculums dilution preparation.
3. 1 box of sterile 200 µl – size micropipette tips
4. 1 box of sterile 1,000 µl – size micropipette tips

5. 1 zip lock plastic bag of 80 pieces of sterile 5 ml – size plastic pasture pipettes
6. 40 sheets of TBI&S record and copy forms and agar proportion record forms
7. 1 TBI&S tests kit handbook
8. 1 standard proportion method handbook.



Table 12 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test				culture result	MTT assay										Proportional							
		Niacin	Nitrate reduction	Catalase test	PNB		direct					Indirect					EMB	SM	SM					
							RIF	INH	EMB	SM	RIF	INH	EMB	SM	RIF	INH				EMB	SM			
16.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
17.	+	+	-	-	-	G	R	R	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
18.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
19.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
20.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
21.	+	+	-	-	-	G	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
22.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
23.	+	+	-	-	-	G	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
24.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
25.	+	+	-	-	-	G	S	S	S	S	S	S	R	S	S	R	S	R	S	R	S	S	S	S
26.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
27.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	R	R	R	S	S	R	R
28.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
29.	+	+	-	-	-	G	S	R	S	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S
30.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
31.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
32.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 12 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test			culture result	MTT assay						Proportional				
		Niacin reduction	Catalase test	PNB		direct			Indirect			EMB	SM	SM		
						RIF	INH	EMB	RIF	INH	EMB					
33.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
34.	+	+	-	-	G	S	R	S	R	S	R	S	R	S	S	R
35.	+	+	-	-	G	S	S	S	S	R	S	S	S	S	S	S
36.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	R
37.	+	+	-	-	G	S	S	S	S	R	S	S	S	S	S	S
38.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
39.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
40.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
41.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
42.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
43.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
44.	+	+	-	-	G	S	S	S	R	R	R	R	R	R	R	R
45.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
46.	+	+	-	-	G	S	S	S	S	R	S	S	S	R	S	S
47.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S

Table 12 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test			culture result	MTT assay						Proportional				
		Niacin reduction	Nitrate reduction	Catalase test		PNB	direct			Indirect			EMB	SM	SM	
							RIF	INH	EMB	SM	RIF	INH				EMB
48.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	R
49.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
50.	+	+	-	-	G	S	S	S	S	S	S	R	R	R	R	S
51.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
52.	+	+	-	-	G	S	S	S	S	S	S	S	S	R	R	R
53.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
54.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
55.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
56.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
57.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
58.	+	+	-	-	G	S	S	S	S	R	R	S	R	S	R	R
59.	+	+	-	-	G	S	S	S	R	R	S	S	R	S	S	S
60.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
61.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
62.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
63.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S

Table 12 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test				culture result	MTT assay						Proportional					
		Niacin reduction	Nitrate reduction	Catalase test	PNB		direct			Indirect			INH	EMB	SM			
							RIF	INH	EMB	RIF	INH	EMB				SM		
64.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
65.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
66.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
67.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
68.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
69.	+	+	-	-	-	G	S	S	S	S	S	S	S	R	R	R	R	S
70.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
71.	+	+	-	-	-	G	S	S	S	S	R	S	S	S	S	S	R	R
72.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	R
73.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
74.	+	+	-	-	-	G	S	S	S	S	R	S	S	S	S	S	S	R
75.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
76.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
77.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	R	R	S	S
78.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
79.	+	+	-	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
80.	+	+	-	-	-	G	S	S	S	S	R	R	R	R	R	R	R	R

Table 12 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test			culture result	MITT assay						Proportional				
		Niacin reduction	Catalase test	PNB		direct			Indirect			EMB	INH	SM		
						RIF	INH	EMB	SM	RIF	INH				EMB	SM
81.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
82.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
83.	+	+	-	-	G	S	S	S	R	R	R	R	R	R	R	R
84.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
85.	+	+	-	-	G	S	S	S	S	R	R	R	R	R	R	S
86.	+	+	-	-	G	S	S	S	S	S	S	R	S	S	S	R
87.	+	+	-	-	G	S	S	S	S	S	R	S	S	R	S	S
88.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
89.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
90.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
91.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
92.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
93.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
94.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S
95.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S

Table 12 (cont.)

Sp. No.	CONCE	Biochemical Test			culture result	MTT assay										Proportional	
		Niacin	Nitrate reduction	Catalase test		PNB	direct					Indirect					
							RIF	INH	EMB	SM	RIF	INH	EMB	SM	RIF		INH
96.	+	+	-	-	G	S	S	S	S	S	S	S	S	R	R	R	R
97.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
98.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
99.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
100.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S

Note: PBN = para-nitrobenzoic acid, RIF = rifampicin, INH= Isoniazid, EMB=Ethambutal, SM=Streptomycin

- G = Growth
- S = Susceptible
- R = Resistant
- + = Positive
- = Negative



Table 13 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test				culture result	MTT assay						Proportional					
		Niacin reduction	Nitrate reduction	Catalase test	PNB		direct			Indirect			EMB	SM	SM			
							RIF	INH	EMB	RIF	INH	EMB				RIF	INH	EMB
15.		+	+	-	-	G	C	C	S	S	S	S	S	S	S	S	S	S
16.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
17.		+	+	-	-	G	R	S	S	R	S	S	R	S	S	S	S	S
18.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
19.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
20.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
21.		+	+	-	-	G	R	R	R	S	R	R	S	R	R	R	R	S
22.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
23.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
24.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
25.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
26.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
27.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
28.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
29.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
30.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S

Table 13 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test			culture result	MTT assay						Proportional					
		Niacin reduction	Nitrate reduction	Catalase test		PNB	direct			Indirect			EMB	SM	SM		
							RIF	INH	EMB	RIF	INH	EMB				RIF	INH
31.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
32.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
33.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
34.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
35.	+	+	-	-	G	S	R	R	S	R	R	S	S	R	R	S	S
36.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
37.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
38.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
39.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
40.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
41.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
42.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
43.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
44.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
45.	+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S
46.	+	+	-	-	G	R	R	R	R	R	R	R	R	R	R	R	R

Table 13 (cont.)

Sp. No.	CONCEN TRATE SMEAR	Biochemical Test				culture result	MTT assay																
		Niacin reduction	Nitrate reduction	Catalase test	PNB		direct					Indirect											
							RIF	INH	EMB	SM	RIF	SM	EMB	INH	RIF	SM	EMB	INH	SM				
47.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
48.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	s
49.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
50.		+	+	-	-	G	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	s

**Note :** PBN = para-nitrobenzoic acid, RIF = rifampicin, INH= Isoniazid, EMB=Ethambutal , SM=Streptomycin

**Direct MTT:** PBN 100 µg/ml, RIF 0.2 µg/ml , INH 0.04 µg/ml, EMB 1 µg/ml, SM 0.4 µg/ml

**Indirect MTT and proportion :** PBN 500 µg/ml, RIF 1 µg/ml , INH 0.2 µg/ml, EMB 5 µg/ml, SM 2 µg/ml

G = Growth

S = Susceptible

R = Resistant

+ = Positive

- = Negative



## **BIOGRAPHY**



## BIOGRAPHY

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**Work Place** The Office of Diseases Prevention and Control 9<sup>th</sup>  
Phitsanulok

**Position** Medical technologist

**Work Experiences**

2009	Training course Public Private Mix in TB control, Indonesia
2008	International training course of Regional Workshop on Strengthening Laboratory Service for TB and STIs/HIV Laboratory Diagnosis
1998	Trainer of TB, Parasite laboratory diagnosis
1995	Work on <i>Mycobacterium tuberculosis</i> laboratory diagnosis

**Education Background**

2007	BS (Medical technology), Haw-cheawchalemprakit University, Samutpakan, Thailand.
2003	BS (Computer science), Pibulsongkram Phitsanulok, University, Phitsanulok, Thailand.
1997	BA(General technology), Sukhothaitammaraj University, Nontaburi, Thailand

