Comparision Drugs Susceptibility Testing of *Mycobacterium tuberculosis* between Direct and Indirect MTT Assay

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Out-lines

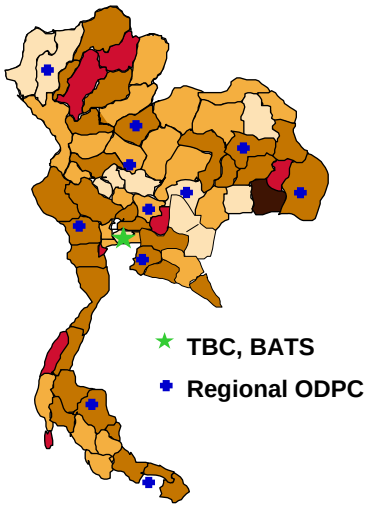
- Situation
- Introduction
  - TB
  - MTT assay
- Objectives
- Scope of study
- Material & method
- Result
- Conclusion & Discussion

National Situation Assessment

Population	66m
Global rank	18
Incidence rate (all cases)	142/100,000
Incidence rate (new M+)	62/100,000
Prevalence rate	208/100,000
Mortality rate	21/100,000
TB case HIV+	17%
New cases, MDR	0.9%

Sources:NTP 2009

National Situation Assessment



Distribution of new smear positive

(RATE / 100,000 POP .)

- 1 to 15
- 16 to 30
- 31 to 45
- 46 to 60
- 61 to 75
- 76 +

Sources:NTP 2009

Introduction (con.)

Laboratory diagnosis

- Direct smear
- Culture

New technology for diagnosis

- Automation culturing
- Molecular technique
  - * Genetic probe
  - * PCR
  - * DNA fingerprint
  - * DNA sequencing
- Antigen detection (Serodiagnosis)
- Electrophoresis (* HPLC * GLC)

Introduction (con.)

Drug resistance

- Single - drug resistance
- Multi - drugs resistance (Isoniazid + Rifampicin)
- Extensively – drug resistance
  (capreomycin, kanamycin and amikacin)

Drug Susceptibility Test

- Direct Susceptibility Test
- Indirect Susceptibility Test

Introduction (con.)

- Direct Susceptibility Test
  - Directly from clinical sample: Smear "positive"
  - Isoniazid and Rifampicin
  - Turnaround Time : 4 wks
  - Cost: 120 ฿ / test

Introduction (con.)

- Indirect Susceptibility Test:
  - From pure culture (3-4 week)
  - Turnaround Time: 7-8 wks
  - M7H10 agar or LJ
  - Cost: 250 ฿/ test

Introduction (con.)

MTT ASSAY

3-(4,5-dimethylthiazol – 2-yl)-2,5-diphenyl tetrazolium bromide (growth rate)

- yellow color (normal)
- reduce by enzyme dehydrogenase (Violet)
- spectrophotometer (570 nm)
- MTB growth
- Cost 120 ฿/test

TBI&S test kit
From the Department of Microbiology
Faculty of Medicine, Siriraj hospital

Introduction (con.)

MTT ASSAY

- PNB (p – nitrobenzoic acid)
- Drug
  - Isoniazid 0.2,1 µg/ml (INH)
  - Streptomycin 2,10 µg/ml (S)
  - Ethambutal 5,10 µg/ml (E)
  - Rifampicin 1 µg/ml (R)

Introduction (con.)

MTT ASSAY

- Primary identify (MTB & NTM)

- Drug resistance result

Comparision Drugs Susceptibility Testing of *Mycobacterium tuberculosis* between Direct and Indirect MTT Assay

Objectives

To compare the drugs Susceptibility Testing of *Mycobaterium tuberculosis* between direct and indirect MTT assay

Scope of the study

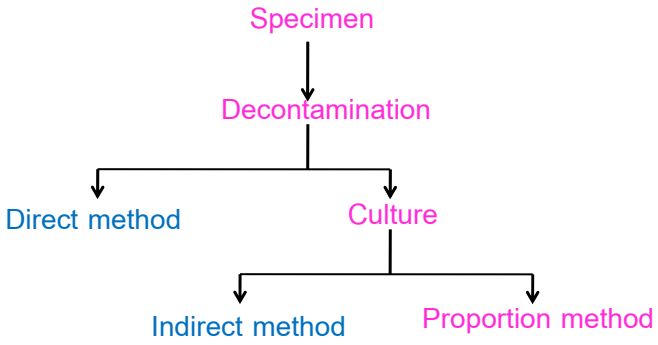
150 AFB positive sputum for Mycobacteria at the office of diseases prevention and control 9th Phitsanulok.

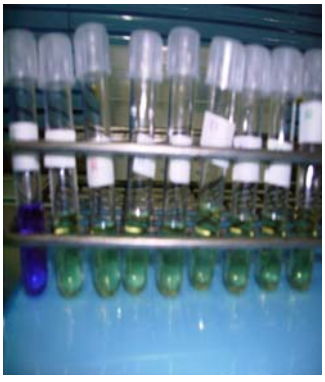
1 June 2007 – 1 March 2009

Laboratory of Allied Health Sciences Faculty ,Naresuan University

The office of diseases prevention and control 9th Phitsanulok.

Materials & methods





Result

Methods	Results			Total
	Resistant	Susceptible	Contamination	
7 days				
Direct MTT	5 (5%)	95(95%)	0	100
Indirect MTT	23(23%)	77(77%)	0	100
Proportion	28(28%)	72(72%)	0	100
14 days				
Direct MTT	5 (10%)	43(86%)	2(4%)	50
Indirect MTT	5(10%)	45(90%)	0	50
Proportion	5(10%)	45(90%)	0	50

Distribution of Drug Susceptibility result by Direct and Indirect MTT

Direct MTT	Indirect MTT			Total
	Resistant	Susceptible	Contamination	
7 days				
Resistant	5	0	0	5
Susceptible	18	77	0	95
Contamination	0	0	0	0
total	23	77	0	100
14 days				
Resistant	5	0	0	5
Susceptible	0	43	0	43
Contamination	0	2	0	2
total	5	45	0	50

The resistant result in either methods

Methods	Resistant			
	Streptomycin	INH	RIF	Ethambutol
7 days				
Direct MTT	3	4	2	1
Indirect MTT	12	13	8	12
Proportion	21	19	10	14
Total	36	36	20	27
14 days				
Direct MTT	1	4	3	4
Indirect MTT	1	4	3	4
Proportion	1	4	3	4
Total	3	12	9	12

The correlation of AFB results and drug resistant in either methods

Drug resistant by methods	AFB result				Total
	AFB 3+	AFB 2+	AFB 1+	Scanty AFB	
7 days					
Direct MTT	2/8	2/63	1/18	0/1	5
Indirect MTT	5/8	14/63	4/18	0/1	23
Proportion	5/8	15/63	8/18	0/1	28
14 days					
Direct MTT	4	1	0	0	5
Indirect MTT	4	1	0	0	5
Proportion	4	1	0	0	5

Conclusion & Discussion

- Medium mix in solution
- Concentration specimens
- 2-3+ AFB positive
- Time period

Conclusion & Discussion

- Direct MTT assay screening for the drug susceptibility test
- Time periods 14 days
- 100 % sensitivity and specificity.

Conclusion & Discussion

- Simple assay
- Low cost
- Rapid test for drugs Susceptibility Testing

Thank You

Home