

## CHAPTER II

### DIAGNOSIS

#### A. Bacteriology

The diagnosis of pulmonary tuberculosis (PTB) is established when tubercle bacilli are identified in the sputum, bronchial aspirates and pleural fluid or tissue. For majority of patients who have PTB, the diagnosis can be readily made by sputum examination. The staining characteristics of *M. tuberculosis* allow its identification in clinical specimens. A definitive stain is using carbol fuchsin reagent. Culture adds to the diagnostic yield and also permits the specific identification of acid fast bacilli and the study of drug susceptibility of the organisms.<sup>(3,5)</sup>

At the Sto. Tomas University Hospital, BACTEC, a Middlebrook 7H12 culture medium for mycobacteria has been used since February, 1990. The 7H12 liquid medium contains a <sup>14</sup>C labeled substrate specific for mycobacterial growth. Instead of colony counting for about 3 weeks, the growth is monitored radiometrically and results are reportable within 4-5 days.

### Principles of BACTEC procedure<sup>(6)</sup>

The BACTEC TB medium is an enriched Middlebrook 7H9 broth base. Mycobacteria use a  $^{14}\text{C}$  labeled substrate present in the medium releasing  $^{14}\text{CO}_2$  into the atmosphere above the medium. When the 7H12 medium vials with growth are tested on the BACTEC 460 instrument, the  $^{14}\text{CO}_2$  is aspirated from the vial and its radioactivity is determined quantitatively in terms of numbers on a scale from 0 to 999. These numbers are designated as Growth Index (GI) . The increase in the GI output everyday is directly related to the rate and amount of growth in the medium.

If an inhibitory agent is added into the medium, inhibition of metabolism is reflected by reduction of  $^{14}\text{CO}_2$  when compared to a control which has no inhibitory agent. This principle applies to drug susceptibility testing and differentiates TB from mycobacteria other than tuberculosis (MOTT) .

The BACTEC procedure describes only one concentration of each drug for susceptibility testing. However, other concentrations, or more than one concentration of each drug maybe tested.

Preliminary studies indicate the following comparative concentrations of drugs in BACTEC and conventional methods.

**Equivalent Drug Considerations (ug/ml)**

| DRUG         | BACTEC | CONVENTIONAL |      |
|--------------|--------|--------------|------|
|              | 12 B   | Middlebrook  | LJ   |
|              |        | 7H10         |      |
| Streptomycin | 2.0    | 2.0          | 4.0  |
|              | 6.0    | 10.0         | -    |
| INH          | 0.2    | 0.2          | 0.2  |
|              | 1.0    | 1.0          | -    |
| Rifampin     | 2.0    | 1.0          | 40.0 |
| Ethambutol   | 2.5    | 5.0          | 2.0  |
|              | 7.5    | 10.0         | -    |

Published studies have reported that results obtained by the BACTEC method compared well with the conventional proportion method or the resistance ratio method. The accuracy and reproducibility of the method has also been evaluated with excellent results.

The increased volume of BACTEC TB medium (12B) with PANTA supplement was evaluated at 3 different sites. The following are the data comparing 12A, 12B LJ and 7H10 or 7H11 media.

## 1. TOTAL SPECIMENS : 2736

TOTAL AFB CULTURE POSITIVE 219 (all media combined)

| MEDIUM       | TOTAL ISOLATES | % POSITIVE |
|--------------|----------------|------------|
| 12 B         | 195            | 89         |
| 12 A         | 178            | 81         |
| 7H10 or 7H11 | 151            | 69         |
| LJ           | 134            | 61         |

## 2. Analysis of Possible Combinations of Two Media

12 A plus LJ Number of Isolates

|                       |          |
|-----------------------|----------|
| Both Positive         | 127      |
| Positive in 12 A only | 51       |
| Positive in LJ only   | <u>7</u> |
| Total Positives       | 185      |

12 B Plus LJ

|                       |          |
|-----------------------|----------|
| Both positive         | 130      |
| Positive in 12 B only | 65       |
| Positive in LJ only   | <u>4</u> |
| Total Positives       | 199      |

12 A Plus 7H10 OR 7H11 :

|                               |           |
|-------------------------------|-----------|
| Both positive                 | 141       |
| Positive in 12 A only         | 37        |
| positive in 7H10 or 7H11 only | <u>10</u> |
| Total Positives               | 188       |

12 B plus 7H10 or 7H11 :

|                               |          |
|-------------------------------|----------|
| Both positive                 | 145      |
| Positive in 12 B only         | 50       |
| Positive in 7H10 or 7H11 only | <u>6</u> |
| Total positives               | 201      |

LJ Plus 7H10 or 7H11 :

|                               |           |
|-------------------------------|-----------|
| Both Positive                 | 111       |
| Positive in LJ only           | 23        |
| positive in 7H10 or 7H11 only | <u>40</u> |
| Total positives               | 174       |

The data indicate that 12 B medium offers a 9.6% improvement over 12 A medium.

Outlines of the procedures for BACTEC TB system overview, primary isolation, TB differentiation, indirect and direct drug susceptibility tests are seen on Appendices E<sup>1</sup>, E<sup>2</sup>, E<sup>3</sup>, E<sup>4</sup>, E<sup>5</sup>.

### **B. Serology**

Enzyme-linked immunosorbent assay (ELISA) techniques offer the potential for readily applied serologic test for PTB especially in children and in extra pulmonary disease where sputum examination is not available.<sup>(3)</sup>

### C. Radiology

The chest roentgenogram is an important tool for the diagnosis and evaluation of PTB. Although there is truly no radiographic picture absolutely typical of PTB, other studies show that the chest x-ray is still a useful procedure to screen population where PTB is common. Multinodular infiltrates in the apical posterior segments of the upper lobes and superior segments of the lower lobes are the most typical lesions of PTB.<sup>(7,6,4)</sup> When tuberculosis becomes inactive or heals, fibrotic scarrings become apparent.<sup>(8)</sup> Fibrotic lesions may develop calcification. Tuberculous activity therefore maybe judged only after the films are serialized.

Radiologic classification of PTB is defined as<sup>(4)</sup> :

- a) Minimal: includes those of slight to moderate density but does not contain demonstrable cavitations. It involves a small part of one or both lungs but should not exceed the volume of lung on one side which is present above the second chondrosternal junction and the spine of the fourth or the body of the fifth thoracic vertebra. It also applies to lesions that can not be seen on x-ray but are associated with the confirmed finding by culture of tubercle bacilli.
- b) Moderately advanced: maybe present in one or both lungs, but the total extent should not exceed the following: disseminated lesions of slight to

moderate density which may extend throughout the total volume of one lung, or the equivalent in both lungs; dense and confluent lesions that are limited in extent to 1/3 the volume of one lung; total diameter of cavitation must be less than 4 centimeters.

- c) Far-advanced: describes lesions that are more extensive than moderately advanced.

#### **D. Tuberculin Test**

Using a 5 tuberculin unit purified protein derivative (5 TU-PPD), intracutaneous tuberculin test, usually applied on the forearm, is recommended as a screening test for prior mycobacterial infection.

However, in the Philippines, the use of PPD as a diagnostic test in Filipino adults is still a controversy. Because seventy percent (70%) of Filipino adults are PPD-positive (32%: naturally infected; 40%: BCG immunized), as a diagnostic tool, PPD becomes significant when it is negative.<sup>(9)</sup> Hence, it can not be used as a sole criterion for diagnosis of PTB.

#### **National Trend**

The National TB Program Plan used tuberculin testing, radiologic examination and bacteriologic analysis to measure the magnitude of tuberculosis in the country. The

population was divided into two main groups: non-infected population and infected population. The latter was subdivided into tuberculin reactors, TB patients with positive chest radiograph and TB patients with bacteriologic exam. As a whole, there are 4 groups. Philippine population in the year 1987 when the study was conducted was 57,356,042. Based on the data, the National Tuberculosis Program Plan estimated the number of TB cases.<sup>(2)</sup> This is seen on Table 1.

**TABLE 1<sup>(2)</sup> ESTIMATED NUMBER OF TB CASES BY BACTERIOLOGY, X-RAY, NUMBER INFECTED WITH TUBERCLE BACILLI BY TUBERCULIN TESTING FOR 1987**

|   |       | Population |
|---|-------|------------|
| A. Bacteriologic Examination                          |       |            |
| 1. Culture positive                                   | 0.86% | 493,261    |
| 2. Sputum positive                                    | 0.66% | 378,549    |
| B. X-ray Examination                                  |       |            |
| 1. Total with radiographic abnormality                | 2.9%  | 1,663,325  |
| a. Minimal cases                                      | 1.72% | 986,523    |
| b. Moderately advanced cases without cavitary lesions | 0.69% | 395,757    |
| c. Moderately advanced cavitation                     | 0.14% | 80,299     |
| d. Far-advanced with cavitary lesions                 | 0.14% | 80,299     |
| e. Far-advanced with out cavitation                   | 0.14% | 80,299     |
| C. Tuberculin Testing                                 |       |            |
| 1. TB infection among general population              | 32.4% | 18,583,357 |
| D. Symptom Inquiry                                    |       |            |
| 1. TB symptomatics                                    | 8%    | 4,588,483  |