RAPID METHODS FOR CULTURE OF MYCOBACTERIA

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Tuberculosis remains a major health problem in many parts of the world. Rapid and accurate detection of *M. Tuberculosis* is essential not only to speed up the treatment of patients but also to control the disease in the population. Bacteriological investigations play a key role in the diagnosis of different forms of tuberculosis.

Microscopy

Traditionally the demonstration of acid fast bacilli (AFB) in smears from clinical specimens, though confirms the diagnosis of tuberculosis with a specificity of almost 100%, its sensitivity ranges only from 45-75% since the limit of detection with this method is 5000-10,000 bacteria per ml. of clinical specimens. And also microscopy neither can detect viability of AFB nor identify the mycobacterial species. Hence it is recommended that the results of microscopy should be confirmed by culture, identification and drug susceptibility test in order to institute appropriate treatment and better management of patients.

Culture and Drug Susceptibility

Traditional approaches: Isolation of mycobacteria from clinical specimens has traditionally depended on the use of solid media such as the egg-based Lowenstein-Jensen (LJ) medium, Middlebrook 7H11 agar, Middlebrook 7H9 broth and Kirchner's liquid medium. The major constraint of this procedure is the slow growth of mycobacteria which necessitates a mean incubation period of 4 weeks on these conventional media. The drug susceptibility tests to the antituberculosis drugs require an additional 4 period before results can be obtained.

Modern approaches: Today, there are fewer rapid methods for the culture of Mycobacteria. These include Microcolony detection on solid media, the Septi-Chek AFB method, Mycobacterial growth indicator tube (MGIT) system, radiometric Bactec 460 TB method and Bactec MGIT 960 method.

Microcolony counting method: In this method, plates poured with thin layer of Middlebrook 7H11 agar medium are inoculated and examined microscopically on alternate days for the first 2 weeks and less frequently thereafter. For microscopic examination, the plates are inverted on the stage of a conventional microscope and the objective is focussed on the surface of the agar through the bottom of the plate. In

less than 7 days, microcolonies of slow growing mycobacteria such. as *M. Tuberculosis* can be detected using this method. Though this method is less expensive and requires about half the time needed for conventional culture, the recovery of mycobacteria is less efficient and it is labour intensive[1].

Speti-Chek AFB system: It consists of a liquid phase 7H9 broth with three solid media, the middle brook 7H11 agar, modified egg medium and chocolate agar. This biphasic medium is presented in a self contained CO_2 environment. This non-radiometric approach has the potential to expedite processing, obviate CO_2 incubation requirements and facilitates early detection of positive cultures. This method requires about three weeks of incubation. The unique advantage of this technique is the simultaneous detection of *M. Tuberculosis*, Non-tuberculous Mycobacteria, other respiratory pathogens and also contaminants. It has been reported from multicentric studies conducted by different groups that this system gives a better culture yield when compared to other methods including Bactec[2].

Mycobacterial Growth Indicator Tube (MGIT) system: It is a non-radiometric broth method for the growth and detection of mycobacterial isolates from clinical specimens. The MGIT system consists of a Middlebrook 7H9 broth and a fluorescent compound embedded in silicone. Antibiotic and growth supplements are added before inoculation. Positive cultures are detected visually because of the metabolic depletion of oxygen, which otherwise quenches fluorescence. Instrumentation is not required, and multiple tubes can be inspected simultaneously. The tube is examined under UV light where the growth is indicated by an unmistakable orange fluorescent glow. In several studies conducted elsewhere, it was found that the culture sensitivity of this method in both smear positive and smear negative samples compared well with the Bactec 460 were and better than the conventional egg based method[3].

Radiometric BACTEC 460 TB method; Middle brook et al developed this technique that is specific for mycobacterial growth, wherein C^{14} labelled palmitic acid in 7H12 medium is used. This system detects the presence of Mycobacteria based on their metabolism rather than their visible growth. When the ^{14}C-labelled substrate present in the medium is metabolised, $^{14}CO_2$ is produced and measured by the BACTED 460 instrument and reported in terms of Growth Index (GI) value. In addition to detecting Mycobacteria, Bactec system is also useful in the identification of *M. tuberculosis* using the specific inhibitor p-nitro-alpha-acetylamino-beta-hydroxypropiophenone (NAP). The NAP differential procedure has been shown to be a rapid and reliable method for identifying *M. Tuberculosis.* Using the same system, drug susceptibility test can be performed for all the antituberculosis drugs when sufficient Growth index (GI) is observed. Mycobacteria in clinical samples can be detected in half the time that is observed. Mycobacteria in clinical samples can be detected in half the time that is needed for conventional culture methods.

A comparison of the BACTEC radiometric method with the conventional culture and drug susceptibility testing methods on isolates from clinical specimens in pulmonary and extrapulmonary tuberculosis, childhood TB and TB in HIV-infected individual was undertaken at our Centre. In the case of pulmonary TB, the rate of isolation of positive cultures was significantly faster with the BACTEC method, with 87 percent of the positive being obtained by 7 days, and 96 percent by 14 days. While there was no difference in the total number of positive cultures by the two methods in smear positive pulmonary tuberculosis, in smear negative pulmonary TB, the BACTEC method yielded more number of positive cultures. In extrapulmonary TB, HIV-TB and childhood TB, although the BACTEC method did not yield additional positives, the detection of positives was considerably faster than that by the conventional method, in which the degree of growth was also scanty. The agreement in drug susceptibility tests was 94 percent for streptomycin and isoniazid, 99 percent for rifampicin and 91 percent for ethambutol. Further, most of the drug susceptibility test results became available within 8 days by the BACTEC method. By facilitating early diagnosis, the BACTEC method may prove to be cost effective in a population with a high prevalence of tuberculosis, particularly in the extrapulmonary and paucibacillary forms of the disease[4].

Bactec(R) MGIT 960 Mycobacteria Detection System

It is an automated system for the growth and detection of Mycobacteria with a capacity to incubate and continuously monitor 960 MGIT culture tubes. This culture tube contains the same Middlebrook 7H9 broth base, OADC enrichment and PANTA antibiotic mixture as does the manual MGIT, except the final volume here being 7 ml. After a 0.5 ml inoculum of the processed specimen was added, the tubes are Incubated at 37°C in the BACTEC MGIT 960 instrument, and are monitored automatically every 60 minutes for increase in fluorenscence. Growth detection is based on the AFB metabolic O_2 utilisation and subsequent intensification of an O_2 quenched fluorescent dye contained in a tube of modified MGIT. A series of algorithms are used to determine presumptive positivity and alert the operator to the presence and location of positive tubes.

In a multi-centre evaluation of the BACTEC MGIT 960 system, three high-volume testing sites in USA compared the growth and recovery of AFB to that of the BACTEC 460 TB and conventional culture. The overall recovery of Mycobacteria comprising of 14 species on a total of 1599 clinical specimens from both pulmonary and non-pulmonary origin compared well in this study. In brief, out of a total of 147 AFBs isolated; 80 were *M. tuberculosis* complex (MTB), 30 were *M. avium* complex (MAC) and the remaining other Mycobacterium species. Comparison of average time of detection between paired specimens, the BACTEC 460 TB and BACTEC MGIT 960 systems were 8.7 versus 8.6 for MAC and 13.4 versus 15.5 days for MTB respectively.

According to these investigators the BACTEC MGIT 960 system exhibits greater potential as a rapid, accurate and cost effective method for a high volume AFB laboratory[5].

Conclusion

Today, many new techniques are available for the detection and identification of Mycobacteria. However, detection of AFB by direct microscopy on 2-3 samples of sputa is the easiest and quickest diagnostic procedure that can detect majority of the open cases of pulmonary tuberculosis in developing countries such as India. Sputum culture by conventional method can be done for patients who do not respond to treatment and simultaneous direct sensitivity on those sputa to determine rapidly the sensitivity status to rifampicin and isoniazid for better management of individual patients. In larger settings based on the availability of the infrastructure facility, indirect sensitivity and identification of species can be done provided their quality assurance is ensured by a referral laboratory. Faster culture methods such as BACTEC, MGIT, Nucleic acid amplification techniques described involve prohibitive expenditure in terms of instrumentation, expertise and reagents, making them out of reach of most public health laboratories of developing countries especially in India[6].

References:

1. Idigoras P, Peres - Trallero E, Alcorta M, Gutierrez C and Munoz - Baroja I. Rapid Detection *of tuberculous and Non-tuberculous Mycobacteria by microscopic observation of growth on Middlebrook 7H11 agar.* Eur. J. Clin. Microbiol. Infect Dis., 1995, 14: 6-10.

2. lsenberg HD, D'Amato RF, Heiffets L, Murray PR, Scarda Maglia M, Jabos MC, *et al. Collaborative feasibility study of a biphasic system (Roche Septi - Check AFB) for rapid detection and isolation of Mycobacteria.* J. Clin. Microbiol. 1991, 29: 1719-22.

3. Zuhre - Badak F, Kiska, DL, Setterquist S, Cami Hartley, O'Connell, MA and Hopfer RL. *Comparison of Mycobacteria growth indicator tube with BACTEC 460 for detection and recovery of Mycobacteria from Clinical specimens.* J. Clin. Microbiol. 1996, 34: 2236-39.

4. Venkataraman P, Herbert D, Paramasivan CN, *Evaluation of the BACTEC radiometric method in the early diagnosis* of *Tuberculosis.* Indian J. Med. Res. 1998, 108: 120-27.

5. Elliot LB, Rusch - Gerdes S, Ebrahimzadeh A, *Multi-Center evaluation of the BACTEC MGIT 960 system for the growth and recovery of acid fast bacilli 1998,* presented at the 98th General Meeting of the American Society for Microbiologist, Atlanta, GA.

6. Paramasivan CN, *Recent advances in the diagnosis of Tuberculosis.* Hospital Today 1997, 11: 33-6.