EFFICACY OF COMBINED USE OF SOILD AND LIQUID CULTURE MEDIA FOR DIAGNOSIS OF TUBERCULOSIS

The contribution of microbiology laboratory to the diagnosis of *Mycobacterium tuberculosis* involves the detection and isolation of the bacilli, identification of species and determination of susceptibility to the anti-mycobacterial drugs.

Since clinical management of tuberculosis is becoming challenging, the laboratory procedures needed to diagnose and monitor the course of disease have become more complex. Diagnostic procedures for *Mycobacterium tuberculosis* are time consuming, employ reagents & involve techniques not routinely used in study of bacteria of other genera.

However, till date, culture remains the "Gold Standard" for diagnosis of tuberculosis; being more sensitive than Acid Fast Staining and reliably can detect *Mycobacterium* at concentrations of 10-10^2 bacilli/ ml of specimen.

Culture detects tuberculosis cases earlier, before they become infectious. Since culture techniques can detect few bacilli, the efficiency of diagnosing and failures at the end of treatment can be improved considerably. Culture also provides the necessary material for drug susceptibility testing.

Culture of specimens is, however, much more costly than microscopy and requires facilities for media preparation as well as skilled staff. Culture should be used selectively, in the following cases:

1. Diagnosis of cases with clinical and radiological signs of pulmonary tuberculosis where smears are repeatedly negative or doubtful.
2. Diagnosis of extra-pulmonary and childhood tuberculosis.
3. Follow-up of tuberculosis cases who fail a standardised course of treatment and may be at risk of harbouring drug resistant organisms
4. Surveillance of tuberculosis drug resistance as an integral part of the evaluation of control program performance
5. Investigation of high-risk individuals who are symptomatic, eg. Laboratory workers, health care workers looking after multidrug resistant patients

Mycobacteria are isolated using conventional solid and / or liquid growth media from sputum, blood, cerebrospinal fluid and tissue biopsy specimens. Solid culture media are recommend because of development of characteristic colonial morphology, good growth from small quantities of inoculum and relatively low rate of contamination. It has been observed that *Mycobacterium tuberculosis* tends to grow better on egg-based solid media such as Lowenstein-Jensen (L.J.) slants. Contamination of the slants is prevented by the addition of aniline dyes such as Malachite Green. However, the concentration of the aniline dye is critical to achieve the required growth of the bacilli besides preventing growth of the contaminants.

The use of a liquid medium for the detection of *Mycobacterium tuberculosis* is mainly justified by increased sensitivity and detection rate from specimens with low bacilli count along with reduced detection time as compared to solid culture media. Kirchner's Liquid Media (K.L.) is most useful and least expensive liquid media for culture of tubercle bacilli. It has the additional advantage that it can support a large

inoculum besides preventing overgrowth of contaminants by the addition of selective antibiotic cocktails such as PACT (Polymyxin B, Amphotericin B, Carbenicillin, Trimethoprim).

Culturing method based on a combination of liquid together with solid media are currently recommended, internationally. Since this combination attains maximum sensitivity along with reduced detection time and it is recommended for non-repeatable specimens, such as cerebrospinal fluid and biopsy material. Appropriately decontaminated and treated suputm specimens can be inoculated in Kirchner's Liquid Medium and the growth if obtained can be used for Acid Fast Staining or drug susceptibility testing.

An evaluation report published in the Indian Journal of Medical Research, 1987; 86, stated that, Lowenstein-Jensen and Kirchner's Liquid Media when considered together detected 93% of positive tuberculosis cases and are the best combination for isolation of mycobacterium.

Needless to say, the use of a combination of both solid and liquid culture media for diagnosis of tubercle bacilli would indeed increase the positivity of cultures for *Mycobacterium* besides decreasing the detection time.

Bibliography:

- Diagnostics Standards and Classification of Tuberculosis in Adults and children; Am. J. Respir. Crit. Care. Med., Vol.161. pp1376-1395, 2000
- Biotest Bulletin, Vol.5 No.2.
- Use of multiple media for the cultivation of mycobacteria from specimens other than sputum; Indian Journal of Medical Research, 1987; 86,290-294.