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***Research Article***

# UTILITY OF CONVENTIONAL TECHNIQUES FOR DIAGNOSIS OF PULMONARY AND EXTRAPULMONARY TUBERCULOSIS: STUDY FROM A TERTIARY CARE HOSPITAL IN NORTH INDIA

**Atul Kumar and *Aroma Oberoi**
*Department of Microbiology, Christian Medical College and Hospital, Ludhiana*
**Authors for Correspondence*

## ABSTRACT
Though pulmonary tuberculosis (PTB) remains the most common presentation, extra pulmonary tuberculosis (EXTB) is another significant clinical problem and due to paucibacillary nature, remains a challenge for diagnosis. The aim of this study is to evaluate the utility of smear microscopy and culture for detection of Mycobacteria in pulmonary and extra pulmonary specimens. This retrospective study was carried out in Microbiology Department at Christian Medical College Ludhiana, from January 2013 to September 2013. A total of 1636 clinical specimens received for routine mycobacterial cultivation were processed. All clinical specimens were cultured on Lowenstein-Jensen (LJ) medium after homogenization and decontamination by modified Petroff's method. Consequently identified by AFB staining (Ziehl-Neelsen method). Of the 1636 cases, 13 of the cases were of extra-pulmonary (EPTB) origin where as 34 were of pulmonary tuberculosis. By microscopy for AFB 9(19%) samples and by culture (LJ) 14(30%) samples were positive. Smear microscopy and culture together yielded a positivity rate of 24 (51%).This can be concluded from our study, smear microscopy and culture still remains the gold standard for diagnosis of pulmonary and extra pulmonary tuberculosis with limited resources and poor infrastructure in developing countries like India.

***Keywords:*** *Pulmonary Tuberculosis, Concentrated Smear Microscopy, Culture*

## INTRODUCTION
Tuberculosis (TB) continues to be a major public health threat worldwide despite the availability of many highly sensitive diagnostic tools and highly efficacious treatment for decades. It is more of a threat in the developing world. Mycobacterium tuberculosis is a human pathogen, infecting one third of the population and causing 2 million deaths per year. The global TB emergency has been further exacerbated by multi drug resistant (MDR) TB. The global burden of TB was 9 million new cases and 1.4 million died of TB (WHO report 2012). Early diagnosis of TB is crucial both clinically and epidemiologically.

Tuberculosis can involve any organ system in the body. Though pulmonary tuberculosis (PTB) remains the most common presentation, extra pulmonary tuberculosis (EXTB) is another significant clinical problem and due to paucibacillary nature, remains a challenge for diagnosis (Fanning, 1999; Iscman, 2000; Dutt, 1999). Pulmonary tuberculosis (PTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the trachea-bronchial tree. Extra Pulmonary tuberculosis (EXTB) refers to any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs such as pleura, lymph nodes, intestine, genitourinary tract, joint and bones, meninges of the brain etc. Patients suspected of having EPTB should also have their sputum examined for AFB if they have chest symptoms, irrespective of the duration of these symptoms. A patient diagnosed with both pulmonary and EPTB is classified as a case of pulmonary TB. The aim of this study is to evaluate the utility of smear microscopy and culture for detection of Mycobacteria in pulmonary and extra pulmonary specimens.

## MATERIALS AND METHODS
This retrospective study was carried out in Microbiology Department at Christian Medical College and Hospital, a tertiary care hospital of Ludhiana, from January 2013 to September 2013.

***Specimen Processing and Culture***

A total of 1636 specimens were aseptically collected from patients who were suspected to have pulmonary TB disease or extra pulmonary TB on the basis of their presenting symptoms. A suspect was defined as an individual if he/she had persistent cough for more than three weeks, and/or evening rise of temperature for more than two weeks. The collected specimens were transported in Department of Microbiology, Christian Medical College and Hospital, Ludhiana for AFB microscopy and culture.

Direct smears were prepared by taking a small portion of the purulent part of the sputum with a sterile loop. The specimens were then processed by Petroffs method. In this method sputum or other samples is mixed with equal volume of 4% sodium hydroxide (NaOH) and is incubated at 37ºC with frequent shaking for about 30 minutes. It is then centrifuged at 3,000 rpm for 30 minutes. The supernatant fluid is poured off and the deposit is neutralised by adding 8% hydrochloric acid in presence of a drop of phenol red indicator. The deposit is used to prepare smear (concentrated smear) and to inoculate into Lowenstein-Jensen (L-J) culture media. NAOH acts as a strong mucus digester and the smear processed by it has less debris and a greater concentration of AFB (Kent *et al.*, 1985). This method has been found to increase the sensitivity of microscopy substantially (Apers *et al.*, 2003).

***AFB Smear and Microscopy***

Smears made from original specimens and/or from the concentrated specimens were air dried, heat fixed, and stained by the ZN staining technique. The stained slides were examined under oil immersion (1,000x lens objective), and they were reported negative when no AFB were seen in at least 100 microscopic fields. Smears were graded positive (Diseases IUATaL, 1996) for any of the following observations:

when 1 to 9 AFB were seen in 100 microscopic fields (scored as scanty positive), when 10 to 99 AFB were seen in 100 fields (scored as 1+), when 1 to 10 AFB were seen per field in at least 50 fields (scored as 2+), and when more than 10 AFB were seen per field in at least 20 fields (scored as 3+).

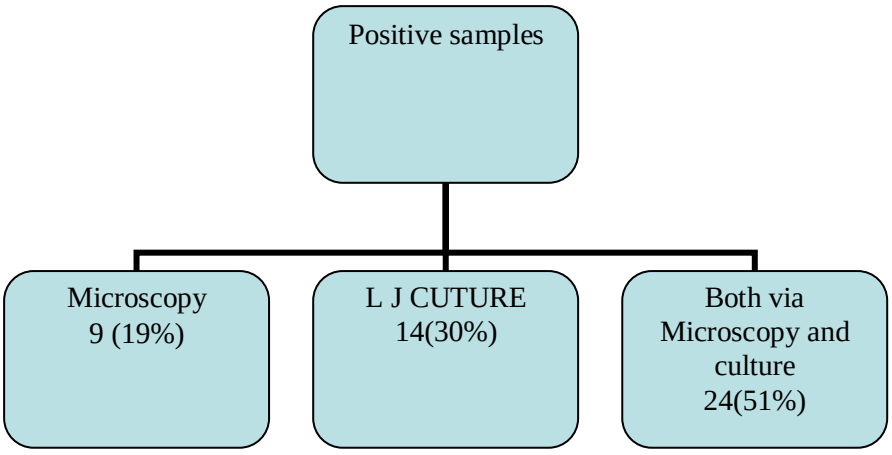
All the specimens inoculated into L-J media were incubated at 37°C for 6 to 8 weeks in a vertical position for the better development of individual colonies. When small and buff coloured colonies grew on LJ medium, the sample was considered as positive. Contaminated cultures (e.g. growth of moulds, and also those in which the medium had liquefied or turned dark green) were discarded.

**RESULTS**

Out of the 1636 specimens, 13 of the cases were of extra-pulmonary (EPTB) origin where as 34 were of pulmonary tuberculosis.

Out of 1636 specimens 9 (19 %) were found to be AFB positive and 1627 (81%) were found AFB negative when smear prepared from the concentrated specimens. Among the 1636 specimens, 14(30 %) were found to be positive on culture. 24(51%) were found to be positive in both concentrated smear microscopy and culture.

***Comparison of Zn Staining and the Gold Standard Culture Method***



## DISCUSSION

TB is a major public problem, majority of TB cases occur in low and middle income countries (WHO, 2009). In high TB burden countries, infrastructure for the diagnosis is not adequate; ZN staining is the only diagnostic technique. In spite of new technologies such as TB culture, Line probe Assay and Gene X pert.

AFB microscopy is believed to be the most practical and fastest technique in establishing a diagnosis of pulmonary TB, especially in developing countries where most of the TB cases live (Huebner *et al.,* 1993; Aber *et al.,* 1980) Studies have shown that direct smear microscopy is highly specific in settings where TB is more prevalent (Albert, 2004; Suarez *et al.,* 2002). Though AFB microscopy is simple, inexpensive and provides rapid result, it has some limitations. The threshold for detection of AFB in sputum samples under optimal conditions is between $10^4$ and $10^5$ bacilli per ml. The sensitivity and specificity of AFB microscopy is low when compared to culture method. In some studies it has been shown that this technique has a low sensitivity, 22-43% for a single smear (Toman *et al.,* 2005) and up to 60% under optimal conditions (Siddiqi *et al.,* 2003; Apers *et al.,* 2003). When compared with that of cultures. In this study nine specimens was found to be positive in concentrated smear microscopy but negative in culture. Sensitivity is even more reduced if samples are of poor quality, which is often the case in children and HIV-coinfected patients (Getahun *et al.,* 2007; Corbett *et al.,* 2003). Although all mycobacterial species are acid fast, this assay is highly specific for M. tuberculosis in countries where TB is endemic (Steingart *et al.,* 2007).

Microscopy clearly has many advantages when it comes to speed and feasibility, and if sensitivity could be improved it has the potential to become an even more valuable tool for National TB Control Programmes (NTPs) around the world. In the last decade many researchers have suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with chemical reagents and then concentrated by centrifugation or sedimentation prior to acid-fast staining (Kent *et al.,* 1985; Heifets *et al.,* 1994) In this Petroffs method has been found to increase the sensitivity of microscopy substantially (Apers *et al.,* 2003). However, it requires some level of staff training, increases time needed for diagnosis, and requires some level of biosafety arrangement to ensure the security of the lab personnel.

The use of sputum smear as a screening procedure for the diagnosis of pulmonary TB has recently been criticized following the finding by several large laboratories that up to 55% of specimens with positive smear failed to grow in culture while 30% are smear negative but culture positive(Uy *et al.,* 1988; Bass *et al.,* 1990). In this study among the 1636 specimens, 14(30 %) were found to be positive on culture. 24(51%) were found to be positive in both concentrated smear microscopy and culture.

### *Conclusions*

This can be concluded from our study, smear microscopy and culture still remains the gold standard for diagnosis of pulmonary and extra pulmonary tuberculosis with limited resources and poor infrastructure in developing countries like India.

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