

ORIGINAL ARTICLE

**IMPORTANCE OF ZIEHL-NEELSEN SMEAR AND CULTURE ON
LOWENSTEIN JENSEN MEDIUM IN DIAGNOSIS OF PULMONARY
TUBERCULOSIS**

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ABSTRACT

Background: Ziehl-Neelsen (ZN) smear is widely used for rapid diagnosis of Tuberculosis in developing countries but culture on Lowenstein Jensen (LJ) medium is more sensitive and cheaper than modern diagnostic techniques in the present settings.

Objective: To evaluate the validity of ZN smear and reliability of acid-fast bacilli culture on LJ medium.

Design and Settings: This comparative study was carried out in PMRC TB Research center in collaboration with Institute of Chest Medicine King Edward Medical University, Mayo Hospital Lahore from January, 2009 to December, 2011.

Patients and methods: Study subjects included patients visiting TB OPD clinic and wards of Mayo Hospital. Lahore and other leading hospitals of Lahore. A total of 8102 specimen were processed for both smear and culture. The smears were stained with ZN method using 1% carbolfuchsin, 25% sulphuric acid and 0.3% methylene blue and were observed under 100-x oil immersion lens. Cultures were inoculated on LJ medium after digestion and decontamination of clinical specimens.

Results: Of total 7333 study subjects, 7055 were pulmonary specimens and among them 800 (11.33%) were smear positive while 1092 (15.47%) were culture positive. Out of 800 smear positive pulmonary tuberculosis cases 664 (83%) were found to be positive on LJ culture. A total of 428 (6.06%) pulmonary cases were negative on the smear but were found to be positive on LJ culture for *Mycobacterium tuberculosis*.

Conclusion: Although AFB smear is rapid, cheap and specific test for early diagnosis of TB but its sensitivity is low and culture on LJ medium is still thought to be gold standard although takes longer time to grow and provides us with positive growth to do drug sensitivity testing.

Key words: *Mycobacterium tuberculosis*, AFB, ZN Stain, Pulmonary TB.

Introduction:

Tuberculosis (TB) is humanity's greatest killer out of control in many parts of the world. The disease, preventable and treatable, has been grossly neglected and no country is immune to it¹. Poor compliance and poverty is among the leading factors that prevent its complete eradication. According to World Health Organization (WHO), there were 291307 new cases and 54721 deaths each year due to TB in Pakistan². Globally TB is responsible for 1.6 to 2.2 million deaths annually and the situation has further worsened with increasing incidence of multi-drug resistance TB³

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The diagnosis of tuberculosis infection is vital both clinically and epidemiologically⁴. A presumptive diagnosis is crucial to guide treatment, to limit the person to person spread of the disease and to assess the degree of activity of the disease⁴.

Acid-fast bacilli (AFB) microscopy, which is a means of detecting/screening of pulmonary tuberculosis, has been used worldwide as a mainstay of case finding⁴. Zheil Neelsen staining is a cheap and specific test which takes about 1 to 2 hours for reporting; however it is less sensitive and requires a large number of bacilli (up to 10,000 bacilli/ml) in the specimen. Moreover, it cannot distinguish Mycobacterium tuberculosis from Mycobacterium other than tuberculosis and is therefore, used for screening only⁴. This technique is widely used in Pakistan and other developing countries⁵.

Culture on Lowenstein Jensen (LJ) medium remains the gold standard for the diagnosis of TB however the facility is not available on its full extent in developing countries^{6,7} hence required special procedures and need skilled workers. Culture on LJ medium is time consuming but cheaper than radiometric and molecular based techniques and a handy approach in the diagnosis of TB in developing countries⁸.

Since, in all the major healthcare settings of Pakistan, LJ media and ZN staining is widely used to diagnose the infection of tuberculosis and to check the response of ATT on tuberculosis. Keeping in view, this study was undertaken to evaluate the validity of AFB smear microscopy and to compare it with AFB culture on LJ medium in the present setting.

Materials and methods:

This comparative study was carried out in PMRC TB Research Centre in collaboration with Institute of Chest Medicine King Edward Medical University, Mayo Hospital, Lahore from January 2009 to December 2011. A total of 7333 specimens from pulmonary sites were included in this study.

Study subjects included patients visiting TB OPD Clinic and wards of Mayo Hospital and other leading hospitals of Lahore. Symptomatic suspects of pulmonary TB with fever, fatigue, anorexia and weight loss and clinically suggestive of TB were asked to submit their respective samples.

Direct and concentrated smears were prepared from clinical specimens after treating with 4 % NaOH (sodium hydroxide) for decontamination and digestion of clinical specimens. Sterile phosphate buffer PH 6.8 is added to neutralize the effect of NAOH and the samples were concentrated by centrifugation at 3000 g for 15 minutes. Supernatant was discarded and sediment was re-suspended in small amount (1-2 ml) of phosphate buffer and inoculated on the slants of LJ medium. The smears were stained with ZN method using 1% Carbol-fuchsin, 25% sulphuric Acid and 0.3% methylene blue. A minimum of 100 oil fields was observed to declare negative smear. Smear is considered positive if it contains at least 3 AFB in observed 100 oil fields for this study. The results were reported according to WHO/ International Union against Tuberculosis and Lung Diseases (IUATLD) as no AFB per 100 high power fields reported as negative, 1-9 AFB per 100 high power fields reported as actual count per 100 high power field, 10-99 per 100 high power fields reported as 1+, 1-10 AFB per high power field in at least 50 fields reported as 2+ and more than 10 AFB per high power field in at least 20 fields is reported

as 3+. Culture is considered positive if it contains only 1 colony, however results were reported as less than 50 colonies, reported exact number of colonies, more than 50 and less than 100 colonies 1+, 100 to 200 colonies 2+ and more than 200 colonies were reported as 3+. A known positive and a known negative slide were included with each run and each batch of staining.

An experienced microbiologist rechecked the random positive and negative smears for internal quality assurance. Random ZN smears are also sent to National TB Control Program (NTP) at each quarter for external quality assurance. LJ media were tested by inoculation of known ATCC strain of H37 Rv. Random slants of LJ media inoculated with sterile distilled water were also incubated from each batch as negative controls.

Results:-

A total of 7333 specimens were processed for smear and culture out of which 278 (3.94%) were contaminated in the culture therefore excluded from the study. Of the remaining 7055 pulmonary specimen's gender wise frequencies of the subjects are shown in table below.

Table I: Year and gender wise frequencies of 7055 pulmonary cases.

year	Pulmonary specimens		Total
	Male	Female	
2009	1678	893	2571
2010	1029	997	2026
2011	1439	1019	2458
Total	4146	2909	7055

Of the 7055 pulmonary specimens 800 (11.33%) were smear positive while 1092 (15.47%) were culture positive. Out of 800 smears positive pulmonary cases 664 (83%) were found to be positive on LJ culture. A total of 428 (6.06%) pulmonary cases were negative on the smear but were found to be positive on LJ culture for *Mycobacterium tuberculosis* as shown above in table II.

Table II: Comparison of AFB smear with culture results in 7055 pulmonary cases.

site of specimens	Smear positive	Culture positive	Smear positive/culture positive	Smear negative/culture positive
Pulmonary	800 (11.33%)	1092 (15.47%)	664 (83%)	428 (6.06%)

ZN smear sensitivity of the pulmonary specimens is considerably high and had following positive predictive value (PPV) and negative predictive value (NPV) as shown in table III.

Table III: Sensitivity, specificity, PPV and NPV of AFB smear microscopy for pulmonary specimens.

Nature of specimen	Sensitivity	Specificity	PPV	NPV

Pulmonary	71%	97%	98%	93%
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Discussion:

Acid-fast microscopy is believed to be the most practical and fastest technique in establishing a presumptive diagnosis of pulmonary tuberculosis. The sensitivity and specificity of AFB smear microscopy and culture varies depending upon the nature of the specimen, its quality, quantity, bacterial content and the extent of viable organisms⁸.

Overall Smear positivity of AFB for pulmonary specimen in the present study is 11.33% and it is little low as compared with International Union against Tuberculosis and Lung Disease (IUTALD)⁹. Smear positivity of pulmonary specimens is (11.33%) and this study is not in agreement with the study that reported the smear positivity of 20.25% in pulmonary specimens¹⁰. The sensitivity of AFB microscopy (71%) for pulmonary specimens in this study is almost similar to that reported by other studies^{11, 12}. However one study also reported the high sensitivity of AFB smear microscopy up to 75%¹³. Another study conducted in the same center reported sensitivity of 66.23% for pulmonary specimens⁸ which is little low as compared to the present study. This could be due to the fact that most of the specimens received in our study came from patients suspected clinically and radiologically to have pulmonary tuberculosis. Three sputum smears for acid-fast bacilli are recommended for proper diagnosis in pulmonary suspects of TB¹⁵. However, WHO has proposed two smears for the diagnosis of TB in countries having functional external quality assurance⁸.

Culture using LJ medium has been the gold standard for the diagnosis of tuberculosis for many years in the developing countries⁷. An overall AFB culture positivity in the present study was 15.47% and is little higher than the study that revealed the culture positivity of 12.3%¹⁴. While others have reported a culture positivity of 48.9% and 47.1% respectively^{12, 15}.

Culture positivity in the present study is significantly high as compared to AFB smear microscopy as about 5000 to 10000 AFB/ml of specimen is needed to yield positive result by AFB smear microscopy while the advantage of culture on LJ medium is that it has the sensitivity of 80-85%, very specific and being able to detect as few as 10 bacteria per milliliter of specimen^{16, 17}.

The study concludes that although AFB smear is rapid, cheap and specific test for diagnosis of TB but its sensitivity is low. It is thus evident that culture on LJ medium is more sensitive and is documented to be gold standard. It is cost effective than radiometric and molecular methods and can therefore be a useful tool for developing countries.

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