ORIGINAL ARTICLE

Comparison of detection of Acid Fast Bacilli in clinical samples by AFB Smear microscopy and Culture in the diagnosis of Tuberculosis in a tertiary care setting.

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Abstract

Ziehl Neelsen 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 is a comparative study, carried out in PMRC TB Research Centre King Edward Medical University Mayo Hospital Lahore from January, 2006 to December, 2008.

Patients and Methods: Study subjects included patients visiting TB OPD Clinic and Wards of Mayo Hospital and other leading hospitals of Lahore. A total of 6738 specimen were processed for both smear and culture. The smears were stained with ZN method using 1% Carbol Fuchsin, 25% Sulphuric Acid and 0.3% Methylene Blue and were observed under 100X oil immersion lens. Cultures were inoculated on LJ medium after digestion and decontamination of clinical specimens.

Results: 313 (4.65%) were contaminated in the culture therefore excluded from the study, of remaining 6425 pulmonary and extra-pulmonary specimens 964 (15.01%) were smear positive and 2060 (32.06%) were culture positive. Smear positivity of extra-pulmonary specimens were very low (3.97%) as compared to Culture of the same sample (21.18%). 1103 (20.27%) smear negative specimens were found out to be positive by culture on LJ medium.

Conclusion: Although AFB smear is rapid, cheap and specific test for diagnosis of TB, its sensitivity is low and culture on LJ medium is more reliable and cheaper than radiometric and molecular based diagnostic techniques.

Key Words: Mycobacterium tuberculosis, Ziehl-Neelsen staining (ZN), Lowenstein-Jensen medium (L.J), tuberculosis (TB)

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Introduction:

Directly observed short treatment (DOTS) recommended by World Health Organization (WHO) has been expanded throughout the health services of Pakistan since May 2005[1]. Aim of this programm is to detect at least 70% of new smear positive cases and to cure at least 85% of smear positive pulmonary tuberculosis (TB) patients[2]. However extra-pulmonary which carries about 15% to 20% of total TB load in South Asian countries is rarely positive by smear microscopy[3]. Even then the detection of AFB by Zheil Neelsen (ZN) smear remains the most widely used and rapid diagnostic test for TB in developing countries[4].

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[5] hence required special procedures and need skilled workers. ZN smear microscopy is a recommended method especially for developing countries in the diagnosis of TB due to its low cost, rapidity and specificity but a high number of organisms are required to yield a positive smear. 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 countries. This study has been undertaken to evaluate the validity of AFB smear microscopy and to compare it with AFB culture on LJ medium in the present settings.

Materials and Methods:

This comparative study was carried out in 2009; retrospective data from January, 2006 to December, 2008 was used for analysis. A total of 6738 specimens of pulmonary and extra-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 and extra-

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 % Noah (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. In case of tissues they were ground in sterilized mortar and pastel before decontamination and digestion. Body fluids like CSF, Synovial fluid, Pleural fluid, Ascitic fluid and bone marrow that are collected aseptically and were expected to have no contaminants, inoculated without decontamination. 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[5] in observed 100 oil fields for this study. The results were reported according to WHO/International Union of 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 then 50 colonies, reported exact number of colonies, more than 50 and less then 100 colonies 1^+, 100 to 200 colonies 2^+ and more then 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) after each quarter for external quality assurance. LJ media were tested by inoculation of known ATCC strain of H37Rv. Random slants of LJ media inoculated with sterile distilled water were also incubated from each batch as negative controls.

Results:

A total of 6738 specimens were processed for smear and culture out which 313 (4.65%) were contaminated in the culture therefore excluded from the study. Of the remaining

6425 specimens 5646 (87.88%) were from pulmonary and 779 (12.12%) were from extra-pulmonary sites. Males were pre-dominantly included in this study in both pulmonary and extra-pulmonary samples.

Table 1

Year and gender wise frequencies of 5646 pulmonary and 779 extra-pulmonary specimens received.

Year	Pulmonary Specimens		Extra-pulmonary Specimens		Total
	Male	Female	Male	Female	
2006	1153	892	197	148	2390
2007	904	651	177	71	1803
2008	1161	885	109	77	2232
Total	3218	2428	483	296	6425

Table 2

Comparison of AFB smear with culture result in 5646 pulmonary and 779 extra pulmonary specimens.

Nature of specimen	Smear positive N (%) *	Culture positive N (%)	Smear positive Culture positive N (%) **	Smear negative Culture positive N (%)***
Pulmonary	955 (16.91)	1896 (33.58)	929 (97.27)	967 (20.61)
Extra-pulmonary	31 (3.97)	165 (21.18)	29 (93.55)	136 (18.18)

*Percentage is calculated from total number of cases, ** percentage is calculated from total smear positive, ***percentage is calculated from total smear negative cases.

Of the 5646 pulmonary specimens 955 (16.91%) were smear positive while 1896 (33.532%) were culture positive while for extra pulmonary specimen smear positivity was seen in 31 out of 779 (3.97%) While culture positivity was seen in 165 out of 779 (21%). It is also seen that smear positivity extra-pulmonary specimen is quite low as compared to pulmonary specimen. Out of 955 smear positive cases 929 (97.27%) pulmonary and out of 31 smear positive cases 29 (93.55%) extra-pulmonary cases were found to be positive on LJ culture. 967 (20.60%) pulmonary and 136 (18.18%) extra-pulmonary cases that were negative on the smear were also found to be positive on LJ culture for MTB.

Table 3

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AFB smear microscopy

Nature of specimen	Sensitivity	Specificity	PPV	NPV
Pulmonary	66.23%	99.31%	98.65%	79.50%
Extra-pulmonary	21.16%	99.68%	98.80%	81.87%
Total	65.14%	99.36%	98.66%	79.83%

ZN smear sensitivity of the pulmonary specimens is significantly high ($p<0.01$) as compared to extra-pulmonary specimens however the test is specific and has same PPV and NPV.

Discussion:

Diagnosis of TB depends on the history, physical and radiographic evidence or the presence of AFB in ZN smears and cultures[6]. Sensitivity and specificity of AFB smear microscopy and culture varies depending upon the nature of specimen, its quality, quantity, bacterial content and viability of the organism[7].

Overall Smear positivity of AFB in the present study is 15.34% is in agreement with International Union against Tuberculosis and Lung Disease (IUTALD) guidelines[8]while in a study carried out during the year 2006 in same setting it was (11.51%) [9] which is comparatively low. Smear positivity of pulmonary specimens (16.91%) is significantly high as compared to extra-pulmonary specimens (3.97%) in this study and is in agreement with the studies that reported the smear positivity of 3.9% in extra-pulmonary specimens[10, 11]. This study is not in agreement with the study that reported the smear

positivity of 20.25% in extra-pulmonary specimens[12]. The factors responsible for low smear positivity in extra-pulmonary specimen are their paucibacillary nature; inadequate sample and apportioning of samples for various diagnostic tests result in non uniform distribution of micro-organism [13].

When sensitivity was calculated taking culture as gold standard the sensitivity of extra-pulmonary smears (21.16%) is seen significantly low as compared to the sensitivity of pulmonary specimens 66.23%. Much lower sensitivity of AFB smears (0-6%) has been reported for extra pulmonary specimens in other studies[14]. The sensitivity of AFB microscopy for pulmonary specimens in this study is similar to that reported by other studies [15], [16], however one study also reported the high sensitivity of AFB smear microscopy up to 75%[17]. An over all AFB culture positivity in the present study was 32.07% and is much higher than the study that revealed the culture positivity of 12.3%[10]. While others have reported a culture positivity of 48.9% and 47.1% respectively[12, 18].

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[19, 20].

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. It is thus suggested that facilities for carrying out culture of mycobacterium tuberculosis be made available at district level by TB control program.

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