

## Original Article

### **Detection of Mycobacterium tuberculosis in clinical samples by smear and culture**

Roohi Aftab, Farzana Amjad, Rukhshan Khurshid, Nazifa Ahmed  
From Department of Pathology and Biochemistry, Fatima Jinnah Medical College, Lahore.

Correspondence: Dr Roohi Aftab, Fatima Jinnah Medical College, Lahore.

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#### **ABSTRACT**

**Objective:** To compare the smear stained by Ziehl-Neilsen (ZN) and Lowenstein-Jensen (LJ) medium for the detection of Mycobacterium tuberculosis in clinical samples from different categories.

**Materials / Methods:** This laboratory based retrospective study was carried out at Sir Ganga Ram Hospital /Fatima Jinnah Medical College, Lahore over a 5 year period between January 2001 and June 2006. A total of 798 clinical samples were collected from patients with a provisional diagnosis of tuberculosis. A ZN stain of smear and culture on LJ medium were performed for the detection of Mycobacterium. The specimen categories were sputum, pus, lymph node aspirate, urine and endometrial curetting.

**Results:** Out of 798 specimens received over the study period, only 46.3% (n=369) were respiratory whereas 53.7% (n=429) were non respiratory tract category samples. Among these, 3.578% were positive in ZN stain while 11.65% were positive on culture. Out of a total of 369 respiratory tract category samples, 38(10.3%) sputum samples were positive for AFB on both ZN and culture. Among the non respiratory tract category, 47(28.2 %) pus, 26(31%) lymph node aspirates, 05(15.6%) urine, 05(3.42%) endometrial curetting were positive.

**Conclusion:** Fifteen percent of clinical samples belonging to 05 different categories of specimens with a provisional diagnosis of tuberculosis, tested positive for Mycobacterium by both Zn stain smear and culture on LJ medium. Among these, 3.57% were positive for AFB on ZN smear and 11.65 % were positive on culture on LJ medium. These conventional techniques have proved to be reliable testing tools for detection of Mycobacterium tuberculosis in our setting. (Rawal Med J 2008;33:134-136).

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

## **INTRODUCTION**

Diagnosis of Tuberculosis (TB) depends on the history, physical and radiographic evidence or the presence of AFB in acid fast smears and cultures.<sup>1</sup> Detection of AFB in direct smears prepared with concentrated sputa, urine and specimens of other body fluids has considerable clinical and epidemiological value and remains the most widely used rapid diagnostic test for TB in most developing countries.<sup>2</sup> While new radiometric and molecular diagnostic techniques have been developed and are widely used in the developed world, it is estimated that between 60% and 70% of all TB cases are diagnosed by means of sputum smear examination.<sup>3</sup>

Mycobacterium tuberculosis has been found in various body fluids.<sup>4</sup> Culture remains the gold standard for the definitive diagnosis of tuberculosis as it allows testing for drug susceptibility.<sup>5</sup> Conventional method of ZN staining of smears for detection of acid fast bacilli (AFB) has been frequently used for the diagnosis of tubercular bacterial infection in developing countries.<sup>6</sup> The aim of this study was to compare the smear stained by Ziehl-Neelsen (ZN) method and Lowenstein–Jensen (LJ) medium for the detection of Mycobacterium tuberculosis in clinical samples from different sites.

## **MATERIALS AND METHODS**

This laboratory based retrospective study was conducted at Sir Gang Ram Hospital/Fatima Jinnah Medical College, Lahore from January 2001 to June 2006. A total of 798 clinical samples were collected from patients of both genders and all ages with a provisional diagnosis of tuberculosis. A ZN stain smear and LJ media based cultures was performed for the detection of Mycobacterium. The specimen categories were sputum, pus, lymph node aspirate, urine and endometrial curetting. All the sputum samples were decontaminated and concentrated using N Acetyl-L-Cysteine-Sodium Hydroxide procedure recommended by the Center of Disease Control and Prevention.<sup>7</sup> The sputum, urine and other specimens were prepared using the conventional centrifugation method and the smears were stained with carbol fuchsin (ZN). The sputum, urine and other specimen were inoculated on to LJ media and incubated at 37°C in 5% CO<sub>2</sub> for up to eight weeks.

## **RESULTS**

Out of 05 types of 798 specimens, only 46.3% (n=369) were respiratory whereas 53.7% (n=429) were non respiratory tract category samples. Sputum was the commonest sample received (table 1). Among these, 25(3.1%) were positive for ZN stain while 96(12.3%) were positive on culture (table 2). ZN stain was positive in 8.6% males while only 1.96% females gave a positive ZN smear (table 3). In contrast, 64 females and 29 males gave a positive culture results (table 4).

**Table 1. Distribution of 798 samples.**

	Males	Females	Total
Sputum	91	278	369
Pus	4	118	167
Lymph node	26	58	84
Endometrial Curetting	0	146	146
Urine	20	12	32
Total	186	612	798

Out of a total of 369 respiratory tract categories 38(10.3%) sputum samples were positive for AFB on both ZN and culture. Among the non respiratory tract category, 47(28.2 %) pus, 26 (31%) lymph node aspirates, 5(15.6%) urine, 05(3.42%) endometrial curetting were positive. The rate of detection was highest in lymph node aspirates, followed by pus and sputum (table 2).

**Table 2. Comparison of ZN positive and Culture positive samples.**

	ZN	Culture	Total	Percentage
Sputum	9	29	38	10.30
Pus	9	38	47	28.14
Lymph node	3	23	26	30.95
Endometrial Curetting	1	4	5	3.42
Urine	3	2	5	15.63
Total	25	96	121	15.16

## **DISCUSSION**

We found 121(15.16 %) specimens were positive. Among these, 25 (3.1%) were AFB smear positive with ZN stain and 96(12.03%) were AFB positive on culture with L-J medium. In lymph node biopsies from clinically suspected lymphadenitis rate of positive culture and AFB smear was reported to be 13.6% and 28.4% respectively.<sup>8</sup> In another study, bacteriological and/or histological confirmation of tuberculosis was obtained in 88% of the cases.<sup>9</sup> A major number of the sputum specimens were of females who remain at increased risk of developing active tuberculosis being a disadvantaged and marginalized population due to social problems in Pakistan.

**Table 3. Distribution of ZN Positive among 798 samples.**

Group	Zn positive	Zn negative	total	Percentage
Male	16	170	186	8.60%
Female	12	600	612	1.96%
Total	28	770	798	3.51%

Our data indicate that in the diagnosis of TB, culture has greater sensitivity than ZN. The ZN method has commonly been used around the world, particularly in developing countries, because of its simplicity and low cost.<sup>3</sup> A disadvantage of the technique is that it may sometimes yield false positive results. However, most of these can be prevented by re-staining the smear.<sup>10</sup> Our study showed that the rate of detection of Mycobacterium was highest in lymph node and then pus and sputum. In contrast, a study found that the sputum is the major infectious sample, followed by biological fluids.<sup>10</sup>

**Table 4. Distribution of Culture Positive Cases among 798 samples.**

Group	Positive	Negative	Total	Positive %age
Male	29	157	186	15.59%
Female	64	548	612	10.46%
Total	93	705	798	11.65%

ZN smear examination has been reported to have sensitivity of 33.79% and a specificity of 100%, while for LJ media, sensitivity was 48.9% and specificity was 100%.<sup>11</sup> ZN stain has been used as the primary procedure for detection of Mycobacterium.<sup>12</sup> However, a study found that besides bacteriology, histopathology is a complimentary diagnostic tool for detection of TB granuloma in tissues.<sup>12</sup> Although AFB microscopy, and LJ culture remain the cornerstone of the diagnosis of TB, these traditional methods are either slow or their sensitivity is quite low, especially with clinical samples that contain small number of organisms. Thus, there is an urgent need to promote the use of Bactec for early detection and drug susceptibility and real time PCR for even more rapid diagnosis of TB.<sup>8,11</sup> In conclusion, we found over 15% of clinical samples positive for Mycobacterium by both Zn stain smear and culture on LJ medium.

## REFERENCES

1. Powell DA, Hunt WG. Tuberculosis in children: an update. *Adv Pediatr.* 2006;53: 279-322.
2. Truffot-Pernot C, Véziris N, Sougakoff W. Modern diagnosis of tuberculosis. *Presse Med.* 2006;35 (11 Pt 2):1739-46.

3. Uilukanligil M, Aslan G, Tasci S. A comparative study on the different staining methods and number of specimens for the detection of acid fast bacilli. *Mem. Inst. Oswaldo Cruz* [online]. 2000, vol. 95, no. 6 [cited 2008-02-20], pp. 855-85.
4. Erbaycu AE, Taymaz Z, Tuksavul F, Afrashi A, Güçlü SZ. What happens when oral tuberculosis is not treated? *Monaldi Arch Chest Dis.* 2007;67:116-8.
5. Bello AK, Njoku CH. Tuberculosis: current trends in diagnosis and treatment. *Niger J Clin Pract.* 2005;8:118-24.
6. Goel MM, Ranjan V, Dhole TN, Srivastava AN, Mehrotra A, Kushwaha MR, et al. Polymerase chain reaction vs. conventional diagnosis in fine needle aspirates of tuberculous lymph nodes. *Acta Cytol.* 2001;45:333-40.
7. Kent PT, Kubica GP. Public health Mycobacteriology: Guide for the level 111 laboratory. US Department of Health and Human Services, Centers for Disease Control, USA, 1985.
8. Singh HB, Singh P, Jadaun GP, Srivastava K, Sharma VD, Chauhan DS, et al. Simultaneous use of two PCR systems targeting IS6110 and MPB64 for confirmation of diagnosis of tuberculous lymphadenitis. *J Commun Dis.* 2006;38 : 274-9.
9. Umopathy KC, Begum R, Ravichandran G, Rahman F, Paramasivan CN, Ramanathan VD. Comprehensive findings on clinical, bacteriological, histopathological and therapeutic aspects of cutaneous tuberculosis. *Trop Med Int Health.* 2006;11:1521-8.
10. Hamze M, Majzoub MN. Search for acid-alcohol resistant bacilli in 1222 pathological specimens. *J Med Liban.* 1997;45:21-4.
11. Negi SS, Khan SF, Gupta S, Pasha ST, Khare S, Lal S. Comparison of the conventional diagnostic modalities, bactec culture and polymerase chain reaction test for diagnosis of tuberculosis. *Indian J Med Microbiol* 2005;23:29-33.
12. Perenboom RM, Richter C, Swai AB, Kitinya J, Mtoni I, Chande H, et al. Diagnosis of Tuberculous lymphadenitis in an area of HIV infection and limited diagnostic facilities. *Trop Geogr Med.* 1994;46:288-92.