

ENHANCED DIAGNOSTIC YIELD OF AURAMINE-O STAINING AND MYCOBACTERIA GROWTH INDICATOR TUBE OVER ZIEHL-NEELSEN STAINING AND LOWENSTEIN JENSEN MEDIA IN EXTRA-PULMONARY TUBERCULOSISKawal Preet Kaur¹, Bharti Arora²**HOW TO CITE THIS ARTICLE:**

Kawal Preet Kaur, Bharti Arora. "Enhanced Diagnostic Yield of Auramine-O Staining and Mycobacteria Growth Indicator Tube Over Ziehl-Neelsen Staining and Lowenstein Jensen Media in Extra-Pulmonary Tuberculosis". *Journal of Evolution of Medical and Dental Sciences* 2014; Vol. 3, Issue 28, July 14; Page: 7787-7791, DOI: 10.14260/jemds/2014/2972

ABSTRACT: BACKGROUND & OBJECTIVES: Tuberculosis has affected mankind for over 5000 years, and it still continues to be a leading cause of morbidity and mortality. As for any disease of contagious nature, early diagnosis and treatment remains the mainstay of therapy. This holds true for extrapulmonary tuberculosis as well. Therefore, this study was undertaken to compare the Auramine-O staining with Ziehl-Neelsen (ZN) staining and Lowenstein Jensen (L-J) media with Mycobacteria Growth Indicator Tube (MGIT) for diagnosis of extrapulmonary tuberculosis. **METHODS:** A total of 83 extra-pulmonary samples were processed. All the samples were stained by both ZN and Auramine-O method. The samples were inoculated on L-J media and in MGIT after performing the NALC-NaOH (N-Acetyl-L-cysteine NaOH) procedure according to standard protocol. All the isolates obtained were identified biochemically using standard procedures. **RESULTS:** ZN staining failed to detect acid-fast bacilli in any of the extra-pulmonary samples, while Auramine -O staining detected 5 of the positive cases. Growth on L-J media was obtained in 4 samples while MGIT was positive in 8 samples. **INTERPRETATION & CONCLUSIONS:** We found out that Auramine-O staining was better than ZN staining and MGIT was better than L-J media for the extra-pulmonary tuberculosis.

KEYWORDS: Tuberculosis, HIV, MDR, MGIT, LJ media, NALC-NaOH.

INTRODUCTION: Tuberculosis is the leading cause of death from a curable infectious disease. TB has affected mankind for over 5000 years, and it still continues to be a leading cause of morbidity and mortality. The bacilli was discovered more than a century back by Sir Robert Koch in 1882 and effective drugs for treatment were available for more than half a century, globally more than 1.3 million people die of the disease every year. Nearly one third of the world's population is infected with TB bacilli, approximately 10% of them have a life time risk of developing TB disease.^[1]

Recently acquired infections with Mycobacterium tuberculosis do not invariably follow the classic slowly progressive course of secondary disease. Primary progressive tuberculosis or the rapidly spreading miliary type of tuberculosis is the rule rather than the exception in patients with profound immunosuppression such as patients with AIDS.^[2]

As a result of haematogenous dissemination in HIV- infected individuals, extrapulmonary tuberculosis is seen more commonly today than the past. In order of frequency, the extrapulmonary sites most commonly involved are the lymph nodes, pleura, genito-urinary tract, bones, joints, meninges, peritoneum and pericardium. However, virtually any organ system may be affected.^[3]

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As for any disease of contagious nature, early diagnosis and treatment remains the mainstay of therapy. This holds true for extrapulmonary tuberculosis as well, in which case special consideration must be taken to advocate the use of highly sensitive, specific, cost effective and comparatively rapid method for isolation of *Mycobacterium tuberculosis* for the diagnosis. This kind of scientific approach will minimize the spread of infection by a faster achievement of diagnosis and hence earlier initiation of anti-tubercular therapy.

Therefore, this study was undertaken to compare the Auramine-O staining with Ziehl-Neelsen staining and Lowenstein Jensen media with *Mycobacteria Growth Indicator Tube* for diagnosis of extrapulmonary tuberculosis.

MATERIAL AND METHODS: This prospective study was conducted over a period of one year (1st August 2009 to 31st July 2010) in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. A total of 83 extra-pulmonary samples were received.

All the samples were subjected to staining by Ziehl-Neelsen and Auramine-O method. The samples were inoculated on Lowenstein-Jensen media and in *Mycobacteria Growth Indicator Tube* (MGIT) after performing the NALC-NaOH procedure according to standard protocol. All the isolates obtained were identified biochemically using standard procedures.^[2]

RESULTS: Results obtained are depicted in the tables and graphs that follow:

Samples (n=83)	Positive Samples (n=8)	Negative Samples (n=75)
Pleural Fluid (n=26)	2 (7.69)	24 (92.31)
Pus / Wound Swab (n=19)	4 (21.05)	15 (78.95)
Endometrial Biopsy (n=18)	0	18 (100.00)
Soft Tissue (n=6)	1 (16.67)	5 (83.33)
Drain Fluid (n=3)	0	3 (100.00)
Lymph Node Aspirates (n=3)	1 (33.33)	2 (66.67)
Ascitic Fluid (n=2)	0	2 (100.00)
Cerebrospinal Fluid (n=2)	0	2 (100.00)
Pericardial Fluid (n=2)	0	2 (100.00)
Semen (n=1)	0	1 (100.00)
Bone Marrow Aspirates (n=1)	0	1 (100.00)

Table 1: Distribution of Various Samples

Figures in parentheses indicate percentages

Samples (n=8)	Smear Positive		Culture Positive	
	ZN Staining (n=0)	Auramine Staining (n=5)	L-J media (n=4)	MGIT (n=8)
Pleural Fluid (n=2)	0	1	1	2
Pus/ Wound Swab (n=4)	0	2	1	4
Soft Tissue (n=1)	0	2	2	1
Lymph Node Aspirates (n=1)	0	0	0	1

Table 2: Distribution of Positive Samples

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ZN staining failed to detect acid-fast bacilli in any of the extra-pulmonary samples, while Auramine –O staining detected 5 of the positive cases.

Growth on L-J media was obtained in 4 samples while MGIT was positive in 8 samples. We also biochemically identified all the 8 isolates. Out of the 8 isolates obtained 7 isolates were slow growers taking an average time of 32.88 ± 12.07 days for growth. All the isolates were non-chromogens. One isolate was a rapid grower, in which growth was obtained on 4th day. On the basis of the biochemical identification, all the slow growers were identified as *Mycobacterium tuberculosis* while the rapid grower was identified as *M. chelonae*.

DISCUSSION: Tuberculosis is one of the oldest diseases known to mankind. It still afflicts a large number of the human population. There has always been a constant need for newer diagnostic methods to enable the earlier diagnosis and eradication of this disease. Direct microscopic examination is still the only available diagnostic tool in many resource-poor settings.

Presently, two types of stains are used in clinical mycobacteriology laboratories. One type is carbolfuchsin (Ziehl-Neelsen [ZN] or Kinyoun methods), and the other is fluorochrome (either auramine or auramine-rhodamine).^[4] We also routinely performed both these staining procedures on all the samples.

However, the standard method of sputum examination, that is, ZN staining is not sensitive enough and a large number of these suspected cases miss diagnosis.^[5] It is of particular importance in the diagnosis of extrapulmonary disease, in which the acid fast bacilli are present in scanty number or may not be detected at all. Hence, ZN staining alone cannot be relied upon for the diagnosis of tuberculosis.

Similar results were obtained in our study. ZN staining failed to detect acid-fast bacilli in any of the extra-pulmonary samples, while Auramine –O staining detected 5 of the positive cases. Since fluorescence staining method is more sensitive and rapid, using this method in clinical laboratories with large specimen numbers is recommended.^[6] This staining method was of a massive advantage at our centre, where the specimen number is large.

In response to altered clinical and epidemiologic situations, several changes in laboratory practice have evolved over the past several years. There is need to identify MTB complex (slow strains) from NTM (fast growers) to enable prompt eradication of the infecting strain from the host.^[7] New techniques and revised algorithms for the recovery, identification and susceptibility testing of mycobacteria are being implemented in many clinical laboratories in view of changes in the clinical manifestations and epidemiology of tuberculosis. Therefore, the use of broth culture medium for the early recovery of mycobacteria is now highly recommended.^[2]

We compared one of the broth based methods i.e. MGIT with the conventional L-J media. Growth on L-J media was obtained in 4 samples while MGIT was positive in 8 samples. Thus MGIT had a much higher isolation rate. Many other studies have demonstrated a higher isolation rate of MGIT as compared to L-J media.^[8,9,10]

Studies have found out a much lower incidence of non-tuberculous mycobacteria (NTM) in India. A study from CMC Vellore found out *M. tuberculosis* to be the predominant isolate (96.1%) as compared to only 3.9% NTM.^[11] These findings are consistent with our study. We also found out *M. tuberculosis* to be the predominant isolate (87.5%). Even in HIV positive patients, only 1 out of 23 isolates was found to be atypical while the other 22 were all *Mycobacterium tuberculosis* isolates.^[12]

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We detected cases of extra-pulmonary tuberculosis which would have been otherwise missed with the conventional methods of ZN staining and culture on L-J media.

Hence, the use of newer and rapid methods of culture is highly recommended in view of the rising number of tuberculosis, especially extra-pulmonary tuberculosis which poses additional diagnostic difficulties.

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Date of Submission: 26/06/2014.
Date of Peer Review: 27/06/2014.
Date of Acceptance: 05/07/2014.
Date of Publishing: 11/07/2014.