DOI: 10.14260/jemds/2015/960

ORIGINAL ARTICLE

COMPARISION BETWEEN DIFFERENT CONVENTIONAL METHODS FOR THE DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS

Suryamani Chintapalli[1], P. Balamurali Krishna[2], P. Sivajyothi[3], B. Manjula[4]

HOW TO CITE THIS ARTICLE:
Suryamani Chintapalli, P. Balamurali Krishna, P. Sivajyothi, B. Manjula. "Comparision between different Conventional Methods for the Diagnosis of Mycobacterium Tuberculosis". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 38, May 11; Page: 6631-6635, DOI: 10.14260/jemds/2015/960

ABSTRACT: BACKGROUND: The identification of infectious case is a crucial first step for Tuberculosis Control Programmes worldwide. Now a days Molecular Methods are available for rapid diagnosis but they are too costly and most of the people may not be able to effort for this. Therefore there is an urgent need to improve quality of the smear microscopy. **OBJECTIVES:** The USP method was compared with the two commonly used conventional methods of smear microscopy namely direct smear microscopy and the microscopy by modified Petroff's method. **MATERIALS & METHODS**: Two samples from each patient were taken from 210 patients of presumptive tuberculosis. One smear was made for direct Ziehl-Neelsen staining and two smears were made after processing by two concentration methods i. e modified Petroff"s and USP solution. LJ media were inoculated for culture after processing by both concentration methods. **RESULTS**: Among 195 cases 90 were culture positive by either method. Out of 90 culture positive samples 80.21% were direct smear positive, 91.2% were 4% NaOH smear positive and 95.83% were USP smear positive. Diagnostic accuracy for direct smear was 87.21%, for modified Petroff"s was 92.21%, and for USP it was 96.21%. **CONCLUSION**: The present study evaluated the smear microscopy by USP method with the two conventional methods, direct microscopy and microscopy by modified Petroff"s method. The study concludes that USP method is more sensitive than other two conventional methods for diagnosis of Tuberculosis.

KEYWORDS: Modified Petroff"s method, tuberculosis, USP, Ziehl-Neelsen staining.

INTRODUCTION: Tuberculosis is an important global public health problem. According to the World Health Organization (WHO), approximately nine million new cases and two million deaths are reported worldwide annually.[1,2] Early & rapid diagnosis is crucial for successful disease management as well as effective control of the disease. In most of the centres diagnosis depends exclusively on the detection of acid fast bacilli in the sputum by smear microscopy.[3]

Most laboratories use smears of unconcentrated sputum (direct smears) with Ziehl Neelsen staining which is a less sensitive method as 5-10,000 bacilli per ml are required to get reproducible results.[4] It is estimated that less than 20% of approximately 8 million predicted annual cases of TB worldwide are identified as smear positive. The targets of 90% case detection rate and treatment success are not likely to be achieved with the existing methods of smear microscopy.[5]

Therefore there is an urgent and definite need to improve the sensitivity of smear microscopy. There is a novel specimen processing technology called universal sample processing (USP) (5) for TB diagnosis in both pulmonary and extrapulmonary specimens. This enables highly sensitive smear microscopy (with a sensitivity of detection in the order of 300-400 AFB/ml of specimen) and culturing of the tubercle bacilli. The present study was undertaken to evaluate the performance of the USP method in a clinical setting with different specimens and compared with the two most commonly used conventional methods of smear microscopy namely direct smear

microscopy and the smear microscopy by concentration method (modified Petroff's method) for detection of TB bacilli.

MATERIALS & METHODS: In this prospective cross-sectional study total of 210 sputum samples of presumptive new cases of T.B were included. The present study was conducted at Culture & Drug Susceptibility Testing Laboratory (Intermediate Reference Laboratory for Tuberculosis) under RNTCP Programme located at Government Hospital for Chest & Communicable diseases, Andhra Medical College, Visakhapatnam during a period of 6 months from June 2012 to November 2012.

According to RNTCP India, two samples, one early morning and other spot sample from each patient, were collected in a universal container. All the received sputum specimens were divided into 3 parts. One part was taken for direct smear preparation on a new slide and Ziehl Neelsen staining[5] was performed. Second & Third portions were processed for decontamination using the modified Petroff's method and USP method. Modified Petroff's method consists of 4% NaOH as a decontaminating reagent. USP solution consists of 4 M guanidinium hydrochloride (GuHCL) (Amresco), 50mM Tris-CI, (Sigma), 25 mM EDTA (SRL), 0.5% Sarkosyl (Sigma) and 0.2 M mercaptoethanol.[6]

The USP smear microscopy was performed as per the method described by Chakravorty et al. In brief, the sputum samples were mixed with 2-3 volumes of the USP solution, which contained the following chemicals: 4-6 M guanidinum hydrochloride (a chaotropic agent which disrupts the hydrogen bonds), 50 mM Tris chloride (pH 7.5), 25 Mm Ethylene Diamine Tetra Acetic acid (EDTA), 0.5% sarkosyl, and 0.1-0.2 M Beta mercaptoethanol These ingredients together brought about mucolysis and acted as detergents. Thus by their action the chemicals used lysed all the cells in the sputum samples except the tubercle bacilli. Then the samples were homogenized by vortexing or shaking by hand, incubating for 15 minutes at room temperature. To the homogenate, thus obtained 5-15 ml of sterile water was added and centrifuged at 9000x g for 20 min. The sediment formed was mixed well and a part (10%) of the sediment was used for making the smear and a part of it was used for culture. The smear was dried, subjected to ZN staining and observed under the microscope.

Smears were made from sediments and Ziehl-Neelsen staining was performed. Then Lowenstein-Jensen media (LJ media) were inoculated with 5 mm loop full of the centrifuged sediments processed by the two concentration methods separately. Bottle caps were tightened to minimise evaporation and drying of media.All cultures were incubated at 37°C for 12 weeks.

Out of 210 samples 15 were contaminated by both methods probably due to improper exposure time with decontaminating agent and not using the cold centrifuge for centrifugation. Hence these cases were excluded from the study. The USP method was evaluated for its sensitivity/efficiency using these 195 sputum specimens by the use of smear microscopy and culture.

STATISTICAL ANALYSIS: Statistical analysis was done using culture as gold standard test for diagnosis of T.B. A positive LJ slant by any one method of processing (Modified Petroff or USP method) for the same specimen was considered as true positive. LJ slant that were negative by both methods for the same specimen were considered true negative samples. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy on the basis of culture.

DOI: 10.14260/jemds/2015/960

RESULTS:

	Positive (n=195)	Negative (n=195)	%	%	%	%	Diagnostic Accuracy (%)
	True False	True False	Sensitivity	Specificity	PPV	NPV	
Direct	73 2	102 18	80.21	98.07	97.33	85.00	87.21
USP	92 4	95 4	95.83	95.95	95.83	95.95	96.21
M. Petroff	83 3	101 8	91.20	97.11	96.51	92.66	92.21

Table 1: ANALYSIS OF SMEAR MICROSCOPY FOR DETECTION OF TUBERCULOSIS BY DIFFERENT METHODS

Detection of T. B. by Modified Petroff Method: Of the 120 negative samples by the direct smear method (smear negative samples), the 4% Na0H method detected 11 additional positive samples. In our study 4% NaOH method had 91.20% sensitivity as compared to the direct method which had 80.21% sensitivity: the 11% enhancement in sensitivity was highly significant P<0.001) Diagnostic accuracy of four percent NaOH method was also more (92.21)% than direct method 87% (Table-1). There was also the concentrating effect in smear status. The smear grade status was enhanced by the 4% NaOH smear method: slides graded as scanty, 1+, or 2+ by the direct smear microscopy generally graded as 1+, 2+, or 3+ by 4% Na OH smear microscopy.

Detection of T.B by USP Method: Of the 120 negative samples by the direct smear method (smear negative samples) the USP method detected 21 additional positive samples out of them 2 were 2+, 5 were 1+ and rest 14 smears had scanty bacilli. In our study USP method had 95.83% sensitivity as compared to direct method which had 80.21%) sensitivity. The 16% enhancement in sensitivity was highly significant (p<0.001) (Table-1).

The USP Smear Method was Simultaneously Compared with the Modified Petroff: Smear microscopy. 10 specimens which were smear negative by the 4% NaOH method were detected positive by the USP method, all of these specimens which were missed by the modified Petroff method of smear microscopy had small number of bacilli, as they predominantly belonged to the scanty or 1+ category. Thus the USP method showed an enhancement in sensitivity over that of the modified Petroff method.

In our study diagnostic accuracy of USP method was best (96%) as compared to other methods. There was also concentrating effect in smear status. The smear grade status was enhanced by the USP smear method, slides graded as scanty, 1+, or 2+ by the direct smear microscopy were generally graded as 1+, 2+, or 3+ by the USP smear microscopy. Among 195 specimens, 75 specimens were positive by both the direct and USP methods of smear microscopy. Of these only 33 belonged to the 3+ category by the direct smear method, in contrast to 44 by the USP method. Thus 10% of the specimens that were positive by the direct smear method were upgraded to the 3+ category by the USP method. The same trend was observed between the USP method and modified Petroff smear microscopy. Among 86 specimens positive by both the modified Petroff and USP methods of smear microscopy, 8 were upgraded to the next category by the USP method.

DOI: 10.14260/jemds/2015/960

Evaluation between Cultures by USP and Modified Petroff Methods: Total number of specimens, culture positive by either method were 96. Out of which 86 were positive by USP method or modified Petroff method. Out of 86 samples 77 samples were positive by both culture methods. Out of 96 culture positive samples 80.2% were direct smear positive, 91.2% were 4% NaOH smear positive and 95.8% were USP smear positive samples

DISCUSSION: Chakravorty and Tyagi[6] described a new methodology of microscopy by USP method that can be reliably applied to all types of clinical specimens for diagnosing tuberculosis in laboratories with diverse infrastructure capabilities. In other study USP method exhibited a highly significant enhancement in sensitivity compared to direct method and NALC-NaOH method of microscopy.[7] In our study USP method was definitely exhibited a highly significant enhancement in sensitivity compared to direct method. It detected 21 additional positive samples proving that the direct smear method sometimes fails to detect specimens with high bacterial loads due to technical errors during smear preparation or faulty reading of slides. The USP method also has significant enhancement in sensitivity as compared to modified Petroff method. Diagnostic accuracy of USP method is more as compared to modified Petroff method.[8]

In culture methods also USP method showed more positives when compared to modified Petroff method.[9] In our study contamination rate by modified Petroff method was 2.03% which is acceptable, contamination rate by USP method was only 1.01%, so it has more sensitivity than other methods.

CONCLUSIONS: The present study evaluated the smear microscopy by USP method with the two conventional methods, direct microscopy and microscopy by modified Petroff method. The study concludes that USP method is more sensitive than conventional methods and it may be routinely used in laboratories to diagnose Tuberculosis.

ACKNOWLEDGEMENTS: This study would not have been possible without the expert guidance of Dr. P. Balamuralikrishna. We convey our sincere thanks to the Superintendant of Government Hospital for Chest and Communicable diseases, Dr. Sambasivarao for providing the study samples. We also like to thank Dr. Vasundharadevi, District Tuberculosis Control Officer. We would also thank our colleagues, paramedical staff and technicians of Culture and Drug Susceptibility Testing Laboratory for Tuberculosis.

REFERENCES:

1. Deivanayagam CN, Rajasekharan S, Venkatesan R, et al prevalence of acquired MDR-TB & HIV co-infection. Indian J Chest Dis Allied Sci 2002: 44: 237-42.
2. T.B India 2013, Revised National Tuberculosis Control Programme annual status report, publisher Central T.B division, New Delhi, 2013.
3. Katoch VM, smear microscopy to diagnose tuberculosis, Indian J Med Res 2006: 123: 735-8.
4. TB Division Directorate General of Health Services Ministry of Health and Family Welfare, Revised National Tuberculosis Control Programme (RNTCP) manual for laboratory technician. New Delhi: Central T.B division Directorate General of Health Services, Ministry of Health and Family Welfare, 2005.
5. TB India 2012, Annual status report Government of India. P. 12-3 Available From: tbcindia.com.

6. Chakravorty S, Tyagi JS. Novel multipurpose methodology for detection of Mycobacteria in pulmonary and extrapulmonary specimens by smear Microscopy, culture & PCR. J Clin Microbiol 2005: 43: 2697-702.
7. Chakravorty S, Dudeja M, Hanif M, Tyagi JS. Utility of universal sample processing methodology, combining smear microscopy, culture and PCR for diagnosis of pulmonary tuberculosis. J Clin Microbiol 2005: 43: 2703-8.
8. Cattamanchi A, Davis JL, Worodria W, Yoo S, Matovu J, Kiidha J, et al. Poor performance of universal sample processing method for diagnosis of Pulmonary tuberculosis by smear microscopy and culture in Uganda. J Clin Microbiol 2008; 46: 3325-9.
9. WHO, Tuberculosis laboratory biosafety manual, GPS publishing Italy 2012.

AUTHORS:

1. Suryamani Chintapalli
2. P. Balamurali Krishna
3. P. Sivajyothi
4. B. Manjula

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Microbiology, Andhra Medical College, Visakhapatnam.
2. Professor and HOD, Department of Microbiology, Andhra Medical College, Visakhapatnam.
3. Junior Resident, Department of Microbiology, King George Hospital. Visakhapatnam.
4. Assistant Professor, Department of Microbiology, Andhra Medical College, Visakhapatnam.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Suryamani Chintapalli,
Assistant Professor,
Department of Microbiology,
Andhra Medical College,
Visakhapatnam.
E-mail: suryamanichintapalli27@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None

Date of Submission: 13/04/2015.
Date of Peer Review: 14/04/2015.
Date of Acceptance: 30/04/2015.
Date of Publishing: 08/05/2015.