International Journal of Research in Medical Sciences
Singh P et al. Int J Res Med Sci. 2015 Sep;3(9):2204-2209
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20150602

Research Article

Comparative study of the smear microscopy, with conventional culture in clinically suspected cases of pulmonary and extra pulmonary tuberculosis

Paramjit Singh, Sapna Chauhan*, Mapary Lakshmikantha, Swati Tiwari, Sachin Sharma

Department of Microbiology, Muzaffarnagar Medical College and Hospital, Muzaffarnagar, UP, India

Received: 09 June 2015
Accepted: 08 July 2015

***Correspondence:**
Dr. Sapna Chauhan,
E-mail: drsapna_chauhan@yahoo.com

ABSTRACT

Background: Tuberculosis is a significant cause of morbidity and mortality in the Indian subcontinent. A major challenge to clinical microbiology is the detection of *Mycobacterium tuberculosis* as accurately as possible.
Objective: Tthe most important tool in the diagnosis of tuberculosis is direct microscopic examination of appropriately stained specimens for acid- fast bacilli and the gold standard for diagnosing tuberculosis is MTB convention culture on L-J media So, the present study was undertaken to compare smear microscopy by Z – N staining with conventional culture on L-J media, in cases of clinically suspected Pulmonary Tuberculosis and Extra Pulmonary Tuberculosis.
Methods: 279 samples were processed within 24 hours of receipt. Samples from non-sterile sites were subjected to decontamination by the modified Petroff's method. Sterile samples were directly processed as per conventional methods. Smear microscopy was done by Z- N staining and culture was done on L- J media. A control organism in the form of *M. tuberculosis H37Rv* was also tested with each batch of clinical isolates.
Result: Results of smear microscopy and conventional culture of pulmonary and extra pulmonary specimens were compared. 22 and 14 more cases were detected by culture as compared to smear in case of pulmonary and extra pulmonary specimens respectively.
Conclusion: From this study we can state that direct microscopic examination of appropriately stained Pulmonary and Extra Pulmonary specimens for acid fast Bacilli is an important tool in the diagnosis of tuberculosis. The Technique is simple, inexpensive and fast .However many Paucibacillary cases may be missed on smear microscopy. Thus specimens from all suspected cases of Pulmonary and Extrapulmonary Tuberculosis should be subjected to conventional culture on LJ media. This is the Gold Standard for Diagnosing Tuberculosis.

Keywords: Tuberculosis, Conventional culture, ZN staining, Pulmonary, Extra pulmonary

INTRODUCTION

From *Phthisis* to *Pott's* poetic consumption, tuberculosis has reached statistically alarming proportions, especially in developing countries. Tuberculosis is a disease of antiquity. Skeletal remains of prehistoric humans dating back to 8000 BC, found in Germany, have shown evidence of the disease. Ancient Hindu and Chinese scripts also have documented the existence of this disease.[1] In its present shape, tuberculosis is a global emergency with almost one third of the world's population being affected resulting in nearly 1.5 million deaths per annum.[2] India has 2% of the land area of the world and 15% of its total population, but a disproportionately high TB burden (30%). One person dies from TB in India every minute. Tuberculosis remains a serious public health problem with an annual incidence of 2 million out of which nearly 1 million are

infectious smear positive pulmonary cases.[3] One smear positive patient infects up to 20 contacts annually in absence of chemotherapy.[4]

In order to have effective control, a prompt diagnosis and effective treatment would help break the chain of transmission. However, the disease with its protean manifestations and chronic progression poses problems in specific diagnosis. Diagnosis becomes difficult especially in HIV infected individuals, where the clinical manifestation is atypical.

A major challenge to clinical microbiology is the detection of Mycobacterium tuberculosis (MTB) easily and accurately. Isolation of *Mycobacterium tuberculosis* remains the gold standard for the laboratory diagnosis of tuberculosis. The conventional solid media take a minimum of 6-8 weeks, thus leading to late diagnosis. Substantial improvements in the mean time to detection and the total number of isolates recovered can be realized by using a broth based system such as BACTEC (BBL) or the broth/agar system SEPTI-CHECK AFB (BBL). This expedites growth of *Mycobacterium tuberculosis* from the smear positive and most of smear negative specimens within 2-3 weeks. The advent of molecular tools for detecting specific nucleotides of *Mycobacterium tuberculosis* complex by direct amplification methods/ polymerase chain reaction, provide a precise rapid final diagnosis.[5] These techniques are not cost effective in a developing country like ours. Also these tests are yet to be standardized for all specimens and are not recommended for smear negative sputum samples.[5,6] They have low specificity and can lead to false positive diagnosis.[6] For over 100 years now, the only rapid test for presumptive diagnosis of tuberculosis was the smear examination of the patient's specimen for acid-fast bacilli. Smear form a non-processed sputum specimen can be positive if specimen contains more than 10,000 AFB/ml. The sensitivity of this method can be increased to detect even 1,000 AFB/ml, if the specimen is concentrated through appropriate processing and, the smear examined by means of fluorescent microscopy.[7] In spite of this, the sensitivity is not beneficial thus leading to missed diagnosis. Also, though smear negative cases are considered less infectious than smear positive cases, they have nevertheless been shown to transmit disease.[8] The traditional methods of demonstrating the bacilli on direct smear examination and the isolation of the organism in culture on the conventional LJ medium remain the mainstay in the diagnosis of tuberculosis, especially in developing countries. This is in part due to the fact that the spectacular recent advances in the diagnosis and drug susceptibility testing, including the radiometric, the polymerase chain reaction (PCR) based methods and various other serological, chromatographic and molecular techniques are all time, money and technology intensive and are beyond the reach of the average Mycobacteriology laboratory.

METHODS

Inclusion criteria

- Clinically suspected fresh cases of Pulmonary or Extra Pulmonary Tuberculosis.

Exclusion criteria

- Patient receiving anti Tuberculosis treatment or
- Patients who had received anti Tuberculosis treatment in the previous month, or
- Sputum samples suggestive of saliva only.

Study design: prospective

Setting: Military hospital Namkum, and Muzaffarnagar medical college and hospital.

Study period: July 2010 to October 2014.

Bacteria: The NCTC strain of *M. Tuberculosis H37Rv* was used as control organism.

Samples: A total of 279 samples from 279 different patients were processed for microscopy by Ziehl-Neelsen stain, and culture on Lowenstein-Jensen medium. Of these 183 were pulmonary samples and 96 were extra-pulmonary samples.

Distribution of samples processed was as follows

Distribution of samples processed was as follows: Sputum (n= 172), Bronchoalveolar lavage (n= 11) FNAC (n=36), pus(n=33), pleural fluid (n=21), pericardial fluid (n=03) tissue biopsy(n=03)

Collection and transport of samples

Accurate rapid microbiological diagnosis of tuberculosis and other mycobacterial infections begins with proper specimen collection and rapid transport to the laboratory.[9,10] The clearly labeled specimen were transported to the laboratory quickly.

Processing: All the samples were processed within 24 hours of receipt in the laboratory. The sample collection of sputum was preferable in the morning, shortly after the patient awakened, since Mycobacteria were in the highest concentration. The patients were instructed to expectorate deep respiratory specimen with no nasal secretion or saliva. Irregular & intermittent release of Mycobacteria into the bronchial lumen from the tubercular cavities results in variable pattern of recovery. Therefore, sputum collected on 3 consecutive days was processed. All specimens were transported promptly to the laboratory and refrigerated if processing was likely to be delayed.

Digestion, decontamination and concentration

Before culture, clinical specimens from non-sterile body sites must be subjected to pretreatment involving homogenization, decontamination, and concentration.[2,9,10] This procedure will eradicate more rapidly growing contaminants such as normal flora (other bacteria and fungi) but not seriously affecting the viability of mycobacteria. However, the efficacy of these procedures is highly influenced by the time of exposure to the reagent used for decontamination, the toxicity of that reagent, the efficiency of centrifugation, and the killing effect of heat buildup during centrifugation.[11] There is evidence that even the mildest decontamination methods used such as N-acetyl-L-cysteine-NaOH method can kill about 33% of the mycobacteria in a clinical specimen, whereas more overzealous methods can kill up to 70%.[10]

Concentrated samples were used for preparing 2 smears and inoculating 3 Lowenstein-Jensen [LJ] slants [2 containing glycerol and one containing sodium pyruvate]

The smears made were stained by Ziehl–Neelsen staining technique. They were screened for Acid Fast bacilli by two independent observers .All smears were graded according to CDC method[7] and the results were recorded. The LJ bottles were incubated at 37^0C for 8 weeks [in candle jar for the first week]

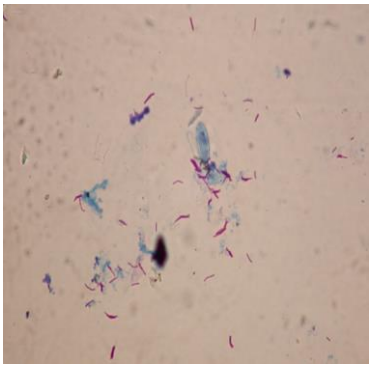

Figure 1: Acidfast bacilli (Z-N stain).

Figure 2: MTB culture on L-J media.

Reading of culture results

Lowenstein Jensen slopes were observed for any visible growth daily for the first week and then weekly up to 8 weeks. The colony morphology and the time period required for any visible growth was noted. Subsequently, the isolates were confirmed by Ziehl Neelsen staining and were reported as positive if showing the presence of acid fast bacilli.

Identification of *M. tuberculosis* was done as per following parameters:

- Growth rate slow, Growth temperature 35^0-37^0 C only, No pigmentation, Niacin positive, Catalase negative at 68^0C, No growth on LJ Medium containing p-nitro benzoic acid.[12,13]

RESULTS

Of the 183 pulmonary specimens, which include 171 sputa and 12 bronchoalveolar lavages, smear positivity was found in 81 specimens and culture positivity in 103 specimens. There were 10 specimens which were smear positive but culture negative.

Table 1: Comparison of smear and culture in pulmonary specimens (n=183).

Smear	Culture Positive	Culture Negative	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Smear Positive	81	10	91				
Smear Negative	22	70	92				
Total	103	80	183	78.6	87.5	89	76

PPV: Positive Predictive Value, NPV: Negative Predictive Value

Table 2: Comparison of smear and culture in extra-pulmonary specimens (n=96).

Smear	Culture Positive	Culture Negative	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Smear Positive	18	7	25				
Smear Negative	14	57	71				
Total	32	64	96	56.3	89	72	80.3

When culture is taken as the gold standard, the sensitivity, specificity, PPV, and NPV for smear is 78.6%, 87.5%, 89% and 76% respectively.

22 more cases were detected by culture as compared to smear.

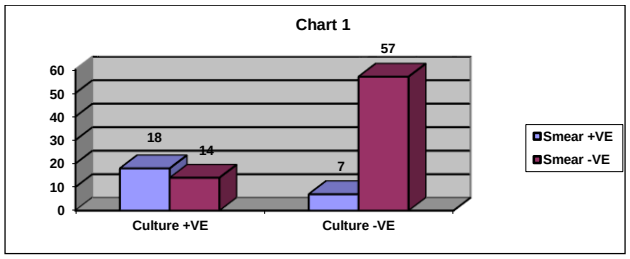

Figure 3: Comparison of smear and culture.

While analyzing 96 extra-pulmonary samples 18 cases were smear and culture positive while 57 specimens were both smear and culture negative. In 7 cases smear were positive but culture were negative, and in 14 smear negative cases conventional culture were positive. Considering the conventional culture on LJ media as gold standard, the sensitivity, specificity, PPV and NPV of smear examination were 56.3%, 89%, 72% and 80.3%, respectively.

DISCUSSION

Tuberculosis is a leading cause of death all over the world. Rapid and accurate diagnosis of symptomatic patients is the cornerstone of tuberculosis control strategies. Efforts are going on all over the world so that the speed and quality of Mycobacteriology diagnostic services can be upgraded. The role of the laboratory is important in the management of a case of tuberculosis and it has to respond to the new challenges related to rapid evolution in the epidemiologic situation and development of new technologies and therapies. Tuberculosis continues to be a global public health problem despite the various advances in diagnosis and therapy in the latter half of the twentieth century. This disease places a huge strain on the already overburdened health care system especially in the developing world. Tuberculosis is responsible for over one-fourth of all avoidable deaths due to infectious disease, especially in the productive age group. Mortality remains the highest in the developing world, which contributes three quarters of the global disease burden. Emergence of multi-drug resistance (MDR) strains and co-infection with HIV has further compounded the problem by accelerating the development of the active disease, delaying cure and causing outbreaks.[1] To add fuel to the fire, the time taken to obtain the laboratory confirmation further delays the implementation of infection control procedures and appropriate therapy.

For most TB patients worldwide, especially, the vast majority in disease endemic areas, microscopic examination of the stained sputum smear is the primary laboratory tool. The advantages of AFB microscopy are well known; it is inexpensive to perform, is very specific in high prevalence settings, and detects the most infectious subset of patients. However, short-comings of AFB microscopy seriously limit both the extent and quality of its applications, and ultimately, this has its impact on TB control. The yield of the result of AFB microscopy depends upon the studious attention of a trained and motivated microscopist. It is also notoriously insensitive. This problem is made critical by rising incidence of smear negative disease associated with HIV infection. Currently less than 20% of the roughly 8 million predicted annual cases of tuberculosis are identified as smear positive.

Smear negative patients are also responsible for transmission of disease, although to a lesser degree than sputum smear positive patients; they may later become smear positive if left untreated. In paucibacillary disease, it is often difficult to establish a rapid and definitive diagnosis. Culture of digested and decontaminated sputum is a more sensitive test than sputum microscopy, but culture results are not readily available for at least several weeks. This causes serious delay in both diagnosis and treatment, with the opportunity for further disease transmission.[4] Lawn et al summarized the potential costs of delays in the diagnosis of TB; "Delay causes prolongation of the period of infectivity in the community, and may result in the disease state being more advanced at presentation, with consequent increases in acute morbidity, late sequelae and overall mortality" .[4] Additional problems of diagnosis are that people who do not have TB are misdiagnosed as having this disease and are put on 'trial treatment'. In other instances patients may already be cured but treatment continues. There is currently no reliable way of determining whether they are cured. The laboratory thus has an important role to play in the management of tuberculosis patient.

The diagnostic modalities should have certain desirable features *viz* sensitivity, specificity, predictive value, speed, reproducibility, cost effectiveness, safety, simplicity, robustness and easy application. Ideally, the tests should be quantitatively, at least in some measures so that the infectiveness of the individual case can be measured. This is especially important for decisions to isolate hospitalized patients and to provide preventive therapy to contacts. Diagnostic modalities must also be tailored to needs of the population and epidemiology of TB in that region.

Rapid diagnostic tests for tuberculosis have been developed and are in routine use in the industrialized world. However, these tests with their high reagent costs and requirement for sophisticated equipment may not be appropriate for use in developing countries.

While the conventional methods for the cultivation of mycobacteria on solid media such as Lowenstein Jensen medium and Middlebrook agars are relatively straight forward, they do require several weeks of incubation for the detection of organisms, and may lack sensitivity when used alone. Substantial improvements in the mean time to detection and the total number of isolates recovered can be realized by using a broth based system such as BACTEC (BBL) or the broth/agar system SEPTI-CHECK AFB (BBL).

Culture was used as the "gold standard" for comparison. Discrepant results were resolved using clinical information, radiological findings, histopathological reports where available, initiation of antituberculosis treatment, and response to therapy. A total of 300 samples were included in this study. Of these 204 were pulmonary samples and 96 were extra pulmonary. Twenty one sputum samples had to be excluded from the study due to reasons mentioned before.

Pulmonary specimens

There were 10 other patients who were smear positive but culture negative. Of these, 8 showed scanty bacilli on microscopy and 2 were graded 1+. Repeat sputum samples in 8 of these 10 specimens recorded similar findings. Based on smear, clinical and radiological findings both these patients were started on ATT.

Muzaffar et al. have reported 7.8% of their smear positives that tested culture negative. These results were considered as true negatives.[13] Albert et al. have reported 35 of their 171 smear positive specimens as culture negative. Of these 4 patients (8 specimens) were started on TB treatment based on clinical evidence of disease.[14] In our study we had 10 such cases which were smear positive but tested culture negative (5.5%).

Extrapulmonary specimens

There were 25 smear positive specimens in our study. Out of these 18 were true positives and 7 were false positives when co-related with clinical evidence of disease. The overall smear positivity in our study was only 26% in spite of careful observation of over 300 fields by two independent observers. Shenai et al. reported lower sensitivity, specificity in their study.[15] The smear positivity for extrapulmonary specimens has varied between 20% to 80% in different studies.

Most of the discrepancies are due to poor sensitivity and specificity of smear microscopy. Of these 7 cases, 3 were smear positive and 4 were smear negative. Of the 3 positive cases, one was a sample of pericardial fluid from a case of TB pericarditis who responded to antituberculosis treatment. The other sample was that from a case of pleural effusion with clinical evidence of TB who also responded to antituberculosis treatment. These cases are thus true positives. The reason why these 3 samples were negative for culture is difficult to explain. It is possible that the use of a more enriched media like Middlebrook 7H9 or 7H11 could have facilitated growth of *M. tuberculosis.*[16]

CONCLUSION

Microscopic examination of respiratory specimens for acidfast bacilli (AFB) plays an important role in the initial diagnosis of tuberculosis. From this study we can state that the most important tool in the diagnosis of Tuberculosis is direct microscopic examination of appropriately stained Pulmonary and Extra Pulmonary specimens for acid fast Bacilli. The Technique is simple, inexpensive and fast .However many Paucibacillary cases may be missed on smear microscopy. Thus specimens from all suspected cases of Pulmonary and Extrapulmonary Tuberculosis should be subjected to conventional culture on LJ media. This is the Gold Standard for Diagnosing Tuberculosis.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Haas DW. Mycobacterium Tuberculosis. In: Mandell GL, Bennett JE and Dolin R, editors. Mandell Douglas and Bennett's Principles and practice of infectious Diseases. 5th edition. New York. Churchill Livingstone, 2000; 2576-2607.
2. The World Health Report 2014: Office of Publications, World Health Organization, Geneva. 2014; 13-14.
3. Khatri GR. DOTS Progress in India: 1995-2002. Tuberculosis 2003;83:30-4.
4. Walker D. Economic Analysis Of Tuberculosis Diagnostic Tests in Disease Control: How it can be modeled and what additional information is needed? Int J Tuberc Lung Dis 2001;5(12):1099-108.
5. Catanzaro A, Davidson BL, Fujiwara et al. Rapid Diagnostic Tests for Tuberculosis: what is the appropriate use? Am J Respir Crit Care Med 1997; 155:1804-1814.
6. Barnes PF. Rapid Diagnostics Tests for Tuberculosis: Progress but no Gold Standard. Am J Respir Crit Care Med 1997; 155:1497-1498.
7. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology 5th edn. Philadelphia: Lippincott – Raven Publishers; 1997: 893-952.
8. Behr MA, Warren SA, Salamon Het al. Transition of *Mycobacterium tuberculosis* from patients smear negative for acid-fast bacilli. Lancet. 1999;353:444-9.
9. American Thoracic Society and Centers for Disease Control and Prevention. Diagnostic Standards and

Classification of Tuberculosis in Adults & Children. Am J Resp Crit Care Med. 2000;161:1376-95.
10. Somoskovi A, Mester J, Hale YM, Parsons LM, Salfinger M. Laboratory Diagnosis of Nontuberculous Mycobacteria. Clin Chest Med 2003;23:585-97.
11. Kent PT, Kubica GP. Public Health Mycobacteriology: A guide for the level III laboratory. Atlanta: Department of Health and Human Services. 1985.
12. Perkins MD. New Diagnostic Tools for Tuberculosis. Int J Tuberc lung Dis. 2000;4(12):S182-S188.
13. Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of the FAST Plaque TB assay for direct detection of *Mycobacterium Tuberculosis* in sputum specimens. Int J Tuberc Lung Dis. 2002;6(7):635-40.
14. Albert H, Heydenrych A, Brookes R, et al. Performance of a Rapid Phage Based-Test, FASTPlaqueTB^{TM} , to diagnose Tuberculosis from sputum specimens in South Africa. Int J Tuberc Lung Dis. 2002;6:529-37.
15. Shenai S, Rodrigues C, Mehta AP. Evaluation of a New Phage Amplification Technology for Rapid Diagnosis of Tuberculosis. Indian J Med Microbiol. 2002;20(4):194-9.
16. Cohn ML, Waggoner RF, McClatchy JK. The 7H11 medium for the cultivation of Mycobacteria. Am Rev Resp Di.s 1968;98:295-6.

Cite this article as: Singh P, Chauhan S, Lakshmikantha M, Tiwari S, Sharma S. Comparative study of the smear microscopy, with conventional culture in clinically suspected cases of pulmonary and extra pulmonary tuberculosis. Int J Res Med Sci 2015;3:2204-9.