DOI: JCDR/2012/3507:1901

Original Article

Microbiology Section

Rapid Slide Culture: Relevance to the Modern Day Diagnosis of Tuberculosis

SANJEEV H., KARNAKER VIMAL K., RAI REKHA, PAI ASHA K.B., GANESH H.R., KRISHNAPRASAD M.S.

ABSTRACT

Background: Tuberculosis is probably the most important infectious disease of human beings. Early diagnosis of tuberculosis and initiating prompt treatment has been the mainstay in interrupting the transmission of the infection to others in the community.

Aim: The present study was aimed at evaluating and comparing the different tests which are available for the diagnosis of tuberculosis and at determining their feasibility as diagnostic tests in terms of their effectiveness and affordability.

Materials and Methods: 100 sputum samples, 50 samples which were positive for acid fast bacilli (AFB) and 50 samples which were negative for AFB on Ziehl-Neelsen (ZN) staining were included in the study. The sputum was decontaminated and concentrated by the modified Petroff's method and the deposit which was obtained was inoculated onto plain Lowenstein –Jensen (LJ) slants and the rapid slide culture (RSC) medium.

Results: Of the 100 sputum samples which were inoculated onto the RSC and LJ mediums, 64 samples showed growth of *Mycobacterium tuberculosis* on RSC after a mean incubation period of 7 days and 63 samples showed a positive growth on LJ medium after a mean incubation period of 34 days.

Conclusion: RSC is more sensitive as compared to the direct smear examination and as sensitive as the LJ medium for the diagnosis of tuberculosis, but RSC had an advantage of a shorter turn around a period of 7 days.

Key Words: Rapid slide culture, Direct microscopy, LJ culture

INTRODUCTION

Tuberculosis (TB), for many centuries, has been the most important of human infections, in its global prevalence, devastating morbidity and massive mortality. It has been estimated that a third of the world's population is infected with the tubercle bacilli. Each year, more than eight million new cases and three million deaths due to tuberculosis is being reported worldwide [1]. The major brunt of the disease is on the developing nations, which account for more than three fourth of the cases and deaths due to tuberculosis. Fifteen million people suffer from tuberculosis in India and half a million die of the disease every year [2].

Effective control programmes are necessary for controlling the spread of the disease. Poverty, overcrowding, poor infection control practices and lack of financial and technical resources have hindered the effective implementation and the success of the control programmes [3]. This has been further confounded by the emergence of the AIDS pandemic. The co-existence of tuberculosis in the HIV/AIDS patients has resulted in the dissemination of MDR TB and XDR TB into the community. Infection with these multiple drug resistant strains is extremely difficult to treat and it is associated with a high mortality rate [2].

Early diagnosis and effective treatment is necessary for controlling the spread of TB. Sputum microscopy has been the mainstay in the diagnosis of TB [4]. The drawback of the direct smear microscopy is its low sensitivity, which according to published data, ranges from 45 -60% [5, 6].

Conventional culture methods, like the culture on the Lowenstein-Jensen (LJ) medium, though they are the gold standard, are too slow and it takes a minimum of 4-6 weeks for the growth to appear. Newer methods are like the Bactec system, MGIT and molecular methods are all rapid and effective methods, but the high cost and sophistication of these techniques keep them beyond the reach of many laboratories [7].

There is a need for a diagnostic test that is simple, rapid and inexpensive. Rapid slide culture (RSC) is a safe, simple and easy method, with the results being available in a week's time. There have been no systematic studies which were done by using RSC on patients who were suspected of pulmonary tuberculosis [8, 9, 10].

Hence, we undertook this study to evaluate and compare the different diagnostic tests which are available for the diagnosis of tuberculosis and to determine their feasibility as diagnostic tests for the patients in terms of their effectiveness and affordability.

MATERIALS AND METHODS

This study was conducted in the tuberculosis laboratory of the Department of Microbiology, KS Hegde Medical College, Mangalore. Consecutive early morning sputum samples which were obtained from patients with a clinical and radiological suspicion of pulmonary tuberculosis, were screened by using ZN staining. 50 consecutive sputum samples which were positive for acid fast bacilli (AFB) and 50 consecutive samples which were negative for AFB by ZN microscopy were considered for the study.

Each sputum sample was then subjected to fluorescent staining by using the Phenolic auramine dye (Himedia Kit). The sputum sample was then decontaminated and concentrated by using the modified Petroff's method [11]. The deposit, thus obtained, was used for inoculating different culture media:

a) Plain LJ slants
b) Rapid slide culture medium

The Lowenstein-Jensen medium was prepared and inoculated as per the RNTCP guidelines. Two LJ slopes were inoculated for each sample. A control strain of *Mycobacterium tuberculosis*, H37Rv, was inoculated with each batch of media. The LJ slopes were incubated at 37°C for six weeks. The slopes were examined for growth twice a week during the first two weeks, followed by once a week till six weeks. If no growth was seen by six weeks, the slopes were further incubated till the eighth week [11]. The growth which was obtained on LJ medium was identified as *Mycobacterium tuberculosis* by the Niacin test and the aryl sulfatase test [12, 13]. Any growth which was seen within seven days of incubation was considered to be caused due to the rapid growers and it was excluded from the study [8].

For the purpose of RSC, the sputum concentrate was smeared on the lower one third of a sterilized glass slide. The smear was air dried and dipped in 10 ml of human blood medium, so that the smear on the lower third of the slide remained completely immersed in the medium, and it was incubated for 7 days at 37^0C [8].

The human blood medium was prepared by using outdated (but stored for not longer than four weeks) citrated blood which was obtained from the blood bank. The blood was diluted with an equal volume of sterile de-ionized water to cause haemolysis. To the haemolysed blood, anti-bacterial and anti-fungal agents (Polymyxin B: 200,000units/L, Carbenicillin: 100mg/L, Trimethoprim: 10mg/L and Amphotericin B: 100mg/L) were added to make the medium selective. The pH of the medium was adjusted to between 6.5 and 7.5 and it was dispensed in 10 ml quantities in sterile, screw capped, wide mouthed, reagent bottles [8].

On the seventh day, the slide which was incubated in the human blood medium was taken out, dipped in distilled water to wash off the excess blood, and it was dried in an oven at 80^0C for 30 minutes. It was stained by using the ZN method and was examined under the oil-immersion objective. The growth, if any, was graded as 1+, 2+, 3+ and 4+ according to Purohit et al [14]. As a positive control, the H37Rv strain of *Mycobacterium tuberculosis* was processed with each batch of the specimens. If any sputum was found to be positive, it was re-tested by using a sputum aliquot which was stored at -20^0C; this confirmed the reliability of the technique.

RESULTS

The smear and the culture results of a total of 100 patients were analyzed. Of these, 69 cases were found to be positive by one or more tests and 31 were found to be negative by all the tests. The RSC culture was positive in 64 cases, whereas the LJ medium culture was positive in 63 cases [Table/Fig-1].

The correlation between the results of the RSC and the LJ medium cultures in all the patients who were studied, is shown in [Table/Fig-2]. Fifty eight patients showed positive results by both the culture methods and 6 cases were positive only on RSC, while 5 cases showed positive growth only on the LJ medium. In all, 31 cases were negative on culture by any method.

Among the smear positive cases, 48 were positive on the RSC and the LJ medium respectively [Table/Fig-3]. Of these, 47 were positive by both RSC and LJ, and 1 was negative by both the methods.

Among the 50 smear negative patients, 16 were RSC positive and 15 were positive on the LJ medium cultures [Table/Fig-4]. Of these, 11 were positive by both the RSC and the LJ medium culture and 30 were negative by both the methods.

All the patients who were positive on the ZN smear examination were also found to be positive by fluorescent microscopic examination. Among the ZN smear negative cases, fluorescent microscopy detected 3 more cases to be positive for AFB.

In the 100 sputum samples which were inoculated into the LJ medium and RSC, growth was obtained after a mean incubation period of 38 days and 7 days respectively.

DISCUSSION

The early diagnosis and effective treatment of the open cases has been the focus of the tuberculosis control programmes all over the world. In this respect, direct smear examination by ZN staining has been the backbone of any tuberculosis control programme. But the drawback of the ZN smear examination is its low sensitivity. Conventional cultures on the LJ medium, though they are the gold standard, have the inherent disadvantage of time which is required to observe the growth. This may lead to a delay in the start of the treatment, facilitating the spread of the disease, or leading to the unnecessary treatment for the non pulmonary infections [8].

The rapid slide culture, though it was described in the 19th century by Robert Koch, could not be practiced because of the problems

Test	Positive (no.)
ZN Smear	50
RSC	64
LJ	63
All the 3 tests	47
One or more tests	69

[Table/Fig-1]: Distribution of positive results according to test used

		LJ Medium culture		
RSC		Positive	Negative	Total
	Positive	58	06	64
	Negative	05	31	36
	Total	63	37	100

[Table/Fig-2]: Overall correlation between RSC and LJ culture results

		LJ Medium culture		
RSC		Positive	Negative	Total
	Positive	47	01	48
	Negative	01	01	02
	Total	48	02	50

[Table/Fig-3]: Correlation between RSC and LJ culture results in smear positive case

		LJ Medium culture		
RSC		Positive	Negative	Total
	Positive	11	05	16
	Negative	04	30	34
	Total	15	35	50

[Table/Fig-4]: Correlation between RSC and LJ culture results in smear negative cases

of bacterial and fungal contamination of the media. In the recent years, these problems have been overcome with the incorporation of antibiotics and antifungal agents in the media [8]. The rapid slide culture technique, which has thus been improved, has been used to detect *Mycobacterium tuberculosis* and it has been compared with direct microscopy and the cultivation of *Mycobacterium tuberculosis* on the LJ medium for both the sputum positive and negative cases [8]. These studies have shown that RSC is capable of detecting AFB in 8.9%–11% of the sputum negative cases [8, 10]. The rapid slide culture is a safe, simple and easy method, with the results being available in a week's time [7].

In our study, out of the 100 cases of clinically and radiologically suspected cases of pulmonary tuberculosis, 69 cases were positive by any one of the methods i:e smear, RSC or the LJ medium culture.

The RSC was found to be more sensitive than the smear examination in the detection of *Mycobacterium tuberculosis* in this study. *Mycobacterium tuberculosis* could be cultivated on RSC in 16 smear negative cases. Thus, it can be inferred that RSC has a definite advantage over the direct smear examination in the diagnosis of pulmonary tuberculosis. The RSC can be used routinely as a method that compliments the direct smear examination, especially on patients with a clinical and radiological evidence of tuberculosis, but who remain smear negative.

The RSC showed no growth in 2 smear positive and 5 LJ culture positive cases. The reason for these false negative results could be that, a wet smear was immersed into HBM, leading to the washing off of the AFB or the smear was over dried, leading to the loss of viability of AFB. Another reason for the smear positive cases being negative on RSC, could be that the patients were on non-specific antibiotic treatment or that they had received specific anti tubercular chemotherapy, both of which could give false negative culture results. This especially could be the reason in 1 smear positive case which remained negative in both the RSC and the LJ medium culture.

The correlation between the RSC and the LJ culture results in the 50 smear negative cases showed a significant difference. Overall, 20 smear negative cases were found to be positive on either RSC or the LJ medium. Further, RSC was found to be better than the LJ medium in detecting *Mycobacterium tuberculosis* in the smear negative cases. This further emphasizes the importance of culture and the role of RSC in aiding the diagnosis of smear negative pulmonary tuberculosis [8].

Smear examination by fluorescent microscopy had a sensitivity which was slightly superior to that of the ZN method in detecting AFB. This correlated well with the findings of the similar studies [15,16]. However, fluorescent microscopy had the disadvantage of requiring sophisticated equipment and it may not be practical for use in detecting *Mycobacterium tuberculosis*.

The rapid slide culture was found to be as sensitive as the LJ medium in detecting *Mycobacterium tuberculosis*. Sixty four samples showed a positive growth on RSC and sixty three samples grew on the LJ medium. The rapid slide culture, as was evidenced by our study as well as by other studies, was found to be more sensitive as compared to the direct smear examination [8,10]. RSC can be used routinely as a method that complements the direct smear examination. The RSC can be very useful in the early confirmation of *Mycobacterium tuberculosis* and in monitoring the response to the anti tubercular therapy.

CONCLUSION

The rapid slide culture was found to be as sensitive as the LJ medium for the diagnosis of tuberculosis. The seven day turn around period of RSC was a definite advantage over the LJ medium culture. The rapid slide culture can be used as an adjunct to the direct smear examination, especially in patients who are suspected of pulmonary tuberculosis, but remain sputum smear negative by direct microscopy.

REFERENCES

[1] Jureen P, Enstrand L, Eriksson S, Alderborn A, Krabbe M, Hoffner SE. Rapid detection of Rifampicin resistance in Mycobacterium tuberculosis by using the pyrosequencing technique. *J Clin.Microbiol* 2006; 44(6): 1925-29.

[2] ICMR bulletin 2002; 32(8).

[3] Moore AJ, Evans CAW, Gilman RH, Caviedes L, Vivar A, Sanchez E et al. Microscopic- Observation Drug Susceptibility Assay for the diagnosis of TB. *N Eng J Med* 2006;355:1539-50.

[4] Ramachandran R, Paramasivan CN. What is new in the diagnosis of tuberculosis? Part:1 Techniques for the diagnosis of tuberculosis. *Ind J tub* 2003;50:133-40.

[5] Parekh KM, Inamdar V, Jog A, Kar A. A comparative study on the diagnosis of pulmonary tuberculosis by using conventional tools and polymerase chain reaction. *Ind J Tub* 2006; 53:69-76.

[6] Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics for developing countries. *Lancet* 2006; 367:942-43.

[7] Katoch VM. Newer diagnostic techniques for tuberculosis. *Indian J Med Res* 2004; 120:418-28.

[8] Jena J, Neema SK, Panda BN, Rajan KE. Comparative efficacy of the rapid slide culture of M.Tuberculosis and the conventional LJ medium culture in the diagnosis and management of pulmonary tuberculosis cases. *Ind J Tub* 1995; 42:151-54.

[9] Van Deun A, Martin A, Palomino JC. Diagnosis of drug-resistant tuberculosis: the reliability and rapidity of the detection. *Int J Tuberc Lung Dis* 2010; 14(2):131–40.

[10] Nair L, Sudarsana J, Nizamuddin, Karim S, Kumar S. Preliminary report on the rapid slide culture of Mycobacterium tuberculosis. *J Acad Clin Microbiol* 1998;1:151-53.

[11] Manual of Standard Operating Procedures. Revised National Tuberculosis Control Programme. Central TB Division, Directorate General of Health Services, *Ministry of Health and Family Welfare*, Nirman Bhavan, New Delhi.

[12] NTI Monograph Series I. Manual for the establishment and functioning of a tuberculosis culture laboratory 1983;19-25.

[13] Koneman EW, Stephen DA, Janda WM, Schreckenberger PC, Washington CW. In *Colour Atlas and Textbook of Diagnostic Microbiology*. 6th edition, Philadelphia, Lippincot; 2006;1064-1124.

[14] Purohit SD, Gupta ML, Chauhan A, Nanavati V. A new medium for the rapid slide culture of the tubercle bacilli. *Indian J Pathol Microbiol* 1993; 36: 370-75.

[15] Hendry C, Dionne K, Hedgepeth A, Carroll K, Parrish N. Evaluation of the rapid fluorescent staining method for the detection of mycobacteria in clinical specimens. *J Clin Microbiol* 2009; 47:4.

[16] Prasanthi K, Kumari AR. Efficacy of the fluorochrome stain in the diagnosis of pulmonary tuberculosis which is co-infected with HIV. *Ind. J. Med. Microbiol* 2005; 23:179-85.

AUTHOR(S):

1. Dr. Sanjeev H.
2. Dr. Karnaker Vimal K.
3. Dr. Rai Rekha
4. Dr. Pai Asha K.B.
5. Mr. Ganesh H.R.
6. Dr. Krishnaprasad M.S.

PARTICULARS OF CONTRIBUTORS:

1. Corresponding Author.
2. MD, Department of Microbiology, K S Hegde Medical Academy
3. MD, Department of Microbiology, K S Hegde Medical Academy
4. MD, Department of Microbiology, K S Hegde Medical Academy
5. M.Sc, Department of Microbiology, K S Hegde Medical Academy
6. MD, Department of Microbiology, K S Hegde Medical Academy

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Sanjeev H.
Assistant Professor, Department of Microbiology
K S Hegde Medical Academy
Deralakatte, Mangalore-575018
Phone: +91-0824-2204490-92
Fax: +91-0824-22014162
Mobile: 9972212280
E-mail: drsanjeevh@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS:

None.

Date of Submission: **Aug 11, 2011**
Date of peer review: **Oct 15, 2011**
Date of acceptance: **Dec 22, 2011**
Date of Publishing: **May 01, 2012**