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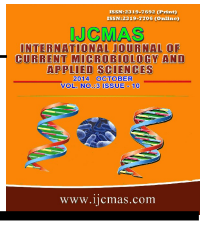

Original Research Article

Comparison of conventional methods with automation and card ELISA test for the diagnosis of Pulmonary Tuberculosis

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Keywords

Rapid slide culture
Lowenstein
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TB Card ELISA.

A B S T R A C T

Pulmonary tuberculosis is a severe, contagious air borne disease caused by Mycobacterium tuberculosis. The discovery of the tubercle bacillus is more than 100 years and all the advances in our knowledge of the disease have been made since then; Tuberculosis still remains one of the major health problems facing mankind, particularly in developing countries. This study is to compare the different methods of culture for Mycobacterium tuberculosis in relation to sensitivity and rapidity. A total of 60 clinical samples of blood and smear negative sputum was obtained from patients of suspected pulmonary TB with strong clinical evidence. Out of 60 samples tested 09 (15%) samples were found to be positive for Rapid slide culture, 08 (13.3%) were positive for growth on LJ media, 09 (15%) were found be BACTEC positive and none of the samples were positive with Card ELISA test. Hence Rapid Slide culture has proved to be a rapid, cheap, effective and technically easy for the confirmation of tuberculosis. Therefore, Rapid Slide Culture is strongly recommended for a developing country like India.

Introduction

Pulmonary tuberculosis is one of the major air borne infectious bacterial disease. It still remains a major worldwide health problem due to increase in drug resistance. Presently, there have estimated 9.5 million incident cases (range, 8.9 million-9.9 million) of TB globally and an estimated 14 million prevalent cases of TB in 2009. An estimated 1.3 million deaths occurred among HIV-negative cases of TB, including 0.38 million deaths among women. In addition, there were an estimated 0.4 million death among incident TB cases that were HIV positive (WHO, 2010).

Currently more people die of tuberculosis than from any other infectious disease. Nearly 95% of all TB cases and 98% deaths due to TB are in developing countries. It also imposes a burden on our economy in terms of current and future output losses, because of premature deaths and ill health. To add to the existing burden, the situation is compounded by the large scale increase of new TB cases associated with increasing HIV infection (Ramachandran and Paramasivan, 2003). The situation is further exacerbated with the increasing incidence of drug resistant TB.

Early diagnosis plays a vital role in control of TB. Diagnosis of Mycobacterial infections however remains an enigma (Negi *et al.,* 2005). Cure of the disease is possible only with correct diagnosis and appropriate treatment. Early and accurate detection of active cases remain an important objective for improved implementation of chemotherapy and for reduction in the spread of the disease. The diagnosis traditionally depends upon identifying the infective organism in secretions of diseased individual (Shah and Rauf, 2001). There are two basic approaches for the diagnosis of the TB. The direct approach includes detection of Mycobacteria and the indirect approach includes measurement of humoral response of the host against TB. The diagnostic modalities should have certain desirable features that are, sensitivity and specificity, speed, reproducibility, cost effectiveness, safety, simplicity and easy application for wider use.

The diagnosis in endemic countries like India depends more on the use of labour intensive, easy to use methodology with minimum infrastructures or equipment. The need is to find a viable alternative for smear microscopy. This method has to have the following desirable features like results as early as possible, simple training, easy interpretation, and allow start of the treatment as early as possible (Ramachandran and Paramasivan, 2003). The Ziehl-Neelsen staining method although rapid and inexpensive, can detect bacilli only when there are more than 10,000 bacilli/ml of the sputum (Shah and Rauf, 2001). The ZN method lacks sensitivity in clinical specimen varying between 30 – 70%, especially when disease occurs in children or co-infected with HIV (Omrani, 2009). On the other hand culturing on Lowenstein-Jensen media are sensitive and can detect 10-100 organisms per sample. However they are time consuming and take 6-8 weeks for the results. Many techniques are available today which have improved the diagnosis and shortened the testing time in the Laboratory. The risk of increasing spread of tuberculosis and development of drug resistance make early diagnosis a matter of utmost concern and improved rapid methods for laboratory confirmation are urgently required (Ahmad and Afghan, 1995).

This prolonged period has prompted many workers to look for quicker method of culture, such as the rapid slide culture technique, BACTEC and Card ELISA test.7 Robert Koch was the first to use rapid slide culture technique using coagulated human serum, he was successful in obtaining the growth of Mycobacterium bacilli in 7 days but contamination hindered further success. The RSC is a rapid, simple and safe method and culture results are available in 7 days. This method has also been successfully employed by Purohit *et al.* (1993)

Materials and Methods

A total of 60 clinical samples blood and smear negative sputum were obtained from patients of suspected pulmonary TB with strong clinical evidence referred from TB and chest department of VIMS and RC from January 2010 to August 2011. The patients were asked to take early morning sputum when he wakes up without brushing the teeth and asked to take a deep breath and expectorate sputum in a given wide mouthed sterile plastic container. Second sample was taken on the spot in the hospital. Collection of blood from AFB negative patients.

Processing of samples: - All the samples were processed in the microbiology department of VIMS. Preliminary ZN test were performed to rule out sputum positive samples. The negative sputum samples were

processed for further studies after concentration by modified Petroff's method. The deposit was examined microscopically and was then inoculated on Rapid slide culture, LJ media and then sent for BACTEC test.

Rapid slide culture method: - Citrated human blood from blood bank not more than 4 week old was mixed with equal volume of sterile distilled water. The mixture was shaken well till blood get haemolysed. The medium was made selective by addition of chemotherapeutics agents like: Polymyxin B -- 200000 units/liter, Carbencillin ---- 100 mg/ liter, Trimethoprim ---- 10 mg/ liter and Amphotericin B --- 10 mg/ liter. The pH of the medium was adjusted at 6.5 – 7.5 and 8-10ml of this media was transported to each of Mac Cartney bottle. Glass slides measuring 3x1 inches split longitudinally were taken. Smears were made over the lower 1/3rd of the 2 splitted slides and smears were air dried. Slides were put into a Mac Cartney bottle containing blood media so that the smear remained dipped in the medium. The inoculated Mac Cartney bottle was incubated at 37□. Slides 1 and 2 were removed on 7th and 13th day respectively. Slides were taken out and dipped in sterile distilled water to remove excess of media on the smears. The slides were then placed in an oven at 80° for 30 min and then stained by ZN staining method.

The growth was recorded as follows.

0	No division of AFB as compared to slide 1
1+	Small clumps of up to 4 bacilli provided these were not present in slide 1
2+	Large clumps of bacilli
3+	Large clumps with some cord formation
4+	Micro colonies with good cord formation

Growth on Lowenstein-Jensen

Lowenstein-Jensen (LJ) medium is most widely used for tuberculosis culture. LJ medium containing glycerol favours the growth of *M.tuberculosis.* After decontamination of the sputum, the deposit was inoculated on L-J media and incubated at 37°. Reading was taken weekly for 5 to 7 weeks and isolates were identified by colony morphology, rapidity of growth and by ZN staining. The growth on LJ media was confirmed by biochemical test viz, Niacin test and Nitrate reduction test.

BACTEC test: Concentrated sputum is sent to the laboratory for BACTEC culture. The components of the automated BACTEC 460 instrument include a scintillation counter, a needle aspiration assembly, and a movable track on which culture bottles can be placed. The track is aligned so that each bottle passes, in turn, beneath the needle aspiration assembly. When the test bottle is immediately below the needle assembly, the needle is lowered through the rubber stopper into the head space. A sample of the head gas is aspirated and delivered to the scintillation counter chamber. If Mycobacteria are present in the inoculum, CO_2 is released into the head space, the amount of which is proportional to the amount of growth in the vial. The amount of radioactivity is measured in the aspirated head gas, which is translated into a numerical value called the growth index. A growth index higher than 10 are considered positive.

TB Card test: Fresh serum is separated from the aseptically collected blood. Remove the test unit from its pouch and place on flat surface. The test unit was labeled with patient's name. Then 2 drops of serum was added into sample well using provided dropper. Then result was recorded

between 10 to 30 minutes. (CTK ONSITE, Onsite Rapid Test, 1 Cassette Test, manufactured by CTK Biotech, Inc. is used.)

Results and Discussion

In the present study a total of 60 Zeihl Neelsen Negative sputum samples and serum samples from the same patients were processed for the diagnosis of pulmonary tuberculosis. Out of 60 samples 09 (15%) were positive by Rapid slide culture, 08 (13.3%) were positive on LJ media, 09(15%) were found to be positive by BACTEC test and none were positive towards Card ELISA test as shown in Table: 01.

The results obtained by some of the workers have been mentioned below: Simpson and Reed (1953) reported that 63.8% were positive by slide culture as compared with 71.6% positive by routine culture method (LJ media). Jena and Rajan (1995) reported that rapid slide culture was found to more sensitive than smear examination and culture, the most sensitive for establishing the diagnosis as well as for primary isolation of MTB. He reported that the sensitivity of RSC (65.2%) is less when compared LJ media (85.1%), as only one sample was processed for the Rapid Slide culture and 3 samples were processed on LJ media.However in our present study Slide culture showed more sensitivity (100%) than LJ Media (88.8%) and our study is comparable with above mentioned studies. Negi *et al.* (2005) reported that sensitivity of 48.9% towards L J media culture and 55.86% towards BACTEC culture. Tuberculosis Research Centre (TRC), Chennai showed that the rate of isolation of positive cultures was significantly faster with the BACTEC method, with87% of the positives being obtained by 7 days and 96% by 14 days. Hence this result can be compared with the present study as BACTEC and Rapid Slide Culture showed the rate of isolation of positive culture within 14 days. Shenai and Rodrigues (2004) reported out of 11 smear negative, clinically diagnosed TB cases, 6 were positive by TB-BACTEC and 4 by LJ. They have also reported that BACTEC medium appeared to be better than LJ media for rapid isolation and identification of *M.tuberculosis.* Hence these results could be comparable with our findings, where out of 60 smear negative clinically suspected TB cases, 9 were positive by TB-BACTEC and 8 by LJ.

According to WHO (2008) report, the sensitivity of TB-Card ELISA test ranged from 1% to 60% and was higher in sputum smear-positive than smear negative patients. They concluded that Rapid Serological TB test had poor specificity when tested in TB suspects from endemic settings. In the present study none of the smear negative samples showed positive reaction hence the present study could be correlated with the report of WHO (2008).

The time required for Rapid slide culture was found to be 10-13 days, 21-28 days for growth on LJ media, 10-12 days for BACTEC and less than 01 hour for Card ELISA test. The time requirement of Card ELISA was found to be very less(< 1 day), followed by BACTEC (12 days), Rapid Slide Culture(12-13) days, and LJ media (21-28) days. This can be compared with that of the reports of Simpson and Reed (1953), Jena and Rajan (1995), Shenai and Rodrigues (2004), Banerjee and Gupta (2003).

The cost for the diagnosis of TB by BACTEC was found to be expensive (Rs.1000), followed by Card ELISA (Rs.300), LJ media (Rs.100) and Rapid slide culture was found to be inexpensive (Rs.20).

Table.1 Comparison of Conventional methods with BACTEC and Card ELISA test for diagnosis of Pulmonary Tuberculosis

Method of diagnosis	Total samples	No of Positive cases	% of Positive cases	No. of Negative cases	% of Negative cases
Rapid slide culture	**60**	**09**	**15**	**51**	**85**
Growth on LJ media	**60**	**08**	**13.3**	**52**	**86.6**
BACTEC	**60**	**09**	**15**	**51**	**85**
Card ELISA test	**60**	**00**	**00**	**60**	**100**

Out of 60 samples tested 09 (15%) samples were found to be positive towards Rapid slide culture, 08 (13.3%) were positive towards growth of LJ media, 09 (15%) were found be positive towards BACTEC and none of the samples were positive toward Card ELISA test.

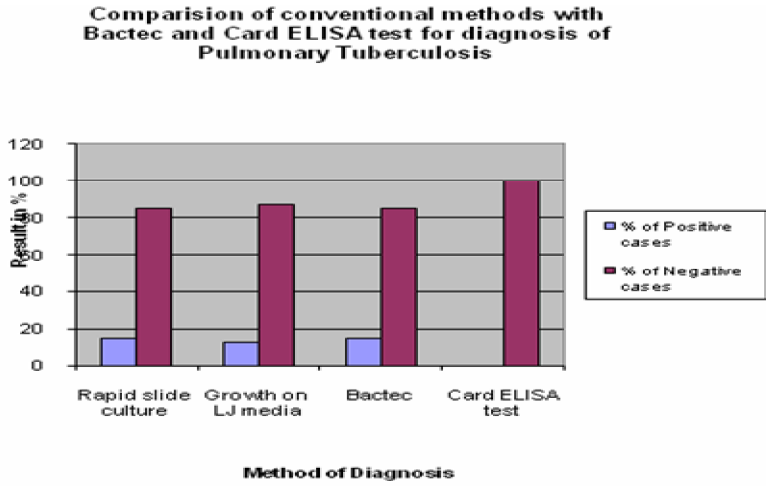

Table.2 Showing different types of grading in Rapid slide culture

Type of grade	No of cases	Percentage
Grade I	**02**	**22.2**
Grade II	**03**	**33.3**
Grade III	**03**	**33.3**
Grade IV	**01**	**11.1**

Out of 09 positive Rapid slide culture, 02 samples showed Grade-I, 03 samples showed Grade II and III respectively and only one sample showed Grade-IV growth.

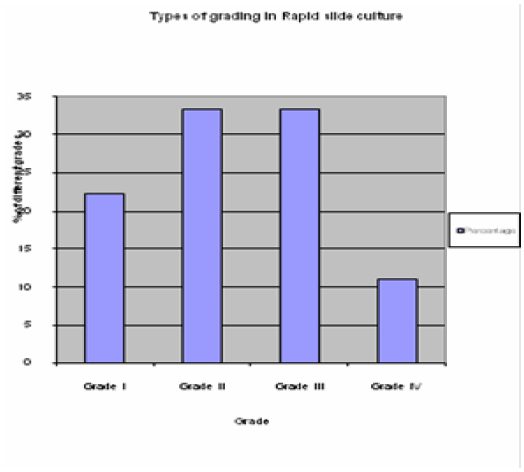

Table.3 Comparison of time required for diagnosis of Pulmonary Tuberculosis

Method of diagnosis	No. of days required
Rapid slide culture	10-13
Growth on LJ media	21-28
BACTEC	10-12
Card ELISA	< 01

The time required for Rapid slide culture was found to be 10-13 days, 21-28 days for growth on LJ media, 10-12 days for BACTEC and less than 01 hour for Card ELISA test.

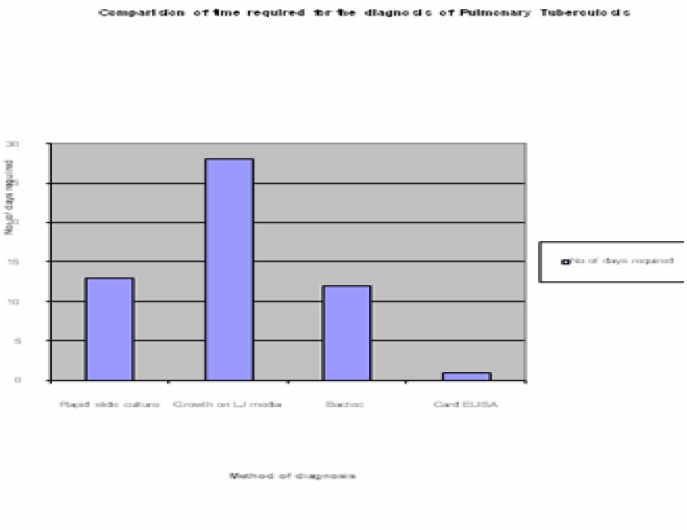

Table.4 Comparison of cost for the diagnosis of Pulmonary Tuberculosis

Method of diagnosis	Cost for each test in Rupees
Rapid slide culture	20
Growth on LJ media	100
BACTEC	1000
Card ELISA	300

The cost for the diagnosis of Pulmonary Tuberculosis was found to be Rs 20 for Rapid slide culture, 100 for growth on LJ media, Rs. 1000 for BACTEC and Rs 300 for Card ELISA test.

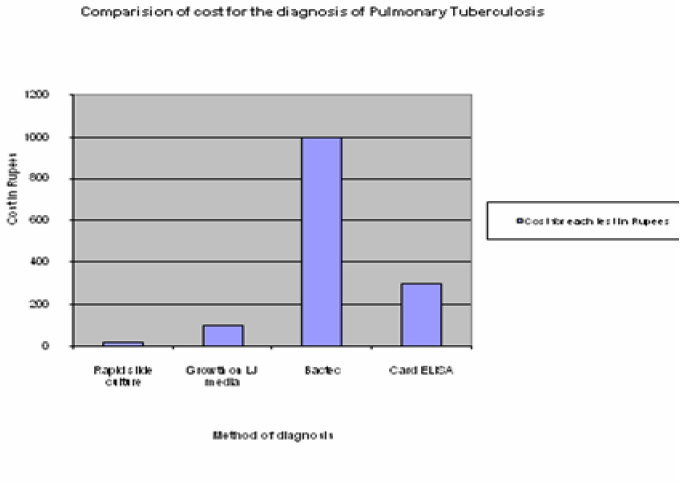

Table.5 showing sensitivity & sensitivity of RSC

	Rapid slide culture				
Growth on LJ media		positive	negative		
	Positive	9 TP	00 FN	TP + FN	09
	negative	00 FP	51 TN	FP + TN	51
	Total	09	51		

Sensitivity of RSC= TP/TP+FN X100, 9/9X100= 100%. Specificity of RSC= TN/FP+TNX100, 51/51X100= 100%.

Table.6 showing sensitivity & sensitivity of LJ media

	Growth on LJ media				
Culture on Bactec		positive	negative		
	Positive	8 TP	01 FN	TP + FN	09
	negative	00 FP	52 TN	FP + TN	52
	Total	08	53		

Sensitivity of LJ media= 8/8+1 x100 = 88.8%.
Specificity of LJ media= 52/1+52 x 100 = 98.1%.

Table.7 showing sensitivity & sensitivity of Card ELISA test

	Card ELISA test				
Culture on Bactec		**positive**	**negative**		
	Positive	**00 TP**	**9 FN**	**TP + FN**	**09**
	negative	**00 FP**	**60 TN**	**FP + TN**	60
	total	**00**	**60**		

Sensitivity of card ELISA= TP/TP+FN X100, 0/0+9 x100.

From this study we can conclude that Rapid Slide culture is found to be equally sensitive as BACTEC, the time consumption is also almost the same. But when cost is taken into consideration Rapid slide culture is found to be very cheap than BACTEC and other methods. However LJ media also shows the same sensitivity of the above mentioned test, the time requirement is long and also costly than Rapid Slide Culture. Hence Rapid Slide culture is proved to be a rapid, cheap, and effective and technically easy for obtaining culture confirmation of tuberculosis. Therefore, Rapid Slide Culture is strongly recommended for developing country like India.

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