



Comparison of the conventional diagnostic techniques, BACTEC and PCR

Aroma Oberoi, Aruna Aggarwal

Abstract

Two hundred and thirty samples from suspected pulmonary and extra pulmonary cases of tuberculosis were processed for detection of Mycobacterium tuberculosis by ZN smear examination, LJ medium culture, BACTEC radiometric culture and Polymerase Chain Reaction tests. A significant difference was seen in the sensitivities of different tests, i.e. 73.9% for PCR tests, 34.78% for ZN smear examination, 52.17% for LJ culture and 58.69% for BACTEC culture. However, there was no significant difference in specificity of different tests ($P > 0.05$). PCR test sensitivity in pulmonary and extrapulmonary clinical samples was 74.0% and 78.5% respectively and found to be significantly higher ($P < 0.05$) when compared with those of other tests. The mean detection time for M. tuberculosis was 24.03 days by LJ medium culture, 12.89 days by BACTEC culture and less than one day by PCR test.

Key words

Tuberculosis, M. tuberculosis, PCR, LJ medium, BACTEC.

Introduction

Tuberculosis remains a major world wide health problem (1). The situation is further exacerbated with the increasing incidence of drug resistant TB (1). Diagnosis of mycobacterial infections, however, remains an enigma. Although acid fast bacilli microscopy, and conventional Lowenstein Jensen culture remain the cornerstone of the diagnosis of this disease, these traditional bacteriological techniques are either slow or their sensitivity is quite low, especially with clinical samples that contain small number of organisms (2). This can affect treatment by either delaying it or causing inappropriate empiric therapy for TB to subjects without mycobacterial infections or with atypical mycobacteria (3).

Several studies have been done to detect M. tuberculosis in respiratory and other clinical samples by amplifying different DNA sequences of M. tuberculosis by polymerase chain reaction test with encouraging results (4,5). The present study was carried out to compare 65 Kda antigen based PCR test in cases

of pulmonary and extrapulmonary tuberculosis with those of conventional ZN (Ziehl Neelson) stained acid fast bacilli (AFB) microscopy and culture by LJ and radiometric BACTEC system.

Materials & Methods

A total of 230 samples were evaluated by all 3 methods. The pulmonary samples included 82 sputum samples from adult pulmonary TB cases, 22 BAL (broncho alveolar lavage) from children with pulmonary tuberculosis, 30 pus, 20 pleural fluid, 10 lymph node aspirate, 18 synovial fluid and synovial tissue from osteoarticular TB, 20 urine, 5 bone marrow aspirate, 10 ascitic fluid, 5 other biopsies, 30 CSF, 5 endometrial biopsy, 1 menstrual fluid and one semen. In addition, 37 sputum samples obtained from nontuberculosis individuals (chronic asthmatics, chain smokers) initially screened by AFB smear examination and chest X ray were also used in the study as negative controls.

From the Department of Microbiology, C.M.C & Hospital, Ludhiana - 141008

Correspondence to : Dr. Aroma Oberoi, Reader, Microbiology Dept. C.M.C & Hospital, Ludhiana - 141008.



Processing of samples

For every clinical sample, two smear one direct and other concentrated after processing by N-acetyl-L-cysteine NaOH (NALC-NaOH) method and other appropriate methods depending on the nature of samples were prepared. Z-N staining was done using standard techniques. Concentrated deposits obtained after processing of sample were inoculated into two bottles of LJ medium and on BACTEC 12B vial(16). One LJ bottle was incubated at room temperature and other at 37°C only. In case of conventional LJ media based cultures, readings were taken on a weekly basis till 8 weeks, where as in case of BACTEC cultures, for first week, bottles were read every day and there after at weekly intervals for six weeks(7). The mycobacterial isolates obtained were subjected to niacin and NAP(P-nitro- Alpha acetyl amino Beta hydroxy-propionophenone) test for speciation of mycobacteria(6,8). For PCR, samples were sent to Lal Path laboratories Pvt.Ltd, New Delhi and results interpreted.

Results

Total of 255, clinical samples with strong clinical suspicion of tuberculosis were subjected to all the tests mentioned. Out of these, 25 samples were found to be contaminated in BACTEC culture. So results of 230 samples were compared. For ZN smear examination sensitivity was 34.78%, 52.17% for LJ media culture and 58.69% for BACTEC culture. In comparison PCR test was found to have a much higher sensitivity of 73.9% (Table - I). Specificity was found to be 100% by ZN smear examination, LJ media culture & Bactec culture but PCR showed specificity of 97.29% (Table II). Sensitivity of PCR test vis a vis three different tests i.e. Smear examination, LJ culture and BACTEC culture result individually as well as in combination are shown in (Table III). PCR test was found to be much more sensitive than smear examination, LJ culture or BACTEC culture ($P < 0.05$). In 65 samples negative by all the other three tests used, PCR test was able to detect 13 positives (20%) and these were not likely to represent false positive result as PCR repeatedly was positive in these samples and these samples belonged to highly suspected cases of tuberculosis who responded to the antitubercular treatment (Table IV).

The mean detection time for M. tuberculosis was 24.03 days by LJ media culture, 12.89 days by BACTEC and

less than one day by PCR test.

The sensitivities of PCR test as well as BACTEC culture method were found to be near similar in both pulmonary and extrapulmonary tuberculosis though smear sensitivity and LJ media based culture was found to be much higher in pulmonary TB as compared to extrapulmonary TB (Table-IV).

Table I - Sensitivity of different tests conducted on samples from suspected cases of Tuberculosis.

Test performed	No. of samples Tested	Results		Sensitivity %
		Neg.	Post.	
ZN smear	230	150	80	34.78
LJ Media	230	110	120	52.17
BACTEC	230	95	135	58.69
PCR	230	60	170	73.91%

Table II - Results of 37 sputum samples from non tuberculosis subjects (negative controls) subjected to different tests.

Test performed	Results		
	Negative	Positive	Specificity
ZN smear	37	0	100%
LJ media	37	0	100%
BACTEC	37	0	100%
PCR	36	1	97.29%

Table - III Comparison of sensitivity of PCR test vis a vis other tests.

Test/Result Category(No.)	PCR Result		Sensitivity of PCR test(%)
	Positive	Negative	
Smear Positive(80)	80	0	100%
Smear Negative(150)	90	60	60%
LJ Positive(120)	115	5	95.8%
LJ Negative(110)	45	55	40.9%
Bactec Positive(135)	130	5	96.29%
Bactec Negative(95)	33	62	34.73%
Smear Negative samples But Positive by either LJ/BACTEC(76)	75	1	98.68%
LJ & BACTEC Positive(101)	100	1	99.00%
LJ & BACTEC Negative(78)	28	50	35.89%
Smear, LJ & BACTEC Positive (53)	53	0	100%
Smear, LJ & BACTEC Negative(65)	13	52	20%

**Table IV -Sensitivity of different tests in pulmonary and extrapulmonary samples**

Nature of Clinical samples	Total samples	Detection rate in number(%)by diff.tests			
		ZN	LJ	Bactec	PCR
Pulmonary (Sputum,BAL)	104	50(48.07%)	55(52.88%)	62(59.61)	77(74.0)
Extrapulmonary (Skin biopsy,Pus CSF,synovial fluid & tissue)	126	26(20.6%)	58(46.03)	80(63.49)	99(78.5)

Discussion

The specificity,sensitivity and speed of PCR test in diagnosis of M.tuberculosis infection evaluated in our study should encourage the use of this method in routine diagnosis and in complicated cases of TB. We compared the performance of conventional tests (ZN stained AFB microscopy, LJ culture) with BACTEC system(automated) and PCR in different clinical samples for diagnosis of Tuberculosis. PCR showed the highest sensitivity 73.9% as compared to other tests as also reported by other workers(1).

By the use of PCR test we were able to detect M.tuberculosis in 60% smear negative samples which were positive by either of the culture methods. Time taken for detection of M.tuberculosis by PCR was less than one day,compared to average 24.03 days required for detection by conventional (LJ) and 12.89 days by radiometric BACTEC technique which is supported by earlier studies(9).

In 3% (7/230) samples ZN smear examination and PCR results were positive but culture was negative, this could be due to the presence of nonviable mycobacteria in the samples as some of the subjects were receiving antitubercular treatment. There was only one false positive result by PCR test which could be due to the ability of the PCR test to detect very low number and even dead bacteria in a sample which can be present in a symptomatic individual (10).

PCR test was also shown to be reasonably sensitive (78.5%) in diagnosis of extrapulmonary TB(11-13).

So this can be concluded from our study that although conventional and radiometric methods are gold standard for diagnosis of tuberculosis yet. Molecular diagnosis of tuberculosis by PCR has a great potential to improve the clinicians ability to diagnose tuberculosis. This will ensure early treatment to patients and prevent further

transmission of disease (11). However further studies are needed for improving sensitivity, specificity and reproducibility of this test and to make it more user friendly and cost effective.

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