Indian J Med Res 108, October 1998, pp 120-127

Evaluation of the BACTEC radiometric method in the early diagnosis of tuberculosis

P. Venkataraman, D. Herbert & C.N. Paramasivan

Tuberculosis Research Centre (ICMR), Chennai

Accepted August 31, 1998

A comparison of the BACTEC radiometric method with the conventional culture and drug susceptibility testing methods on isolates from clinical specimens in pulmonary and extrapulmonary tuberculosis, childhood TB and TB in HIV-infected individuals was undertaken. In the case of pulmonary TB, the rate of isolation of positive cultures was significantly faster with the BACTEC method, with 87 per cent of the positives being obtained by 7 days, and 96 per cent by 14 days. However, while there was no difference in the total number of positive cultures by the two methods in smear positive pulmonary tuberculosis, in smear negative pulmonary TB, the BACTEC method yielded more number of positive cultures. In extrapulmonary TB, HIV-TB and childhood TB, although the BACTEC method did not yield additional positives, the detection of positives was considerably faster than by the conventional methods, in which the degree of growth was also scanty. The agreement in drug susceptibility tests was 94 per cent for streptomycin and isoniazid, 99 per cent for rifampicin and 91 per cent for ethambutol. Further, most of the drug susceptibility test results became available within 8 days by the BACTEC method. By facilitating early diagnosis, the BACTEC method may prove to be cost effective in a population with a high prevalence of tuberculosis, particularly in the extrapulmonary and paucibacillary forms of the disease.

Key words BACTEC radiometric method - drug susceptibility test - *Mycobacterium tuberculosis* isolation

Bacteriological investigations play a key role in the diagnosis of different forms of tuberculosis (TB). The demonstration of acid fast bacilli (AFB) in smears from clinical specimens, though confirming the diagnosis of tuberculosis, needs to be supplemented by isolation of mycobacteria on culture for their identification and drug susceptibility testing in order to institute appropriate treatment. A constraint faced with the conventional culture methods using egg-based or agar-based media is that they take two to eight weeks for detection of mycobacteria. In the case of paucibacillary extrapulmonary specimens it takes even longer[1].

An early result on drug susceptibility is vital for effective treatment of the patient, particularly when resistance to one or more drugs is suspected. Here again the conventional methods using Lowenstein-Jensen (L-J) medium require 4 wk for the results to become available. Further, it is also possible that inspissation of L-J medium and binding of the drugs to proteins could result in loss of potency of the drugs[2]. Although this could be circumvented by the use of agar-based media, these methods also require 3-4 wk before results are obtained. Further, in the conventional methods, lack of standardisation in methodology and definitions of resistance used may

cause errors in the interpretation and validity of the results.

Deland and Wagner in 1969[3] developed a technique for automated detection of bacterial metabolism by measuring radioactive CO, liberated during decarboxylation of C^{14}-labelled substrates in the medium. Cummings and colleagues[4] showed that the same principle could be used for measuring the growth of *Mycobacterium tuberculosis.* Middlebrook *et al* [5] further developed the technique and introduced 7H12 liquid medium containing C^{14}-labelled substrate specific for mycobacterial growth, thus resulting in considerable time saving in the primary isolation of tubercle bacilli from clinical material. It was followed by a modified 7H12 medium designated as BACTEC 12B medium which yielded excellent recovery of mycobacteria from sputum as well as extrapulmonary specimens with considerable reduction in recovery time[6,7]. Employing the same radiometric principle, an evaluation of drug susceptibility testing was coordinated by Snider and others in 1981[8].

At present, it is standard practice among laboratories in the developed nations to use BACTEC as a routine method. However, this system has not been used frequently in developing countries, particularly in India, since the utilisation of this facility requires specialised setting and trained manpower with adequate standardisation. We report here our findings comparing culture and drug susceptibility testing by the BACTEC method with the conventional methods.

Material & Methods

Samples : The following sputum and extrapulmonary specimens from patients attending the Tuberculosis Research Centre, Chennai, for various ongoing studies were included : *(i)* Sputum samples from pulmonary TB patients (130); *(ii)* Samples from extra-pulmonary TB cases (109). This included 35 biopsy specimens, 53 aspirated fluids, 10 urine samples and 11 other specimens such as CSF, pus and bone marrow; *(iii)* Sputum samples from HIV infected individuals (98); and *(iv)* Samples from patients of childhood TB (96). This included 66 sputum samples. The rest comprised either gastric lavage or gl and biopsy material.

Decontamination : The sputum samples were processed by modified Petroff's method[9] while the other specimens were subjected to milder decontamination with 5 per cent sulphuric acid[1].

Conventional methods : The processed specimens were inoculated on to pairs of L-J medium slopes. For extrapulmonary specimens, in addition, one slope of L-J with sodium pyruvate and one bottle of selective Kirchner's liquid medium were inoculated[1].

The inoculated slopes were incubated at 37° C and read weekly up to 8 wk. In the case of Kirchner's medium, the bottles were subcultured on L-J when growth was visible or at the end of 6 wk, if negative, and incubated for a further 8 wk.

Drug susceptibility tests were performed on L-J media after inoculation with one loopful of a standard inoculum (4mg/ml) and interpreted by the resistance ratio method for streptomycin and the absolute concentration method for isoniazid, rifampicin and ethambutol, based on readings at 4 wk [10-12].

The strains were identified as *M. tuberculosis* by standard methods *viz.,* sensitivity to p-nitrobenzoic acid, niacin production and catalase activity at 68°C/ pH 7[13,14].

BACTEC method : The procedures used were as described by Siddiqi[15]. Approximately 0.5- 1.0 ml of the sputum deposit remaining after inoculation of LJ slopes and 0.2 ml of the deposit from extrapulmonary specimens was used for the BACTEC method.

One ml of sterile phosphate buffer, pH 6.8, was added to sputum deposits and 0.5 ml to the deposits from extrapulmonary samples. These were vortexed for 10 sec. PANTA supplement (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) was reconstituted and 0.1 ml was added to each BACTEC 12B vial before it was inoculated with the sample. Prior to inoculation, all the 12B vials were tested on the BACTEC 460 instrument to eliminate vials with high background readings (growth index of > 20) and to establish a CO_2 enriched atmosphere in the vial. Using a new sterile syringe and needle for each specimen, 0.5 ml of the deposit in phosphate buffer was inoculated into one 12B vial each. All inoculated vials were incubated at 37°C.

In the first two weeks after inoculation, the 12B vials were read in the BACTEC 460 TB instrument thrice weekly. After the first two weeks, the 12B vials which remained negative (GI<10) were read once weekly for six weeks before the cultures were reported as negative.

Any time a vial read a GI of 10 or more it was considered as presumptive positive. Such vials were kept in a separate rack and read daily thereafter. When the GI reached 50-100, AFB smear (ZN) and BACTEC NAP (p-nitro- α -acetylamino-ß-hydroxy propiophenone) differentiation tests were done. Obvious turbidity, or sudden increase in GI to high levels indicated contamination. This was confirmed by Gram staining of smear as well as subculture on blood agar medium.

BACTEC NAP TB differentiation test : From the positive vial, 1 ml was transferred to the BACTEC NAP vial after homogenising the culture with a syringe. Both the NAP vial and the control vial were tested on the BACTEC instrument immediately to purge with 5 per cent CO_2. Both vials were incubated at 37°C and tested daily for the next 2-6 days. The daily GI of the NAP vial and the original vial which acted as the control were recorded. The results were interpreted as follows :

1. TB complex *(M. tuberculosis, M. bovis, M. africanum)*
   -two consecutive significant decreases in GI after inoculation in the NAP vial
   -slight, but not significant increase in the first. two days and then a decrease or no increase in GI in the NAP vial.

2. NTM (non-tuberculous mycobacteria)
   -daily GI reading increase to over 400 within 4 days in the NAP vial
   -a slight decrease or no increase in the first 1-2 days after inoculation and then two consecutive daily significant increases following day 2 in the NAP vial.

(Significant increase or decrease meant a 20% or more change from one day to the next).

Drug susceptibility tests - BACTEC method : After the NAP identification test, incubation of the control vial was continued for the original positive vial till the GI reached around 500, at which stage the drug susceptibility tests were set up. If the GI of the control vial was > 800 it was diluted 1:2 in the BACTEC dilution fluid before sensitivity testing.

The lyophilised drugs were reconstituted by addition of 5 ml of sterile distilled water to the respective drug vials and shaken till dissolved to yield stock solutions of streptomycin 240 mg/l, isoniazid 4 mg/l, rifampicin 80 mg/l and ethambutol 300 mg/l. From these, 0.1 ml was added to each of four 12 B vials to give final drug concentrations of streptomycin 6 mg/l, isoniazid 0.1 mg/l, rifampicin 2 mg/l and ethambutol 7.5 mg/l. The remaining stock solutions were stored at -70°C.

Into each of the above test vials, 0.1 ml of the culture was inoculated. For the control vial without the drug, the culture was first diluted 1:100 with BACTEC diluting fluid, and 0.1 ml of the diluted inoculum was used. All the 12B vials used in the test were pretested in the BACTEC instrument to establish 5 per cent CO_2 and also to screen out vials with high background readings. The vials were incubated and read daily for a minimum of 5 days. The difference in GI values from the previous day was designated as delta GI or dGI. Negative dGI indicated a decrease in growth while positive dGI indicated increase in growth. When the control GI reached 30 or more, the results were reported as sensitive if dGI control was more than dGI of the drug vial and as resistant if the dGI of the drug vial was more than dGI control. If the two dGIs were similar, the culture was reported as of borderline resistance.

Statistical methods: Tests of significance were calculated by using McNemar's test.

Results

Sputum samples from pulmonary tuberculosis patients : Of the 130 sputum samples processed, 71 were smear positive and 59 were reported as smear negative. Of the 71 smear positive specimens, 57 were positive by the conventional method as against 55 by the BACTEC method, 50 being positive by both methods (Table I). Three of 11 samples negative by the conventional method were positive by the BACTEC method. No specimen reported negative

Table I. Comparison of culture results of all specimens tested by BACTEC and conventional methods

Type of specimen	Conventional method	BACTEC method			Total
		Positive	Negative	NTM/CT	
Pulmonary TB, smear-positive	Positive	50	0	7	57
	Negative	3	6	2	11
	NTM/CT	2	0	1	3
	Total	55	6	10	71
Pulmonary TB, smear-negative	Positive	2	0	1	3
	Negative	6	35	10	51[+]
	NTM/CT	I	0	4	5
	Total	9	35	15	59
Extra-pulmonary TB	Positive	16	3	1	20*
	Negative	2	68	11	81
	NTM/CT	0	5	3	8
	Total	18	76	15	109
HIV/TB	Positive	4	0	1	5
	Negative	0	72	11	83
	NTM/CT	1	3	6	10
	Total	5	75	18	98
Childhood TB	Positive	9	1	0	10
	Negative	0	69	14	83
	NTM/CT	1	0	2	3
	Total	10	70	16	96

* including 3 positives observed after 5, 6 and 8 wk from selective Kirchner's liquid medium
NTM, Non-tuberculous mycobacteria; CT, contamination
[+] P = 0.04 as compared to BACTEC negative

by the BACTEC method was positive by the conventional method.

Among the smear negative sputum specimens, 3 were positive by the conventional method as against 9 by the BACTEC method, two specimens being positive by both methods. Six specimens negative by the conventional method were positive by BACTEC. Considering the negatives, 51 specimens were negative by the conventional method while only 35 were so by the BACTEC method. The above differences were statistically significant (McNemar's test, P =0.04). The BACTEC method detected more positives among the smear negative specimens, although the number of positives was small.

Considering the rate of growth by the two methods (Table II), it was seen that 45 (87%) of the 52 positives yielded a positive result by the BACTEC method at 7 days or less as against only 4 (8%) by the conventional method at 1 wk, a highly significant difference ($P < 0.001$). Similarly, 50 (96%) were positive by BACTEC method by 14 days compared with 24 (46%) at 2 wk by the conventional method.

Comparing the growth rates with the smear grades in all the 64 specimens positive by the BACTEC method (Table III), it was observed that with heavy smear positives (2+ or 3+), growth was seen within 7 days in all 21, compared with 27 of 43 with scanty or negative smears ($P<0.01$); however, all but one showed a positive result by 21 days.

Extrapulmonary specimens : The culture results of the 109 specimens processed are illustrated in Table I. It might be seen that 18 specimens were positive

Table II. Comparison of the rate of growth by the BACTEC and conventional methods among positives by both methods

Type of specimen	Weeks of growth by conventional method	Days of growth in BACTEC				Total
		1-7	8-14	15-21	>21	
Smear positive pulmonary TB	1	4				4
	2	20				20
	3	18	2			20
	4	2		1		3
	5-7	1	3	1		5
	Total	45	5	2		52
Extra pulmonary TB	3	2	1			3
	4		7			7
	5		1	2		3
	6		1			1
	7-8		1	1		2
	Total	2	11	3		16
Child-hood TB	2	1	2			3
	3		3	1	1*	5
	4			1*		1
	Total	1	5	2	1	9
HIV/TB	4	3	1			4
	Total	3	1			4

*after reprocessing

Table III. Rate of growth (in days) related to smear grades of all sputum samples positive by BACTEC

Smear grade	Days of growth				Total
	1-7	8-14	15-21	>21	
0	5	4			9
1+	22	8	3	1	34
2+	19				19
3+	2				2
Total	48	12	3	1	64

by the BACTEC method as against 20 by the conventional method.

Considering the rate of growth of positive cultures, of the 16 specimens positive by both methods, as many as 13 (81 %) were detected by 14 days by the BACTEC method as against none by the conventional method by 2 wk, the difference being highly significant ($P< 0.001$). On the other hand, a majority of specimens positive by conventional methods were obtained between 3 and 5 wk, in spite of using multiple media for isolation (Table II).

Further, of the 20 positives by the conventional method, 8 (40%) showed a growth of less than 20 colonies, while 9 showed a growth of 20-100 colonies. Three of the positives were obtained after subculture of the Kirchner's medium bottles at the end of 6 wk (results not tabulated).

Sputum specimens from HIV infected individuals : Of the 98 sputum specimens collected from HIV infected individuals, 6 were smear positive while the rest were negative. The culture results of the specimens showed 5 specimens positive by either method of which 4 were positive by both (Table I). While there was no difference in the total number of positives, the rate of growth was much greater with the BACTEC method, all 4 positives being observed

Table IV. Comparison of drug susceptibility test results by BACTEC and conventional method on cultures positive by both methods

Drugs	Conventional method	BACTEC method		Total	
		Sensitive	Resistant	No.	% agreement
Streptomycin	Sensitive	69	1	70	
	Resistant	4	3	7	94
	Both	73	4	77	
Isoniazid	Sensitive	51	4	55	
	Resistant	1	22	23	94
	Both	52	26	78	
Rifampicin	Sensitive	69	1	70	
	Resistant	0	8	8	99
	Both	69	9	78	
Ethambutol	Sensitive	68	1	69	
	Resistant	6	1	7	91
	Both	74	2	76	

at 14 days or less as against at 4 wk by the conventional method (Table 11). Further, of the 5 positives by the conventional method, 4 showed only 1-14 colonies while one specimen gave 20-100 colonies (not tabulated). Thus, although the BACTEC method did not yield any additional positives, the detection time was considerably shorter compared with the conventional method.

Childhood tuberculosis : The culture results of the 96 specimens from this category are presented in Table I. The total number of positives was identical in the two methods. Considering the rate of growth, 6 by BACTEC were positive by 14 days and 8 by 21 days, including one specimen which had to be reprocessed due to contamination. The lone specimen which took over 21 days was also reprocessed. By the conventional method, 3 of 9 specimens showed positive by 2 wk and 8 by 3 wk. The remaining positive was obtained at 4 wk (Table II).

Considering the degree of growth by the conventional method, 70 per cent of the total positives yielded less than 15 colonies while two showed 20-100 colonies. Only one specimen gave confluent growth by the conventional method (not tabulated).

BACTEC drug susceptibility tests : Out of 81 cultures positive by both BACTEC and the conventional methods, susceptibility test results were available for 76-78 strains by both methods. The results are tabulated in Table IV.

For streptomycin 72 of 77 (94%) gave identical results by the two methods, including 3 which were resistant. The remaining 5 strains yielded discrepant results.

Seventy three of 78 (94%) strains yielded identical results for isoniazid, including 22 which were resistant. Four strains resistant by the BACTEC method were classified as sensitive by the conventional method, while one strain sensitive by the BACTEC method was resistant by the conventional method.

For rifampicin 77 of 78 (99%) strains yielded identical results, including 8 resistant, thereby giving an excellent agreement. Sixty nine of 76 (91 %) gave identical results with ethambutol including one strain which was resistant, there being 7 discrepancies.

Speed of availability of drug susceptibility results Of the 78 strains tested by both methods, susceptibility test results by the BACTEC method were available for 68 (87%) strains at 5-8 days, and by 13 days in all the strains, as against 4 wk by the conventional methods (results not tabulated).

Discussion

The major advantage of the BACTEC procedure is the early availability of results. In the case of specimens from patients of pulmonary tuberculosis, it was observed that as many as 96 per cent yielded

a positive result by BACTEC by 14 days. Further, the rate of isolation of these positives was proportional to the bacterial content in the sputum. Roberts *et al* [7] had reported an average detection time of 8.3 days by BACTEC in smear positive specimens, while other workers[6] had reported a mean detection time of 13.7 days for smear negative specimens. In the case of specimens from patients of extrapulmonary TB, HIV/TB and childhood TB, the BACTEC method yielded results much earlier than that by the conventional methods.

Regarding the total number of positives detected by the two methods, there was very little difference in the number of isolates in the case of specimens from all forms of tuberculosis with the exception of smear negative pulmonary TB where the BACTEC method yielded a higher number of positives. This could perhaps be due to the incorporation of a growth promoting substance, polyoxyethylene stearate (POES) in the PANTA reconstituting fluid. This substance is claimed to enhance growth of mycobacteria which grow slowly or poorly[15]. The higher yield on BACTEC could also be attributed to a ten-fold larger inoculum used. Further, since the conventional media yielded scanty growth from extrapulmonary specimens, majority of which had less than 20 colonies, there is a greater chance of these positives being missed in untrained hands due to technical limitations. The BACTEC method is, therefore, more advantageous in these situations.

As regards drug susceptibility tests, an excellent agreement (94-99%) was obtained for streptomycin, isoniazid and rifampicin. This is in confirmity with 98.6 per cent' agreement reported by Roberts *et al* [7] and 98 per cent by Siddiqi and co-workers[16]. In the case of ethambutol, however, the agreement was slightly less, *i.e.*, 91 per cent. Roberts and co-workers[7] had also reported a lower agreement in the case of ethambutol. These differences could be due to the variations in techniques used, the proportion method being used in BACTEC as against the absolute concentration method used in the standard test, the latter being influenced by several factors including the inoculum size. In addition, the BACTEC method was much faster, with 87 per cent of the results being available within 5-8 days, compared with 4-6 wk by the conventional method. The early availability of drug susceptibility results would be very beneficial to patients harbouring drug resistant organisms to enable them to receive effective treatment with appropriate regimens.

Thus, the BACTEC TB system offers a simple automated technique with significant time saving. Introduction of automation has a potential of introducing standardisation in mycobacteriology. At present, a variety of conventional procedures that lack standardisation are in use leading to doubts about the validity of results. A standardised procedure will enable results to be compared throughout the region, especially when surveillance studies are carried out. However, as stated earlier, the only constraint with this technique as well as by PCR based diagnosis, is the cost, both initial and recurring, and the requirement of a specialised setting in a developing country like India. Although the diagnosis by the PCR is much quicker than the BACTEC method, the advantage of the latter remains in detecting the viability of the organisms. However, all constraints faced at present in utilising the recently developed techniques could be partially offset by the ease and early availability of reliable results in a region with high prevalence rates of the disease, particularly in the extrapulmonary and paucibacillary forms of tuberculosis.

Acknowledgment

The research project was supported by the Department of Biotechnology, Ministry of Science and Technology under the Indo-US Vaccine Action Programme (Principal Investigator, Dr C.N. Paramasivan and U.S. Collaborators Dr Robert C. Good. formerly Chief, Respiratory Division, Centre of Disease Control. Atlanta, Georgia, USA and Dr Patricia Charache, Professor of Pathology, Medicine and Oncology, Director, Performance Improvement Programmes, Department of Pathology, The Johns Hopkins Hospital, Baltimore, Maryland, USA). The technical assistance of Shri L. Prabhakaran, Smt. Sulochana Somasundaram and Smt. Gomathi Sekar is gratefully acknowledged. We also wish to thank the Director of this Centre, the Clinical Staff and the field staff of the Epidemiology unit of this Centre for providing the specimens, the Bacteriology department staff for undertaking the conventional tests and Smt. Jothi Segaran for secretarial assistance.

References

1. Paramasivan CN, Vanaja Kumar, Alexander C, Venkatesan P, Somasundaram PR, Prabhakar R. Use of multiple media for the cultivation of mycobacteria from specimens other than sputum. *Indian J Med Res* 1987; *86 :* 290-4.

2. Casal M. Laboratory approaches to mycobacterial susceptibility to antibiotics (Review). *Rev Esp Quimioterap* 1995; *8* : 184-9.

3. Deland FH, Wagner Jr HN. Early detection ofbacterial growth with carbon-14 labelled glucose. *Radiology* 1969; *92* : 154-5.

4. Cummings DM, Ristroph D, Camargo EE, Larson SM, Wagner Jr HN. Radiometric detection of the metabolic activity of *Mycobacterium tuberculosis. J Nucl Med* 1975; *16 :* 1189-91.

5. Middlebrook G, Reggiardo Z, Tigertt WD. Automatable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. *Am Rev Respir Dis* 1977; *115* : 1066-9.

6. Morgan MA, Horstmeier CD, De Young DR, Roberts GD. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. *J Clin Microbiol* 1983; *18* : 384-8.

7. Roberts GD, Goodman NL, Heifets L, Larsh HW, Linder TH, McClatchy JK, *et al.* Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear positive specimens. *J Clin Microbiol* 1983; *18* : 689-96.

8. Snider DE, God RC, Kilbum JO, Laskowski Jr LF, Lusk RH, Marr JJ, *et al.* Rapid susceptibility testing of *Mycobacterium tuberculosis. Am Rev Respir Dis* 1981; *123* : 402-6.

9. Petroff SA. A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. *J Exp Med* 1915; *21* : 38-42.

10. Tuberculosis Research Centre, Madras. Study of Chemotherapy regimens of 5 and 7 months duration and the role of corticosteroids in the treatment of sputum-positive patients with pulmonary tuberculosis in south India. *Tubercle* 1983; *64* : 73-91.

11. Tuberculosis Research Centre, Madras. Ethambutol plus isoniazid for the treatment of pulmonary tuberculosis - a controlled trail of four regimens. *Tubercle* 1981; *62* : 13-29.

12. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, *et al.* Advances in techniques of testing mycobacterial drug sensitivity and the use of sensitivity tests in tuberculosis control programmes. *Bull World Health Organ* 1969; *41* : 21-43.

13. Allen B, Baker RJ. In *: Mycobacteria : isolation, identification and sensitivity testing.* London : Butterworth, 1968 : 17.

14. Kubica GP. Differential identification of mycobacteria. VII : Key features for identification of clinically significant mycobacteria. *Am Rev Respir Dis* 1973; *107* : 9-21.

15. Siddiqi SH. *BACTEC TB system - product and procedure manual.* Becton Dickinson Diagnostic Instruments System, Sparks, Maryland, USA, 1989.

16. Siddiqi SH, Libonati JP, Middlebrook G. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis. J Clin Microbiol* 1981; *13* : 908-12.

Reprint requests : Dr P.R. Narayanan, Director, Tuberculosis Research Centre
Mayor V.R. Ramanathan Road, Chetput, Chennai 600031