INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Official publication of Indian Association of Medical Microbiologists,
Published quarterly in January, April, July and October)
Indexed in Index Medicus/MEDLINE/PubMed, 'Elsevier Science - EMBASE', 'IndMED'

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The journal is printed on acid free paper.

INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

(Publication of Indian Association of Medical Microbiologists)

ISSN 0255-0857 **Volume 25** **Number 4** **October-December, 2007**

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Indian Journal of Medical Microbiology, (2007) 25(4): 383-6

Original Article

A COMPARATIVE STUDY FOR THE DETECTION OF MYCOBACTERIA BY BACTEC MGIT 960, LOWENSTEIN JENSEN MEDIA AND DIRECT AFB SMEAR EXAMINATION

S Rishi, P Sinha, *B Malhotra, N Pal

Abstract

Purpose: To compare BACTEC MGIT 960 (M960) with conventional culture on Lowenstein Jensen (LJ) media and direct acid fast bacilli (AFB) smear examination for the detection of *Mycobacteria* in clinical samples obtained from suspected cases of pulmonary and extra pulmonary tuberculosis (TB). **Methods:** A total of 500 samples were processed for direct AFB smear examination, and culture on M960 and LJ media. **Results:** Two hundred fifty-eight out of 500 (51.6%) isolates of *Mycobacteria* were obtained by combined use of the two culture methods. Two hundred and fifty-three (50.6%) were positive in culture by M960 and LJ media and 28% (140/500) by direct AFB smear examination. The positivity rate of M960 system alone was 34.10% (88/258) and of LJ alone was 1.93% (5/258). Average time to detect growth (TTD) was 9.66 days by M960 and 28.81 days by LJ. **Conclusions:** M960 system is a rapid and sensitive method for early diagnosis of pulmonary and extrapulmonary TB. But for maximum recovery of *Mycobacteria*, a combination of both M960 and LJ media should be used.

Key words: *BACTEC MGIT 960, Lowenstein Jensen media*

Tuberculosis (TB) is responsible for about one third of preventable deaths worldwide.[1] The spread of HIV/AIDS and emergence of multiple drug-resistant TB have further contributed to the worsening impact of the disease.[2] In India, 13 million people are infected and 3.5 million are positive for acid fast bacilli (AFB) with 2.2 million new TB cases being added every year.[3]

Although AFB microscopy and conventional Lowenstein Jensen (LJ) culture remain the cornerstone for the diagnosis of TB, the sensitivity of these traditional methods is quite low, especially in the samples containing small number of organisms.[4] There is a need for rapid, sensitive and accurate detection of these organisms in clinical specimens to hasten the administration of appropriate antimycobacterial therapy and prevent the spread of infection in the community.[5] A variety of manual and automated systems have been developed specifically to reduce the time to detect and identify *Mycobacteria* in clinical specimens.[5]

The present study was carried out to compare one such automated system, i.e., BACTEC MGIT 960 (M960) with conventional culture method, i.e., LJ media and direct AFB smear examination. M960 system is a fully automated, high capacity, non-radiometric, non-invasive instrument, which requires neither needles nor other sharp implements to incubate and monitor 960; 7 mL culture tubes.[6] The culture tubes contain Modified middle brook 7H9 media with fluorescent growth indicator embedded in silicone on the bottom of each tube. This compound is sensitive to the presence of dissolved oxygen in the broth. As the microorganisms grow in the media, oxygen gets depleted, allowing the fluorescence to be detected automatically over time.[5]

Materials and Methods

A total of 500 clinical samples were obtained from suspected cases of TB from different wards and out patient departments of the SMS and other allied hospitals of SMS Medical College, Jaipur, during the year 2004. The samples included sputum (330), cerebrospinal fluid (49), pleural fluid (41), bronchial washings (29), pus (17), urine (8), ascitic fluid (7), endometrium (5), ovarian cyst fluid (4), gastric aspirate (2), lymph node aspirate (2), bone scrapings (1), discharge from sinus (1), granulation tissue (1), menstrual blood (1), stool (1), and tissue from sinus tract (1).

A BBL MGIT tube (from Becton Dickinson) containing 7 mL modified middle brook 7H9 broth was used. Lyophilized MGIT PANTA (containing polymyxin B, azlocillin, nalidixic acid, trimethoprim, amphotericin B) was reconstituted with MGIT growth supplement (containing oleic acid, albumin, dextrose, catalase, polyoxyethylene stearate), and 0.8 mL of this was added prior to sample inoculation to the M960 tube. LJ media was prepared in the laboratory.

Processing of samples

Within 24 h of receiving the samples, direct smear was made, stained by Ziehl Neelsen method and graded as per RNTCP guidelines. For the purpose of digestion and

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Received: 06-05-06
Accepted: 16-06-07

decontamination, a maximum of 10 mL sample was taken in a falcon tube. An equal amount of a mixture containing 50 mL of 4% sodium hydroxide, 50 mL of 2.9% sodium citrate and 0.5% *N*-acetyl cysteine was added to it. The falcon tube was vortexed well and then kept at 37 ℃ for 20 min. After that, neutralization was done by filling the tube up to 50 mL mark with sterile phosphate buffered saline (pH 6.8). The tube was centrifuged for 20 min at 3000 × *g*. Supernatant was discarded and the pellet was reconstituted with 1 mL of sterile phosphate buffer, 0.5 mL of this was added to the M960 tube and two drops on the LJ media. Samples from sterile sites were inoculated as such or after centrifugation, without digestion and decontamination. The inoculated media were incubated at 37 ℃, MGIT tubes were incubated inside the M960 instrument for 6 weeks and LJ media were incubated for 8 weeks and examined every week. M960 instrument detects the growth automatically; flashing red light to indicate instrument positive tubes and green for negative ones. Smears were made from M960-positive culture tubes as well as from M960 negative tubes that had some deposit in them, to confirm the presence or absence of *Mycobacteria*, as per the protocol of Becton Dickinson.

Results

Out of 500 samples processed, 50.6% (253/500) were positive by M960, 33.6% (165/500) by LJ media and 28% (140/500) by direct AFB smear examination. Total 51.6% (258/500) isolates of *Mycobacteria* were obtained by the combined use of these two culture methods. M960 was found to be positive in 98.06% (253/258) cases with average time to detect growth (TTD) around 9.66 days (2-39 days) and that of LJ 63.95% (165/258) with average TTD of 28.81 days (7-48 days). The positivity of direct AFB smear examination was found to be only 54.26% (140/258).

The positivity rate of M960 system alone was 34.10% (88/258) and of LJ alone 1.93% (5/258). Isolation rates of *Mycobacteria* in pulmonary and extrapulmonary samples were 61.83% (222/359) and 21.98% (31/141) by M960, 44.01% (158/359) and 4.96% (7/141) by LJ, respectively. One hundred and forty-two samples were from patients on anti-tubercular therapy. Out of these, 98.59% (140/142) were positive by M960, 69.01% (98/142) by LJ and 69.71% (99/142) by direct AFB smear examination. Details of isolation rates of *Mycobacteria* by M960 and LJ with respect to direct AFB smear results are shown in Table 1.

M960 instrument gave false-negative results for 5/258 (1.93%) samples. Out of these false negatives, two were tube positive but instrument negative. This was observed when smear was made from the deposit present in MGIT tube that had been declared negative by M960 instrument.

In our study, the break through contamination rate was found to be 13.4% (67/500) by M960 and 27.2% (136/500) by LJ. The break through contamination rate is defined as the detection of any instrument positive tube that is AFB smear negative and subculture negative yet Gram stain smear positive for other non acid fast microorganism.[6]

Discussion

The present study demonstrated that M960 system provided better isolation rate of *Mycobacteria* (98.06%) from a variety of clinical samples than the LJ media (63.95%). Various authors have reported similar findings ranging from 80 to 100% for M960 and from 59.7 to 87.2% for LJ.[5-7] In our study, isolation rate by M960 system was 34.11% more than that by LJ method. The additional positivity was seen both in pulmonary and extrapulmonary samples, not only in smear negative but in smear positive samples also. There was a low positivity rate shown by LJ method in our study in comparison to around 69-87.2% reported in literature.[6,7] This could be because of the fact that samples that were grossly contaminated on LJ were considered negative, whereas in M960, since the smears were made from all instrument positive MGIT tubes, it was found that there were samples, which had both contaminants, as well as *Mycobacteria* grown in them. Such tubes were considered positive by M960.

Besides higher isolation rate, even the time to detect *Mycobacteria* was shorter on M960 than on LJ, average being 9.66 days (2-39) with M960 and 28.81 days (7-48) with LJ media. Similar findings have been reported in the literature.[8-13] However in our study, time to detect *Mycobacteria* by M960 was lesser than that reported by others (11.6-14.4 days).[6,7,8,10,11,13] This is probably because large number of samples (28%) were smear positive and incidence of TB is also higher in our country.

Time to detect *Mycobacteria* was directly related to the grade of positivity in direct AFB smear examination as seen in Table 2. In smear positive samples, growth of *Mycobacteria* was detected in average 6.79 days (2-30) by M960.

Table 1: Isolation rate of *Mycobacteria* in relation to acid fast bacilli smear

Type of sample	ZN smear	LJ positive	%	Avg. TTD (in days)	M960 positive	%	Avg. TTD (in days)
Pulmonary	Positive (137)	116/137	84.67	27.09 (7-48)	135/137	98.54	6.79 (2-30)
samples	Negative (222)	42/222	18.91	30.02 (14-48)	87/222	39.18	13.35 (3-32)
Extra pulmonary	Positive (3)	2/3	66.66	28 (28)	3/3	100	8.33 (4-14)
samples	Negative (138)	5/13 8	3.62	32.5 (7-48)	28/138	20.28	17.89 (4-39)

Avg. TTD - average time to detection of growth, Z.N - Ziehl Neelsen, LJ - Lowenstein Jensen media

Table 2: Sample wise details of culture positivity

Samples	AFB smear	No. of samples	M960+	TTD (range in days)	LJ+	TTD (range in days)
Extra pulmonary						
Ascitic fluid	Neg	7	2	15.5 (14-17)	0	
Bone scrapings	Neg	1	1	9	0	
CSF	Neg	49	9	20.77 (12-39)	2	40 (32-48)
Discharge from sinus	Neg	1	0		0	
Endometrium	Neg	5	0		0	
Gastric aspirate	Neg	2	1	15	0	
Granulation tissue	Neg	1	0		0	
Lymph node aspirate	+	1	1	4	1	28
	Neg	1	1	18	0	
Menstrual blood	Neg	1	0		0	
Ovarian cyst fluid	Neg	4	0		0	
Pleural fluid	+	1	1	7	0	
	Neg	40	10	13.5 (4-25)	3	25 (7-47)
Pus	+	1	1	14	1	28
	Neg	16	3	14.6 (10-20)	0	
Stool	Neg	1	0		0	
Sinus tract tissue	Neg	1	0		0	
Urine	Neg	8	1	39	0	
Pulmonary						
Bronchial wash	+	5	5	13.0 (4-30)	3	25.7 (21-28)
	Neg	24	6	15.16 (4-21)	1	28
Sputum	3+	25	23	4.84 (2-13)	23	23.7 (7-35)
	2+	46	46	6.30 (3-14)	42	27.3 (14-48)
	1+	44	44	7.27 (3-24)	38	27.8 (7-48)
	Scanty	17	17	8.52 (2-19)	10	31.1 (21-42)
	Neg	198	81	11.54 (3-32)	41	32 (21-48)
Total		**500**	**253**	**9.66 (2-39)**	**165**	**28.81 (7-48)**

The break through contamination rates in our study (13.4% for M960 and 27.2% for LJ) were higher in comparison to other studies (3.7-10% by M960 and 1.2-21.1% by LJ).[6,8,11,12] This may be attributed firstly to the hot climatic conditions in our country and secondly to the longer transport time of the specimen to the laboratory in some cases, leading to overgrowth of the contaminants.

Thus, M960 was found to be most rapid and efficient system to isolate *Mycobacteria*. However, for maximum recovery of *Mycobacteria*, it is important to use both types of media as 1.93% isolates could be detected by LJ only.

As *Mycobacteria* can be isolated on an average within a week in smear positive samples, the antibiotic susceptibility tests can then be done and results would be available in another 4-13 days (as per BD protocol). This is in contrast to the time taken by LJ method, average 29 days for growth and another 30 days for sensitivity tests. This has great implications in monitoring the treatment failure and multidrug resistant TB patients. Though the cost of M960 culture system is approximately 8-10 times more than that for LJ media, the sensitivity and rapidity of the system are the major advantages that benefit not only the individual patient but also the community by controlling the disease and ultimately its transmission in the society.

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Source of Support: Nil, **Conflict of Interest:** None declared.