

RAPID ISOLATION OF *MYCOBACTERIA*—NEED OF THE HOUR IN OUR SETTINGS

Abdul Hannan, Saadia Chaudhary, Sidrah Saleem, Atika Qayyum*, Muhammad Usman Arshad

Department of Microbiology, University of Health Sciences Lahore, *Microbiologist of Gulab Devi Chest Hospital, Lahore, Pakistan

Background: To compare Bactec MGIT 960 with LJ media in terms of time taken for the initial isolation of *mycobacteria*. **Methods:** A total of 100 AFB (acid fast bacillus) positive sputum samples were processed and inoculated in both the Lowenstein Jensen (LJ) media and mycobacterium growth indicator tube (MGIT) tubes. **Results:** Of the 100 samples, positive growth was obtained from all the samples on both the MGIT and LJ media. In MGIT 53% samples grew in 4 days, 30% in 5 days and 17% in 6 days (Mean=4.6 days) while on LJ media, 44% grew in 30 days, 20% in 35 days and 36% in 44 days (Mean=37 days). Significant difference was observed between two systems with a *p*-value of less than 0.05. **Conclusion:** Bactec MGIT 960 is a much faster and efficient system for the initial isolation of *mycobacteria*.

Keywords: *Mycobacteria*, MGIT, LJ

INTRODUCTION

The increasing incidence of tuberculosis (TB) has made it crucial for the laboratories to use new rapid diagnostic methods to detect *mycobacteria*.^{1,2} Demonstration of acid-fast bacilli (AFB) in a smear made from a clinical specimen provides a preliminary diagnosis of mycobacterial disease, while the isolation of *mycobacteria* on culture provides a definite diagnosis of tuberculosis.³ When conventional culture medium like Lowenstein Jensen is used, several weeks of incubation (4-6 weeks) is required for the isolation of mycobacterium. This results in a delay of both the diagnosis and start of proper treatment.⁴⁻⁶ Although BACTEC MGIT 960 is also a conventional system but it is a fully automated, high capacity, non radiometric, non-invasive instrument fluorescence based system which simultaneously incubates and monitors nine hundred and sixty (960) culture tubes. Besides 7ml of Middlebrook 7H9 liquid media, the MGIT tube has an oxygen-quenched fluorochrome, tris 4, 7-diphenyl-1, 10-phenanthroline ruthenium chloride pentahydrate, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. This depletes the free oxygen, the fluorochrome remains uninhibited, resulting in fluorescence within the MGIT tube when visualized by photo detector inside the station. The detection of growth can also be visually observed by the presence of a non-homogeneous light turbidity or small granular/flaky appearance in the medium.⁷⁻¹¹ In this study we evaluated the performance of Bactec MGIT 960 in terms of rapidity to obtain the initial isolation of *Mycobacteria* and compared it with conventional LJ media.

MATERIAL AND METHODS

Study was done at Department of Microbiology, University of Health Sciences, Lahore. One hundred acid fast bacteria (AFB) positive sputum samples

received from Gulab Devi Chest Hospital Lahore were included in the study.

All specimens were digested and decontaminated by the sodium hydroxide and N-acetyl-L-cysteine (NaOH/NALC) method, with final concentrations of 1% for NaOH and 0.25% for NALC. NaOH-NALC-sodium citrate solution was added in a volume equal to the quantity of specimen in 50 ml falcon tubes (BD). Tubes were vortexed lightly for about 15-30 seconds and allowed to stand for 15–20 minutes. When specimens were completely liquefied phosphate buffer (pH 6.8) was added up to the top ring in the centrifuge tube and was mixed well. Specimens were centrifuged at the speed of 3000 g for 15–20 minutes. After centrifugation, tubes were allowed to set for 5 minutes to allow aerosols to settle down. Then the supernatant was carefully decanted into a container containing a mycobactericidal disinfectant. It was made sure that sediment was not lost during decanting of the supernatant fluid. A small quantity (1–2 ml) of phosphate buffer (pH 6.8) was added into the tube to re-suspend the sediment with the help of a pipette. The re-suspended pellet was used for making smears and for inoculation of culture media.

Onto the surface of an LJ slant, 0.1 to 0.25 ml of each processed specimen was inoculated and was incubated for 4–8 weeks and was examined every week for any visible growth. Prepared tubes of LJ media were obtained from Becton Dickinson (BD USA).

The BACTEC MGIT 960 culture tube contains 7 ml of Middlebrook 7H9 broth base, to which was added an enrichment supplement containing oleic acid, albumin, dextrose, and catalase (BBL MGIT OADC) and an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (BBL MGIT PANTA). After addition of all the enrichments, 0.5 ml of the processed specimen was added.

The tubes were incubated at 37 °C in the BACTEC MGIT 960 and the instrument monitored automatically every 40 min for increase of fluorescence corresponding to any bacterial growth. Any sample which was identified as positive was removed from the instrument, and a ZN smear was prepared and examined for AFB. Final Identification of *Mycobacterium tuberculosis* was done by incorporating paranitrobenzoic acid (PNB) at concentration of 500 µg/ml in the MGIT tubes. Two tubes were labelled, one as GC (growth control without PNB) and other with PNB. The growth of MTB was inhibited at this concentration. Time for detection of mycobacteria was based on the date of the earliest instrument positivity, which correlated with AFB smear positivity. All the procedures were performed inside biological safety cabinet. In house control strains of mycobacteria were used as quality control.

RESULTS

Of the 100 samples, positive growth was obtained from all the samples on both the MGIT and LJ media. Four specimens were found to be contaminated. They were decontaminated again with NALC-NaOH method as discussed above.

In MGIT, 53% samples were grown in 4 days 30% in 5 days and 17% were grown in 6 days (Mean= 4.64±0.76 days). On LJ media, 44% turned out to be positive in 30 days, 20% in 35 days and 36% in 44 days (Mean 36.04±6.25 days). Significant difference between time of isolation of mycobacteria in LJ media and MGIT 960 was observed with a p-value of less than 0.05.

Table 1: Percentage Comparison of time period for mycobacterial growth in MGIT 960 and LJ medium for 100 AFB positive specimens

Time for positive indication (days)	BACTEC MGIT 960 (Mean 4.64±0.76)	LJ medium (Mean 36.04±6.25)
4	53%	0%
5	30%	0%
6	17%	0%
30	--	44%
35	--	20%
44	--	36%

DISCUSSION

The time factor in diagnosing tuberculosis is a matter of great importance. The start of an early and appropriate treatment depends upon the early isolation of mycobacteria because only that will result in further placing drug susceptibility testing and obtaining rapid results. In developing countries like Pakistan, diagnosis of tuberculosis usually depends only on the staining, x-ray and clinical findings. The use of conventional methods like LJ provides only the initial isolation in 4 to 6 weeks and takes another 4 weeks for

susceptibility testing. It not only delays the diagnosis but also results in improper treatment during all the time period. The use of rapid diagnostic methods for the diagnosis of TB is the need of the hour and Bactec MGIT 960 is one such method. In the present study we evaluated the use of Bactec MGIT 960 in our circumstances and compared it with conventional LJ in terms of time taken for the initial isolation of mycobacteria. This is again a time saving factor that the specimens which were contaminated were again turned out to be positive within 4 to 6 days. We obtained excellent results concerning time taken for the initial isolation. A number of studies have been done in the world to evaluate the efficiency of Bactec MGIT and our results exactly tallies in terms of its rapidity as compared to conventional solid media.⁷⁻¹¹

In the diagnosis of tuberculosis time is money. We should promote the use of rapid diagnostic methods and should discourage the use of ATT without performing the culture. The amounts of labour required for proficient operation are a significant consideration in the selection of any microbiology system. The BACTEC MGIT 960 system has very good performance characteristics, is easy to use, and readily fits into the routine mycobacteriology laboratory work. In large-volume laboratories, the 960-culture tube capacity provides a decided advantage over the continuously monitored instruments of lesser capacity.

CONCLUSION

Bactec MGIT 960 is a much faster and efficient system for the initial isolation of *Mycobacteria*.

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Address for Correspondence:

Dr. Sidrah Saleem, Research and Teaching fellow (Microbiology), University of Health Sciences Lahore- Pakistan

Email: sidu_78@yahoo.com