Original Article

Utility of the BACTEC MGIT 960 TB system for recovery of mycobacteria

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ABSTRACT

Background and Objectives: Pulmonary tuberculosis (PTB) remains a public health issue in Nigeria. The rapid diagnosis of PTB is essential for the early initiation of treatment and management of patients. The utility of the BACTEC MGIT 960 TB system was evaluated and compared with the Lowenstein Jensen (LJ) culture method for the recovery of Mycobacteria from sputum samples. **Methods:** A total of 2400 sputum samples submitted to the South East TB Zonal Reference Laboratory, Amachara Umuahia, Nigeria were tested. Samples were decontaminated using the standard N-Acetyl-L-Cysteine Sodium Hydroxide method and concentrated before processing. The processed samples were inoculated into both MGIT 960 tubes and LJ medium and incubated accordingly. **Results:** From all sputum samples, Mycobacteria were recovered from 201 (8.4%) sputum samples by the MGIT 960 system and 175 (7.3%) by LJ culture (p=0.014). The sensitivity for MGIT and LJ culture for mycobacteria were 95.0% and 80.1%, respectively. Among the 201 MGIT-positive cultures, 127 (63.2%) were identified as *Mycobacterium tuberculosis* complex and 74 (36.8%) as Mycobacteria other than tuberculosis (MOTT). The recovery rate of MTBC from LJ-positive samples was 84.0% and MOTT 16.0%. MGIT 960 identified more MOTT than LJ culture (p=0.045). The contamination rate associated with MGIT and LJ culture was 4.1% and 2.5%, respectively (p=0.037). The time to detection of mycobacteria in MGIT 960 and LJ was 14.8 days and 33.2 days, respectively. **Conclusion**: MGIT 960 has good diagnostic accuracy. It provided a more rapid and higher recovery of all mycobacteria than the LJ culture.

Key words: BACTEC MGIT 960, Comparison, Lowenstein Jensen, *Mycobacterium tuberculosis*

Pulmonary tuberculosis (PTB) is a major public health problem with significant morbidity and mortality [1]. Rapid and accurate diagnosis of active PTB, especially in developing countries, is a major challenge to global control of the disease [2,3].

Most developing countries rely on the conventional Lowenstein–Jensen's (LJ) culture and microscopy (Ziehl–Neelsen [ZN] method) for the detection of *Mycobacterium tuberculosis*. The LJ culture has a long duration to detection and ZN has low sensitivity [4].

We, therefore, evaluated the accuracy of the MGIT 960 TB system for the recovery of mycobacteria and compared it with the conventional LJ culture to ascertain its utility.

MATERIALS AND METHODS

Settings

This prospective study was conducted at the South East Zonal TB Reference Laboratory of Amachara Specialist Hospital, Umuahia, Abia State, Nigeria between September 2020 and August 2021. The Reference Laboratory receives samples from all five South-Eastern States TB Control Program for culture and drug susceptibility testing. The sputum samples were from presumptive TB patients who had been tested using Microscopy (ZN Methods).

Study Population

A total of 2400 sputum samples were enrolled for the study. The age and sex of the patients were omitted. We excluded all samples for Line Probe Assay and Drug Susceptibility testing. All samples for LJ culture were included in the study.

Decontamination and Processing of Samples

All samples were decontaminated by the standard N-acetyl-L-cysteine (NALC) – Sodium hydroxide (NaOH) method and concentrated before processing [5]. Each sample was diluted

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with an equal volume of 4% NaOH and 0.5 mL NALC and mixed properly. It was then centrifuged at 3000 rpm for 15 min. The sediment was suspended in 1 mL of sterile phosphate-buffered saline, pH 6.8, and used for further analysis. Exactly, 0.5 mL of the processed sample was inoculated into the MGIT 960 tube and three drops into the LJ medium. Two drops of the processed sample were placed on a slide to make a smear, stained by the ZN method for acid–fast bacilli (AFB).

Detection of Mycobacteria on MGIT 960 TB System

Before inoculation of samples into a 7 mL MGIT tube, the MGIT PANTA antibiotic mixture was reconstituted with 15 mL MGIT growth supplement and mixed to complete dissolution, giving a clear solution. To the 7 mL MGIT tube, 0.8 mL of this enrichment was added followed by 0.5 mL of the processed sample, and the tube was recapped immediately. The contents were mixed by inverting the tube 3 times. All inoculated MGIT tubes were incubated at 37°C in the BACTEC MGIT 960 instrument after scanning the bar code. The tubes were incubated until they were flagged positive by the instrument or for the duration of the recommended 42 days [6]. Tubes signaled positive for growth were removed from the instrument and observed visually under the light. Mycobacterial growth appears grandular in shape, settling at the bottom of the tube while contaminating bacterial growth appears as uniform turbidity in the entire tube.

All MGIT 960 positive tubes were stained by the ZN method for AFB and sub-cultured on Blood agar.

Identification of *M. tuberculosis* Complex (MBTC) by MGIT 960 Using SD Bioline TB Antigen MPT64 RAPID Test

The MPT64 protein detection-based immune-chromatographic test (SD Bioline Kit, Standard Diagnostic Inc, Korea) is a specific antigen that differentiates MTBC from the Mycobacteria other than the tuberculosis (MOTT) group [7,8]. It was performed on AFB-positive MGIT cultures as described by the manufacturer [7]. Results were recorded accordingly.

Isolation and Identification of *M. tuberculosis* by LJ Culture Method

Exactly, two drops of the processed sample were inoculated onto a properly labeled LJ slant, using a sterile pipette. The inoculated slants were incubated at 37°C for up to 8 weeks in a vertical position. The LJ slants were observed daily for the 1st week and thereafter once a week for 8 weeks for the visible appearance of colonies. Contaminated cultures and LJ media which liquefied or turned a dark green were discarded [5].

The slants which showed growth within 1 week of incubation were regarded as rapid growers, that is, MOTT. Colonies developing post 2–6 weeks were considered slow-growing and termed the MBTC. Colonial morphology was also used in characterization [5,9].

Ethical Approval and Patient Consent

Ethical approval was obtained from the Health and Ethics Committee of the Amachara Specialist Hospital, Umuahia, Abia State, Nigeria (Approval number: ASHU/020/0310).

Statistical Analysis

Data generated were analyzed using descriptive statistics and categorical variables expressed in percentages. Differences in proportions were compared using Chi-square. Level of significance was set at $p<0.05$(stating actual value). The sensitivity of each method was calculated.

RESULTS

From all sputum samples, Mycobacteria were found in 201 (8.4%) samples by the MGIT 960 system and 175 (7.3%) by LJ culture (p=0.014). The sensitivity for MGIT 960 system and LJ culture for mycobacteria was 95.0% and 80.18%, respectively. The contamination rate associated with MGIT 960 was significantly higher than LJ culture [4.1% vs. 2.5%, (p=0.037)] (Table 1).

Among the 201 mycobacteria grown by MGIT 960 system, 127 (63.2%) were confirmed by SD Bioline MPT 64 RAPID test as MTBC, and 74 (36.8%) were identified as MOTT. Similarly, of the 174 mycobacteria grown by LJ culture, 147 (84.0%) were identified as MTBC and 28 (16.0%) as MOTT (Table 1). MGIT 960 identified more MOTT than the LJ culture (p=0.045). The time to detection of mycobacteria was 14.8 days in the MGIT 960 and 33.2 days in the LJ culture.

DISCUSSION

Rapid diagnosis of PTB is essential for the treatment, prevention, and control of the disease. The capacity of laboratories to promptly identify cases of *M. tuberculosis* from clinical samples is vital in the management of PTB patients [10,11].

The liquid culture of mycobacteria is more sensitive than smear microscopy and more rapid than classical solid methods [12].

In our study, the sensitivity of the MGIT 960 TB System for mycobacteria was 95.0% compared to 80.1% by LJ culture.

Table 1: Recovery of mycobacteria by MGIT 960 and LJ culture, and identification of MTBC and MOTT from positive MGIT 960 samples and LJ culture

Variable	MGIT 960*	LJ Culture**	p-value
Number of positive samples	201 (8.4)	175 (7.3)	0.014
MTBC	127 (63.2)	147 (84.0)	0.043
MOTT	74 (36.8)	28 (16.0)	0.045
Number of negative samples	2101 (87.5)	2164 (90.2)	0.029
Number of contaminated samples	98 (4.1)	61 (2.5)	0.037

LJ: Lowenstein–Jensen, MTBC: *Mycobacterium tuberculosis complex*, MOTT: Mycobacteria other than tuberculosis, *Sensitivity of MGIT 960=95.0%. **Sensitivity of LJ Culture=80.1%

A study conducted in India showed increased recovery of mycobacteria by MGIT 960 (94.1%) compared to LJ (89.0%) [13]. Several other studies had similarly reported higher recovery rates of mycobacteria ranging from 80% to 100% for MGIT 960 and 44–93% LJ culture [6,12,14].

Exactly, 63.2% of all MGIT 960 positive mycobacteria were identified as MBTC in our study. The LJ culture identified 84% as MTBC. The difference (20.8%) could be attributed to contamination as MGIT 960 medium is rich in proteins.

It was observed that MGIT 960 system detected MOTT (36.8%) more rapidly than the LJ (16.0%). This may be because MOTT survived the decontamination process [15]. In their study in Bangladesh, the MGIT 960 method similarly detected non-tubercular mycobacteria (91.7%) more effectively than the LJ culture [14].

Out of the 2400 sputum samples analyzed, the contamination rate was higher (4.1%) in the MGIT 960 method compared to 2.1% in LJ. This is consistent with the previous studies. Somoskovi *et al*. [16] showed a higher contamination rate with MGIT 960 (9.5%) than with LJ culture (1.3%). Earlier studies reported a higher contamination rate with MGIT 960 [16,17]. In contrast to our findings, a lower contamination rate was reported with MGIT 960 than with LJ [18]. MGIT 960 liquid culture requires careful processing and handling of samples.

The median time to detection of mycobacteria was found to be 13.8 days with MGIT 960 and 33.2 days with LJ method. There was a significantly shorter time with MGIT 960. This is in line with 18.2 days with MGIT 960 and 32.5 days with LJ previously observed [12,19,20].

CONCLUSION

MGIT 960 TB system is a utility method. It provides a more rapid and higher recovery of all mycobacterial (MTBC and MOTT) than LJ culture. It could be used as the initial test for the diagnosis of PTB.

AUTHORS' CONTRIBUTIONS

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; and gave final approval of the version to be published.

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