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Original Article

Evaluation of manual Mycobacterium growth indicator tube for isolation and susceptibility testing of *Mycobacterium tuberculosis* for implementation in low and medium volume laboratories

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ABSTRACT

Background: Manual Mycobacterium growth indicator tube (MGIT) was evaluated for isolation and drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) for its implementation in laboratories with low and medium volume.

Methods: 1018 consecutive clinical specimens were processed using manual MGIT and conventional Lowenstein–Jensen (LJ) culture. Results obtained for culture positivity were analyzed taking combined reference of positivity by either solid or liquid culture. All positive cultures were identified and DST to first line drugs was performed by manual MGIT and 1% proportional method on LJ media. Performance of manual MGIT for DST was compared to conventional DST on LJ media.

Result: Of the total 220 culture positive samples 93.9% were isolated in MGIT while 75.7% in LJ taking combined reference of positivity by either solid or liquid culture. Turn around time for isolation of MTB was significantly less for MGIT as compared to LJ. There was good agreement between manual MGIT and 1% proportional method on LJ media for DST to first line drugs. Turnaround time from inoculation to DST results for smear positive and smear negative cases using manual MGIT was 20.2 and 30.1 days respectively. The total cost for isolation, identification and DST in manual MGIT for smear positive and smear negative cases was INR 2350 and INR 2700 respectively.

Conclusion: It is feasible to implement manual MGIT in low to medium volume laboratory that already has experience with culture provided adequate biosafety measures and appropriate training of laboratory staff are taken care of.

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Introduction

Tuberculosis (TB) remains the leading infectious disease in developing countries. India has the highest burden of TB in the world with an estimated 2.2 million incident cases out of a global incidence of 9.6 million in the year 2014. The problem is further complicated by emergence of drug resistant strains. Worldwide, 3.3% of newly diagnosed TB cases and 20% of previously treated TB cases are estimated to have multidrug-resistant TB (MDR-TB).[1] Laboratory confirmation of TB and drug susceptibility testing (DST) is essential to ensure that patients are correctly diagnosed and appropriately treated.

Smear microscopy for acid-fast bacilli (AFB) is the most commonly used diagnostic test for TB in developing countries. The test, although rapid, cheap and easy to perform, lacks sensitivity, cannot distinguish viable from nonviable bacteria and does not provide any information on drug resistance. Culture of *Mycobacterium tuberculosis* (MTB) remains the gold standard for both diagnosis and DST. Conventional culture on solid media like Lowenstein–Jensen (LJ) medium, while cheap and simple, have the major disadvantage of being very slow requiring 20–56 days for diagnosis and further 4–6 weeks for DST. Therefore, solid culture results often have limited or no impact on patient management.[2]

Culture in liquid media (e.g. 7H9 Middlebrook media) is more sensitive and faster than conventional solid media. In 2007, the WHO endorsed use of liquid culture technology[3] but due to high cost and complexity of commercial automated liquid culture systems its use is limited to few referral laboratories in developing countries. Mycobacteria growth indicator tube – MGIT (Becton-Dickinson, Sparks, MD) commercially available with 4 ml of 7H9 Middlebrook broth base and a fluorescent sensor is intended for the manual isolation and DST of MTB. Few studies done worldwide have found manual MGIT to have comparable results as that of automated liquid culture systems for primary isolation and DST of MTB.[4–6] The method earlier being used to detect fluorescence in manual MGIT tube was transillumination with a 365 nm UV light, e.g. a Wood's lamp. This was quite cumbersome and is now replaced by a handheld fluorescence reader (BACTEC MicroMGIT) which makes the reading of tubes quite simple and objective.

There is no published study on performance of manual MGIT using MicroMGIT reader. The methodology holds promise for low and medium volume laboratories of developing countries, processing up to 10 mycobacterial samples per day, who cannot afford costly equipment and infrastructure required for automated systems. This study was therefore undertaken to compare the recovery rate and time for isolation and DST of MTB from clinical samples by manual MGIT vs the conventional LJ media and evaluate the feasibility for its implementation for use in laboratories with low and medium volume.

Material and methods

This prospective diagnostic study was conducted at a large tertiary care hospital of northern India from December 2013 to March 2015. Pulmonary and extrapulmonary samples received in the laboratory from out patient department (OPD) and inpatients for mycobacteriology culture and DST were included in the study. Samples from patients suspected to have relapse or treatment failure were also included.

All procedures requiring biosafety precautions were performed as per recommended guidelines for mycobacterial work.[7] The laboratory staff was well trained in biosafety procedures and used protective clothing (gloves, cap gown, etc.) and respiratory protection (N 95 masks) at all times. Sample processing, smear preparation, inoculation of media, identification and DST, were performed in a Class II Biosafety Cabinet. The samples and smears after processing were discarded in 5% phenol and inoculated culture media were autoclaved in order to achieve complete disinfection prior to final disposal.

A total of 1018 consecutive clinical specimens (781 sputum, 121 bronchoalveolar lavage, 29 bronchoscopic biopsies, 15 fine needle aspiration cytology (FNAC) aspirates, 9 pus, 57 body fluid aspirates and 6 urine) were received in the laboratory for mycobacterial culture and DST. Specimens were collected in a sterile 15 ml falcon tube with a screw cap. All sterile specimens were concentrated by centrifugation at $3000 \times g$ for 30 min while all nonsterile specimens were digested and decontaminated by the Modified Petroffs N-acetyl-L-cysteine (NALC)-NaOH method before concentration. The pellet obtained was re-suspended in sterile phosphate buffer saline to get a final volume of 2 ml. Resuspended pellet was used for making smears and for inoculation of LJ slopes and MGIT tubes. We inoculated 0.5 ml of the processed specimen into MGIT, and 0.2 ml onto LJ medium slant. All inoculated media were incubated at 37 °C. Positive smears were graded from 1+ to 4+ on Ziehl Neelsen (ZN) stain as per quantitative reporting procedure recommended by Centre for Disease Control and Prevention (CDC).[8]

Commercially available manual MGIT products (Becton-Dickinson, Sparks, MD) were used in the study. Before inoculation, 4 ml MGIT tubes (Catalogue number-245113) were supplemented with 0.5 ml OADC (oleic–albumin–dextrose–catalase) (Catalogue number-245116) and 0.1 ml of reconstituted PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) (Catalogue number-245114) as instructed by the manufacturer. MGIT tubes have an oxygen quenching fluorescent compound embedded in silicon at the bottom to detect microbial growth. The amount of fluorescence emitted is inversely proportional to the oxygen level in the culture medium, indicating the consumption of oxygen by growing microorganisms.

The fluorescence was detected with the help of a handheld MicroMGIT fluorescence reader (Catalogue number-445923). The reader measures 9.2 cm × 14.5 cm × 12.0 cm ($W \times D \times H$) and operates on a standard 9 volt battery (Fig. 1). A calibration tube (Catalogue number-441049) included with the instrument was used to calibrate it before taking readings of the inoculated tubes. The smear positive specimen MGIT tubes were read daily while smear negative specimen MGIT tubes were read weekly till they became positive or for a maximum of six weeks.

LJ medium slants were examined weekly for 8 weeks for the visible appearance of colonies. On the day of detection, all positive liquid and solid media were examined by ZN stain to

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