Note

Thin layer agar compared to BACTEC MGIT 960 for early detection of *Mycobacterium tuberculosis*

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ABSTRACT

We compared the sensitivity and time to detection of growth of *Mycobacterium tuberculosis* in the thin layer agar (TLA) compared to BACTEC MGIT960. The average time for growth of *M. tuberculosis* in TLA and BACTEC MGIT960 was 10.6 and 9.6 days, respectively. The sensitivity of detection of *M. tuberculosis* was 97.3% on TLA and 97% on BACTEC MGIT960 for smear positive samples. TLA showed comparable results to BACTEC MGIT960 and could be an alternative method for low-income countries.

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Tuberculosis (TB) remains a serious public health problem worldwide. According to the last report of the World Health Organization (WHO, 2009) there were 9.27 million new cases of TB in 2007 and among them an estimated 1.37 million were HIV-positive. Sputum smear microscopy is the primary tool for diagnosis of TB in many countries. However, detection of mycobacteria by acid-fast staining lacks sensitivity. On the other hand, culture on Löwenstein-Jensen (LJ) requires several weeks to give results, while the more rapid BACTEC MGIT960 liquid culture or molecular tools are too costly and are not always available in laboratories where economical resources are limited.

In recent years, the development of rapid culture methods has been considered as a priority. The thin layer agar (TLA) method has been described as a low-cost culture method that reduces the time to diagnose TB (Mejia et al., 1999, 2004; Robledo et al., 2006) allowing at the same time the identification of *M. tuberculosis* based on the observation of the colony morphology in the presence of para-nitrobenzoic acid (PNB) (Rastogi et al., 1989; Giampaglia et al., 2007). The objective of this study was to compare the sensitivity and time to detection of growth in TLA and in BACTEC MGIT960.

A total of 284 sputum samples were collected through a collaborative project with "Médecins Sans Frontières" in different settings. Smear microscopy by the Ziehl–Neelsen method was performed on all unconcentrated specimens, and results reported as smear negative or smear positive (classify 1+ to 3+ according to

bacterial load). Sputa were kept refrigerated at 4 °C and sent by courier to the Institute of Tropical Medicine, Antwerp, Belgium, for culture. Sputum samples were decontaminated using the Petroff method (Petroff 1915). Briefly, sputum was decontaminated with sodium hydroxide (NaOH 1N) for 20 min at room temperature with intermittent agitation and then neutralized with hydrochloric acid (HCl 1N). This suspension was then centrifuged at 3000 $\times g$ for 20 min and the pellet was resuspended in sterile distilled water and inoculated in parallel in the BACTEC MGIT960 and in TLA for comparison.

The BACTEC MGIT960 was performed following the manufacturer's instructions (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). Any sample identified as positive was removed from the instrument, and a smear was prepared. All isolates were identified by biochemical methods (IUATLD, 2007). Time to detection of growth was recorded on the date of the instrument positivity.

The TLA plates were prepared as previously described (Robledo et al., 2006) with small modification using a bi-plate Petri dish with PNB. One hundred μl of decontaminated sample was inoculated on the bi-plate Petri dish of 100 mm × 15 mm (Becton Dickinson, Sparks, MD USA) containing 20 ml of Middlebrook 7H11 agar supplemented with 10% OADC (oleic acid, albumin, dextrose and catalase) (Becton Dickinson) plus piperacillin, trimethoprim and amphotericin B (Sigma Aldrich) at 0.05 μg/ml, 0.02 μg/ml and 0.02 μg/ml, respectively. PNB at 500 μg/ml was also incorporated in one compartment of the bi-plate. The plates were sealed with parafilm leaving a space of 1 to 2 cm and incubated at 37 °C in 5% of CO_2. The plates were checked after 24 h for contamination and examined twice a week up to 6 weeks using a low-power objective (10×) for cord formation. A positive culture was identified by the characteristic cord formation of *M. tuberculosis*

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Table 1
Isolation of 210 *M. tuberculosis* isolates according to smear results and time to detection (TTD) in BACTEC MGIT960 and in TLA.

Recovery of mycobacteria							
Smear	Both media	MGIT960	%	TTD (days)	TLA	%	TTD (days)
3+	104	101	97	8	102	98	9
2+	32	30	94	10	32	100	11
1+	64	64	100	11	60	94	12
(−)	10	8	80	14	8	80	17

growth. Non-tuberculous mycobacteria (NTM) were recognized by their lack of cording and ability to growth on PNB. Fungal or bacterial contamination was recognized by rapid overgrowth on the plates. Further identification of the isolates was performed according to standard recommendations (IUATLD, 2007).

Out of 284 samples processed, 60 (21.1%) were found contaminated in BACTEC MGIT960, 49 (17%) in TLA and 14 (4.9%) were negative in both methods. These samples were excluded from the analysis. Out of the remaining 210 positive cultures, 202 (96%) were found positive by BACTEC MGIT960 and 203 (96.6%) by TLA. All isolates were identified as *M. tuberculosis*. Each method was compared against the final diagnosis of *M. tuberculosis*, defined as growth in any culture medium, which was considered as the gold standard for comparison. Sensitivity of each media compared to both media combined for growth of *M. tuberculosis* was 97%, 94%, 100%, and 80% for smear positive 1+, 2+, 3+ and smear negative, respectively, in MGIT960, and 98%, 100%, 94% and 80% for smear positive 1+, 2+, 3+ and smear negative, respectively, when using TLA (Table 1). Samples inoculated in the BACTEC MGIT960 were found to be positive in an average of 8, 10, 11 and 14 days for smear positive 3+, 2+, 1+ and smear negative, respectively. For TLA, positive results were found in an average of 9, 11, 12 and 17 days for smear positive 3+, 2+, 1+ and smear negative, respectively (Table 1). Fig. 1 shows the time to detection of *M. tuberculosis* for each media.

The purpose of this study was to evaluate the sensitivity of the TLA compared to the BACTEC MGIT960 and time to detection of mycobacteria in sputum samples. In this study, the time to detect mycobacteria using TLA was comparable with BACTEC MGIT960. In smear positive samples growth was detected in an average of 10.6 days for TLA and 9.6 days for BACTEC MGIT960. Time to detect mycobacteria in both methods was directly related to the grade of positivity in direct smear examination (Table 1). However, TLA was able to differentiate between *M. tuberculosis* and NTM at the same time of detection, which is a great advantage compared to BACTEC MGIT960. In the automated system, a positive tube should be confirmed for acid-fast bacilli and by biochemical tests, which take at least 2 more weeks. Molecular tools could be used for the identification but they are still quite expensive to be used in routine work. Sensitivity obtained by the TLA was comparable to the BACTEC MGIT960 system with an average of 97.3% for TLA and 97% for BACTEC MGIT960 for smear positive samples and 80% for smear negative samples in both methods. This is the first study that compares the sensitivity of TLA with that of BACTEC MGIT960. Robledo et al. compared TLA and LJ for the diagnosis of TB and showed a sensitivity of 92.6% and 84.7% for TLA and LJ, respectively, with a median time to detection of 11.5 days and 30.5 days, respectively. Prior to performing the TLA culture method, training is required, especially to recognize the typical cord formation characteristic of *M. tuberculosis* that differentiates it from bacterial contamination. The high contamination rate obtained is explained by the fact that sputum samples were collected in the field and mailed by courier to Belgium for culture. The average time between collection and sample processing was 17 days resulting in an increased contamination rate due to overgrowth by
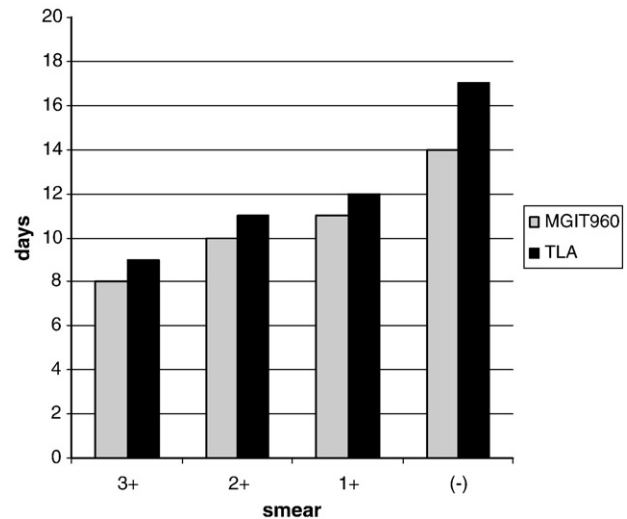

Fig. 1. Time to detection (days) of *M. tuberculosis* culture positive results in TLA compared to BACTEC MGIT960.

contaminants in the sputum. Another method called the microscopic observation drug susceptibility (MODS) assay (Moore et al., 2006) is similar to TLA, but has the disadvantage of requiring an inverted microscope, and the use of liquid medium in plates poses increased biosafety risks due to the generation of aerosols. We determined an approximate cost of TLA of 2.5 euro per isolate compared to 6 euro for BACTEC MGIT960.

In conclusion, TLA has a comparable sensitivity and time to detection of mycobacteria as BACTEC MGIT960 and could be an alternative option for diagnosing *M. tuberculosis* in laboratories in low-resource settings.

Acknowledgements

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