View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

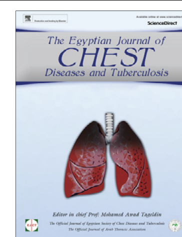
provided by Elsevier - Publisher Connector

Egyptian Journal of Chest Diseases and Tuberculosis (2014) 63, 377–384

The Egyptian Society of Chest Diseases and Tuberculosis

Egyptian Journal of Chest Diseases and Tuberculosis

www.elsevier.com/locate/ejcdt
www.sciencedirect.com



ORIGINAL ARTICLE

Comparative study between using Lowenstein Jensen, Bio-FM media and mycobacteria growth indicator tube (MGIT) system in identification of *Mycobacterium tuberculosis*

Tarek S. Essawy [a,*], Amal M. Saeed [b], Nehad A. Fouad [b]

[a] *Department of Chest Diseases, Faculty of Medicine, Benha University, Egypt*
[b] *Department of Microbiology & Immunology, Faculty of Medicine, Benha University, Egypt*

Received 6 November 2013; accepted 1 January 2014
Available online 7 February 2014

KEYWORDS

Lowenstein Jensen;
Bio-FM media and mycobacteria growth indicator tube (MGIT);
Mycobacterium tuberculosis

Abstract *Background/aim:* Tuberculosis (TB) continues to be one of the most important infectious diseases threatening the human health. Culture systems are used for isolation of tuberculous bacilli as they are more sensitive than smear microscopy. The aim of the work was to evaluate the detection rate and time of *Mycobacterium tuberculosis* by using the Bio FM system and mycobacteria growth indicator tube (MGIT) system in comparison with the Lowenstein–Jensen medium.

Methods: This study was carried out on a total 60 smear acid fast bacilli positive sputum samples obtained from patients attending the Chest Department and Outpatient Clinic of Benha University Hospital, Benha Chest Hospital, from June 2012 to September of the same year. Patients were classified into three groups: Group I: included 40 patients with fresh sputum smear +ve (new cases), Group II: included 13 relapsed cases and Group III: included 7 treatment failure cases. All sputum samples were cultured in parallel on Bio-FM, MGIT and LJ media. The mycobacterial detection rate and time were compared.

* Corresponding author. Tel.: +20 1223528390.
E-mail address: tarek.essawy@yahoo.com (T.S. Essawy).

Peer review under responsibility of The Egyptian Society of Chest Diseases and Tuberculosis.

0422-7638 © 2014 Production and hosting by Elsevier B.V. on behalf of The Egyptian Society of Chest Diseases and Tuberculosis.
Open access under CC BY-NC-ND license.
http://dx.doi.org/10.1016/j.ejcdt.2014.01.001

Results: The mycobacteria recovery rate on MGIT, Bio-FM and LJ was not significantly different (respectively 98.3%, 95% and 93%, $P > 0.05$). The growth of *M. tuberculosis* bacilli was faster on MGIT than on Bio-FM (mean 10.7 ± 2.9 days, 11.1 ± 4.3 days, respectively vs. 23.6 ± 8.1 days for LJ), there was statistically significant difference between LJ and Bio-FM and LJ and MGIT. However there was no statistical significance when comparing MGIT and Bio-FM.

Conclusion: MGIT and Bio-FM are good culture systems for accurate and rapid detection of *M. tuberculosis*.

© 2014 Production and hosting by Elsevier B.V. on behalf of The Egyptian Society of Chest Diseases and Tuberculosis. Open access under CC BY-NC-ND license.

Introduction

In 2010, there were 5.7 million notifications of new and recurrent cases of TB, equivalent to 65% (range 63–68%) of the estimated number of incident cases in 2010. India and China accounted for 40% of the world's notified cases of TB in 2010, Africa for a further 24% and the 22 high-TB burden countries (HBCs) for 82%. At global level, the treatment success rate among new cases of smear positive pulmonary TB was 87% in 2009 [1].

Without treatment, mortality rates are high. In studies of the natural history of the disease among sputum smear-positive and HIV-negative cases of pulmonary TB, around 70% died within 10 years; among culture-positive (but smear-negative) cases, 20% died within 10 years [2].

Although smear microscopy shows the highest rates of disease detection yearly worldwide [3]. Culture systems are used for isolation of tuberculous bacilli as they are more sensitive than smear microscopy. Lowenstein Jensen culture (LJ) is most widely used in low-income countries, it is an egg based medium developed from Jensen's modification of Lowenstein's formula. The inoculation time of the bacilli is up to 8 weeks [4]. LJ medium is commonly used in clinical laboratories to isolate acid fast organisms from sterile and non sterile sources. Bacterial growth on the cultures is considered as positive as soon as colonies appear on LJ medium and it is confirmed by Zeihl Neelsen (ZN) staining. In practice, 40% to 70% of patients with tuberculosis isolated in culture have positive smears [5].

Bio-FM is an enriched Middlebrook 7H9 medium, optimized for rapid mycobacterial growth whose selectivity is enhanced by a selective VCA (vancomycin, colistin and amphotericin B) supplement, containing a colored indicator that allows the detection of positive cultures which become a dark blue to violet color. The aspect of the culture should also permit species identification (*Mycobacterium tuberculosis* or atypical mycobacteria), the results are confirmed by microscopy after ZN staining, then by re-inoculation on LJ medium and by identification by biochemical tests [6].

The mycobacteria growth indicator tube (MGIT) consists of liquid broth medium that is known to yield better recovery and faster growth of mycobacteria. The MGIT contains modified Middlebrook 7H9 broth base. This medium is terminally sterilized by autoclaving. An enrichment, MGIT OADC (Oleic acid, Albumin, Dextrose and Catalase). Addition of the MGIT PANTA (Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin) is necessary to suppress contamination. The MGIT tube contains an oxygen-quenched fluorochrome, tris 4, 7-diphenyl-1, 10-phenonthroline 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. With depletion of free oxygen, the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube when visualized under UV light. The intensity of fluorescence is directly proportional to the extent of oxygen depletion [7].

The aim of the work was to evaluate the detection rate and time of *M. tuberculosis* by using the Bio FM system and mycobacteria growth indicator tube (MGIT) system in comparison with the Lowenstein–Jensen medium.

Subjects, materials and methods

This study was carried out on a total of 60 smear AFB positive sputum samples obtained from patients attending the Chest Department and Outpatient Clinic of Benha University Hospital, Benha Chest hospital, from June to September, 2012. Patients were classified into three groups: Group I: forty (40) patients with fresh sputum smear +ve (New cases), Group II: Thirteen (13) relapsed cases and Group III: Seven (7) treatment failure cases.

The inclusion criteria:

Pulmonary tuberculosis, sputum smear-positive (PTB+).

a. Two or more initial sputum smear examinations positive for AFB, or
b. One sputum smear examination positive for AFB + radiographic abnormalities consistent with active PTB, or according to the history of previous administration of anti tuberculous treatment.

Pulmonary tuberculosis was classified into:

- *New:* A patient who has never had treatment for TB or who has taken antituberculous drugs for less than 1 month.
- *Relapse:* A patient previously treated for TB who has been declared, cured or treatment completed, and is diagnosed with bacteriologically positive (smear or culture) tuberculosis.
- *Treatment after failure:* A patient who is started on a re-treatment regimen after having failed previous treatment. A patient whose sputum smear or culture was positive at 5 months or later during treatment. Also those included in this study were found to harbor a multidrug-resistant (MDR) strain at any point of time during the treatment, whether they are smear-negative or -positive.
- *Treatment after default:* A patient who returns to treatment, positive bacteriologically, following interruption of treatment for 2 months or more [8].

The exclusion criteria: PTB cases on chemotherapy, HIV positive.

The patients were subjected to the following:

- Full history taking (age, sex, family and past history of tuberculosis and history of intake of antituberculous drugs).
- Complete physical examination, plain chest X-ray- P.A. and lateral view if needed.
- Sputum smears for acid fast bacilli (Zeihl Neelsen staining).
- Sputum culturing on Lowenstein–Jensen medium, on Bio-FM medium and MGIT.

Specimens

- *Sputum collection* was in the early morning, patients were instructed to expectorate a deep respiratory specimen with no nasal secretions or saliva. Ideally, the sputum was obtained on 3 consecutive days. 5–10 ml of sample each time was appropriate. For patients who were unable to produce sputum, hypertonic saline (5–15%) was nebulized for induction. All the specimens were collected in clean, sterile containers and transported to the Microbiology & Immunology Department, Benha University in a rapid manner. Specimens were refrigerated when they could not be processed immediately; this prevents overgrowth by other bacteria [9].
- *Sputum liquefaction, decontamination and concentration procedure* was achieved by the N-acetyl L-cysteine sodium hydroxide (NALC–NaOH) method, equal volumes of sodium hydroxide (4%) and sodium citrate (2.9%) solutions were mixed. To this mixture N-acteyl-L-cysteine was added to make a final concentration of 0.5%; 5 ml of this mixture was added to 5 ml sputum; contents were mixed well and kept at room temperature for 15 min. Then these were centrifuged for 15 min at 3000 rpm. Supernatant was discarded in 5% Lysol and sediment was washed with deionized water. The sediment pellet was resuspended in deionized water by vortexing to make a homogeneous inoculums [9,10].
- *Staining:* ZiehI-Neelsen staining technique according to [11]. If any definite red bacilli were seen, the smear was reported as "AFB positive" smear. The number of bacteria present was reported as follows [12].

If no AFB observed that means negative smear, if 1/300 fields that means Doubtful and request other specimen, if 1–9/100 fields that means (1+), if 1–9/10 fields that means (2+), if 1–9/1 field that means (3+) and if more than 9/1 field that means (4+).

Culture of sputum samples

(a). Culture method on LJ [13]. Venkataswamy et al. 2007.
(b). Culture method on Bio-FM medium (BIO-RAD, Marnes la Coquette, France, Ref 70,160–70,161). 0.5 ml was inoculated into a tube of Bio-FM broth. Then it was incubated for 5–6 weeks at 37 °C.

Reading cycle: performed 2–4 times weekly, readings for 3–4 times weeks, then twice a week for another 2 weeks.

Reading – interpretation method (Reading of Bio-FM medium generally was in 2 phases):

1st phase: examination of the sediment and liquid medium without shaking the tube.

- The bottom of the tubes and the liquid medium were carefully examined:
  - If no signs of growth (no cloudiness, turbidity, dark blue/violet grains or flakes): the 2nd phase of reading was done.
  - If signs of growth were present:
- *Dark blue/violet grains or small flakes* that have settled in the bottom of the tube: presumption of MTB.
- *Cloudiness or turbidity*, with a color ranged from dark blue to violet, and tended to partially settle in the bottom of the tube: suspicion of atypical mycobacteria. This possibly was confirmed by the 2nd phase of reading.

2nd phase: examination of the sediment after shaking the tube.

- If no signs of growth (no cloudiness, turbidity, dark blue/violet grains or flakes): negative culture, incubation of the medium continued.
- If signs of growth were present:
- *Dark blue/violet grains or small flakes:* presumption of MTB.
- *Cloudiness or turbidity*, with a color dark blue/violet color: suspicion of atypical mycobacteria.
- The presence of a homogeneous cloudiness (possibly with very slight blue color of the medium), observed after 3 days of incubation, was suggestive of contamination.
- The dark blue color, indicative of growth of mycobacteria, was visible even in the presence of cellular debris or insoluble particles.
- -Counting and a morphological study of colonies were done between the 2nd and the 8th week after inoculation (as recommended).

c. Culture on MGIT (Becton Dickinson) as provided in the package insert:

A lyophilized vial of BBL MGIT PANTA antibiotic mixture was reconstituted with 3 ml of sterile distilled water. The MGIT tube was labeled with specimen number, the cap was unscrewed and aseptically 0.5 ml of MGIT OADC was added, then aseptically 0.1 ml of reconstituted MGIT PANTA antibiotic mixture was added. For best results, the addition of OADC enrichment and PANTA antibiotic mixture was made just prior to specimen inoculation. 0.5 ml of the concentrated specimen suspension was added the tube was tightly recapped and mixed well. Then the tube was incubated at 37 °C. The tube was read daily starting on the second day of incubation using a UV lamp.

Preparation of interpretive negative and positive control tubes:

- *Positive control tube* was prepared by Empty broth from an uninoculated MGIT tube. 0.4% sodium sulfite solution was prepared (0.4 g in 100 ml sterile distilled or deionized water) then 5 ml was added to the tube, the cap was replaced, tightened and the tube was allowed to stand for a minimum of 1 h at room temperature before use.
- *Negative control tube:* An unopened, uninoculated MGIT tube is used as a control.

Reading the Tubes

Tubes were removed from the incubator and placed on the UV light next to a positive control tube and negative control. Normal room light is preferred. MGIT tubes that showed bright fluorescence were visually located. Fluorescence was detected as a bright orange color in the bottom of the tube and also an orange reflection on the meniscus. The MGIT tubes were compared to positive control and negative control tubes.

– The presence of mycobacteria in the medium was confirmed by the presence of AFB after ZN staining, then by re-inoculation of bacteria onto LJ medium and identification by biochemical tests (niacin accumulation and nitrate reduction).

Identification by biochemical tests

(a). Niacin accumulation test [14].

Niacin TB test strips (Becton Dickinson, Sporks, USA) were used, these strips were dipped downward into the test (3–4 weeks old culture on which sterile distilled water was added) and negative control tubes (containing 0.5 ml sterile DW) and left for 5–10 min. Positive test for niacin was indicated by the appearance of a yellow color in the test culture and no color in the control tube.

(b). Nitrate reduction test [15]

A nitrate test strip (Becton Dickinson, Sparks, USA) was used. This strip was dipped into tube containing 0.5 ml of DW, two spades (at least 50 colonies) from a 4 week old mycobacterial culture, then it was incubated at 37 °C for 2 h. Positive nitrate test was indicated by the appearance of a blue color in the top portion of the strip.

Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 software. Qualitative data were presented as frequencies and percentages, while continuous variables were presented as mean and standard deviation. Student's *t*-test was used to compare the mean of two groups of numerical (parametric) data, ANOVA (analysis of variance) (*f* test) was used to compare mean between more than two groups of numerical (parametric) data and post hoc analysis was used to detect significance difference in-between groups. Inter-group comparison of categorical data was performed by using the chi square test (X^2-value). A P value < 0.05 was considered statistically significant.

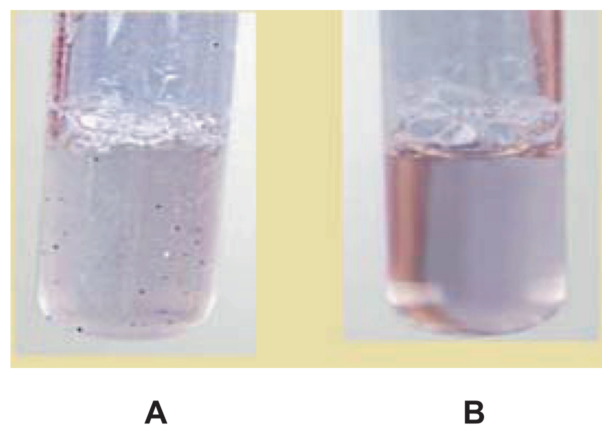


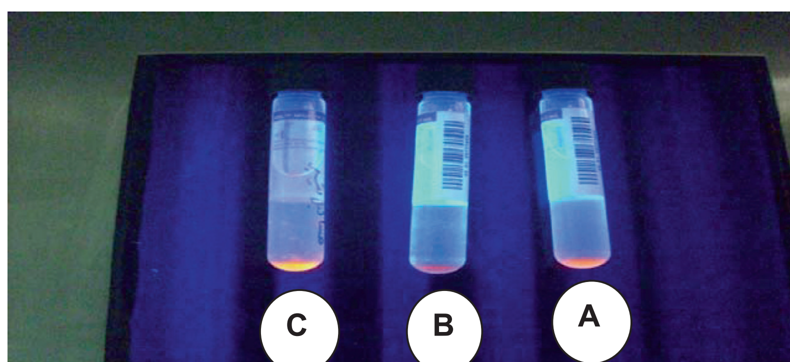
Figure 2 Comparison between positive and negative results on Bio-FM. Dark blue (violet) dots demonstrating positive growth are clearly seen in (A). Clear aspect with no dark blue (violet) dots is seen in (B) reflecting negative results.

Table 1 Distribution of study group according to type of patient.

	No.	%
New cases	40	66.7
Relapsed cases	13	21.7
Treatment failure cases	7	11.6
Total	60	100.0

Table 2 Rates of recovery of *Mycobacterium tuberculosis*.

Culture method	Positive		Negative		X^2	P
	No.	%	No.	%		
LJ	56	93.3	4	6.7	1.8	0.4
Bio-FM	57	95	3	5		
MGIT	59	98.3	1	1.7		



A: positive control B: negative control C: test

Figure 1 Positive result on MGIT as detected by fluorescent orange color.

Table 3 Comparison between LJ, Bio-FM and MAGIT according to mean detection time.

	Mean	Range	Std. deviation	*F*	*P*	Between groups
LJ	23.6	14–40	8.1	86.6	0.000	P_1 = 0.000*
Bio-FM	11.1	7–21	4.3			P_2 = 0.000*
MGIT	10.7	5–20	2.9			P_3 = 0.73

* P_1 between LJ and Bio-FM, P_2 between LJ and MGIT, P_3 between Bio-FM and MGIT.

Results

The results of this study are presented in Tables 1–5 and Figs. 1 and 2.

This study included 60 sputum smear positive tuberculous patients, who were classified into 40 new cases (group I) representing 66.7% of the total cases, 13 relapsed cases (group II) and 7 treatment failure cases (group III) representing 21.7% and 11.6% of the total cases, respectively (Table 1).

The recovery rates of mycobacteria in each cultivation system are summarized in Table 2. MGIT and Bio-FM detected 98.3% and 95% of the isolates, respectively, while LJ detected 93.3%. There was no significant difference between the different cultures for the recovery of MTB (*P* value = 0.4).

The mean detection time of MTB on LJ, Bio-FM and MGIT was 23.6 ± 8.1, 11.1 ± 4.3 and 10.7 ± 2.9, respectively, there was statistically significant difference between the three medias (*P*-value = 0.000). When post hoc analysis was done there was statistically significant difference between LJ and Bio-FM and LJ and MGIT, However there was no statistical significance when comparing MGIT and Bio-FM (Table 3)

The mean detection time on three media in cases with cavitary lesion on chest X-ray was shorter than these cases without cavitary lesions. The mean detection time on Lowenstein Jensen for cases with cavitary lesions was (14.39 days ± 1.94) compared with that for cases with non cavitary lesions which was (22.7 days ± 6.2) and on Bio-FM was (9.3 days ± 2.2) for cases with cavitary lesion and (14.1 days ± 4.2) for cases with non cavitary lesions. While on MGIT mean detection for cases with cavitary lesion was (8.9 days ± 1.9) compared with that for cases with non cavitary lesions which was (12.9 days ± 3.1), and that difference was statistically significant in all culture media (*P*-value = 0.000) (Table 4).

Patients having risk factors for TB whether diseases such as DM, COPD, CRF or receiving long term treatment such as corticosteroid show significant shorter mean detection time on MGIT compared with those on Bio-FM and LJ (*P*-value < 0.05). On the other hand, patients with no risk factors for TB show no significant difference in mean detection times between three media (*P*-value = 0.8) (Table 5).

Discussion

The consequences of tuberculosis on society are immense. Worldwide, one person out of three is infected with *M. tuberculosis* – two billion people in total. TB accounts for 2.5% of the global burden of disease and is the commonest cause of death in young women, killing more women than all causes of maternal mortality combined. TB currently holds the seventh place in the global ranking of causes of death. Unless intensive efforts are made, it is likely to maintain that position through to 2020, despite a substantial projected decline in disease burden from other infectious diseases Smith, 2004 [16].

Active pulmonary tuberculosis is diagnosed by detecting MTB complex bacilli in specimens from the respiratory tract. Although many new (molecular) diagnostic methods have been developed, acid fast bacilli (AFB) smear microscopy and culture are still the "gold standards" for the diagnosis of active TB, especially in low-resource countries De Waard and Robledo [17].

Although considerable efforts have been made to improve the sensitivity of sputum smear microscopy, it still lacks sensitivity, especially in children and HIV +ve people Steingart [18], so culture is used to detect cases with low mycobacterial loads and is also requested in cases at risk of drug-resistant TB for drug susceptibility testing or in cases where disease due to another member of the mycobacterium genus is suspected.

Different culture media are in use for the isolation of mycobacteria. The most common are based on egg and also contain high concentrations of malachite green to overcome contamination with other bacteria. In general, only solid Lowenstein Jensen medium is available in many low income countries, as it is made on site. However, culture on Lowenstein Jensen is long (3 weeks to 3 months for MTB) and time consuming, as it requires large incubators. The Bio-FM broth is an enriched Middlebrook medium that has been on the market for several years Ramarokoto et al. [6].

Table 4 Comparison between LJ, Bio-FM and MGIT as regards mean detection times classified according to presence or absence of cavitary lesion.

Culture method	Cavitary		Non-cavitary		*t*	*P*
	Range	Mean ± SD	Range	Mean ± SD		
LJ	12–20	14.39 ± 1.94	14–40	22.7 ± 6.2	5.5	0.000*
Bio-FM	6–15	9.3 ± 2.2	7–21	14.1 ± 4.2	6.2	0.000*
MGIT	5–14	8.9 ± 1.9	5–20	12.9 ± 3.1	5.1	0.000*

* Significant.

Table 5 Comparison between LJ, MGIT and Bio-FM as regards distribution of positive cases, mean detection times and duration range in days classified according to associated risk factors.

		No.	%	Mean ± SD	Range	*f*	*P*
No associated risk factors	LJ	27	48.2	16.6 ± 5.9	14–40	1.4	0.09
	Bio-FM	27	47.4	16.29 ± 4.2	7–21		
	MGIT	28	47.5	14.1 ± 3.1	5–20		
DM	LJ	11	19.6	20.67 ± 6.9	12–35	4.3	0.008*
	Bio-FM	11	19.3	12 ± 3.3	9–20		
	MGIT	11	18.6	9.3 ± 2.1	6–15		
COPD	LJ	7	12.5	18.8 ± 6.3	11–36	4.2	0.004*
	Bio-FM	8	14.04	11 ± 3.2	7–20		
	MGIT	8	13.6	8.7 ± 1.9	5–12		
CRF	LJ	7	12.5	15 ± 2.4	12–18	3.6	0.01*
	Bio-FM	7	12.3	8.6 ± 2.1	7–12		
	MGIT	8	13.6	6.5 ± 1.1	5–10		
Long term corticosteroids	LJ	7	12.5	13.8 ± 1.3	11–19	3.1	0.03*
	Bio-FM	7	12.3	8.9 ± 1.7	7–13		
	MGIT	7	11.9	6.4 ± 1.5	5–12		

DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; CRF, chronic renal failure.
* Significant.

The aim of the work was to evaluate the detection rate and time of *M. tuberculosis* by using the Bio FM system and mycobacteria growth indicator tube (MGIT) system in comparison with the Lowenstein–Jensen medium.

This study included 60 sputum smear positive tuberculous patients, who were classified into 40 new cases (group I) representing 66.7% of the total cases, 13 relapsed cases (group II) and 7 treatment failure cases (group III) representing 21.7% and 11.6% of the total cases, respectively.

In the present study the mycobacteria recovery rates on MGIT, Bio-FM and L–J were compared. The results indicate that recovery rates from MGIT, Bio-FM are comparable to those obtained from L–J (98.3%, 95% and 93.3%, respectively, $P = 0.4$), it is similar to that obtained by Ramarkoto et al. [6] who found that the detection rate of the Bio-FM system was not significantly different from that on standard L–J culture. The obtained figure is also consistent with Levidiotoul et al. [19] who found that recovery rates from MGIT were comparable to those obtained from LJ (89.65% and 80.46%, respectively, $p > 0.05$).

In this study, the mean time for detection of mycobacteria was 10.7, 11.1, and 23.6 days in MGIT, in Bio-FM and on L–J medium, respectively. The mean time for detection was shorter for MGIT than Bio-FM, and the difference was statistically non significant ($P = 0.73$). The mean time for detection was shorter for MGIT and Bio-FM than L–J and the difference was statistically significant ($P = 0.000$) for both. This result comes true with that reported by Ramarkoto et al. [6] who found that mycobacteria growth was significantly faster on Bio-FM than L–J (mean 13.04 vs. 19.52). Also result of this work agreed with that reported by Rivera et al. [20] who found that mean time for detection of positive culture on MGIT was shorter than that on L–J medium. However the same study reported that the mean time for detection on MGIT was 15.7 ranges (5–56 days) and this was much higher than that reported in the current study as mean time for detection on MGIT was 10.7 ranges (5–20 days) this difference may be due to small number of patients included in it.

It is possible for all laboratories performing mycobacterial culture to use the Bio FM medium. Unlike the other culture systems in liquid medium, it does not require costly specific equipment; the Bio FM system is entirely manual, with visual reading of cultures, which is therefore simple, although only qualitative. Due to its ease of use and the growth time of mycobacteria on Bio FM medium, it can be used for rapid detection of M. tuberculosis. However no one of the three media, were able to detect all the mycobacteria Ramarkoto et al. [6].

Furthermore, the cost of the Bio FM system (for reagents and consumables) is three to four times higher than for LJ, and it can therefore not be used for all samples routinely analyzed in low income countries. Bio FM could be used in combination with LJ for bacteriological diagnosis of extrapulmonary TB, which is usually the most difficult to diagnose using smear microscopy, and the association of two methods, a liquid and a solid medium, would be the optimal solution for mycobacterial detection, as indicated by Heifets et al. [21]. It could also be useful in those countries that are unable to make LJ medium, as well as in laboratories that do not have large volumes of samples to process and which cannot justify using a costly system such as BACTEC.

This work showed that the mean detection times on three media in cases with cavitary lesion on chest X-ray were highly significantly shorter than these cases without cavitary lesions (P-value = 0.000).The mean detection time on L–J for cases with cavitary lesions was (14.39 days ± 1.94) compared with that for cases with non cavitary lesions which was (22.7 days ± 6.2) and on Bio-FM was (9.3 days ± 2.2) for cases with cavitary lesion and (14.1 days ± 4.2) for cases with non cavitary lesions. While on MGIT mean detection for cases with cavitary lesion was (8.9 days ± 1.9) compared with that for cases with non cavitary lesions which was (12.9 days ± 3.1).

The results outlined in the previous section were explained by Palaci et al. [22] who demonstrated the relationship between

quantitative sputum bacillary load and the radiological extent of tuberculous lesions as well as the presence or absence of cavitary lesion in chest X-ray, (more advanced lesions associated with more sputum bacterial loads). It also can be explained by Rathman, et al. [23] and Matsuoka et al. [24] who stated that higher bacterial loads were found in patient's sputum samples associated with shorter detection time. Another study made by Perrin et al. [25] investigated the relationship between detection time of MTB and the presence or absence of cavitary disease by using the BacT/ALERT culture system. The mean detection time was significantly lower in patients with cavities (8.4 days) than in those without cavitation (16.2 days).

This work showed that patients having risk factors for TB whether diseases such as DM, COPD, CRF or receiving long term treatment such as corticosteroid show significant shorter mean detection time on MGIT compared with those on Bio-FM and LJ (P-value <0.05). Also those patients have shorter mean detection times on three media than that have no associated risk factor. This obtained figure is explained by higher bacillary burden at presentation of tuberculosis in diabetic patients Dooley and Chaisson, [26] and due to the prevalence of cavitary disease in diabetics such finding was supported by Guzman et al. [27] and so higher bacterial loads were found in their sputum associated with shorter detection times Rathman et al. and Matsuoka et al. [23,24].

On the other hand, patients with no risk factors for TB show no significant difference in mean detection times between three media (P-value $= 0.8$).

Conclusion

MGIT and BioFM media were better than LJ medium in recovery rate and detection time of mycobacterial growth. And this will facilitate reporting of TB cultures earlier than the conventional LJ method at a more reasonable cost to the patient, thereby improving overall patient management.

Conflict of interest

None.

References

[1] World Health Organization. Global tuberculosis control: WHO report 2011, 20 Avenue Appia, 1211 Geneva 27, Switzerland, 2011.

[2] E.W. Tiemersma, M.J. van der Werf, M.W. Borgdorff, et al, Natural history of tuberculosis: duration and fatality of untreated pulmonary tuberculosis in HIV negative patients: a systematic review, PLoS ONE 6 (4) (2011) e17601.

[3] H. Adler, C. Sraub, R. Frei, Comparison of BacT/ALERT 3D, Lowenstein–Jensen medium and Middlebrook 7H10/7H11 biplate for recovering mycobacteria from clinical specimens, Eur. Clin. Microbiol. Infect. Dis. (2005) 499–500.

[4] M.D. Perkins, J. Cunningham, Facing the crisis improving the diagnosis of tuberculosis in the HIV era, J. Infect. Dis. 196 (Suppl. 1) (2007) S15–S27.

[5] A. Pio, P. Chaulet, Tuberculosis Handbook, second ed., World Health Organization, Geneva, 2003 (WHO/CDS/TB 2003.320).

[6] H. Ramarokoto, T. Rasolonavalona, L. Ratsimba, et al, Evaluation of rapid culture method on liquid Bio FM (BIO-RAD) medium for the isolation of mycobacteria, Int. J. Tuberc. Lung Dis. 11 (8) (2007) 898–903.

[7] Salman H. Siddiq, Sabine Rüsch-Gerdes. Section I: principle of procedure, in: M G I TTM Procedure Manual, 2006, pp. 10–11.

[8] World Health Organization, Treatment of Tuberculosis: Guidelines for National Programmes, third ed., World Health Organization, Geneva, 2003.

[9] G.D. Roberts, G.A. Procop, Pathophysiology, Basic Aspects, Part I. Pathogenesis of Tuberculosis, in: Tuberculosis, fifth ed., Schlossberg, David, Copyright ©2006 McGraw-Hill press, Part I, ch.3, 2006, pp. 53–54.

[10] Murli L. Mathur, Scientist F, 1. Jyoti Gaur, Field Investigator, 1. Ruchika Sharma, Field Investigator, 1 and Aruna Solanki, Professor 2. Rapid culture of *Mycobacterium tuberculosis* on blood agarin resource limited setting, Dan. Med. Bull. 56 (2009) 208–210.

[11] M.A. Darrow, Staining the tubercle organism in sputum smears, Stain Technol. 24 (1948) 93–94.

[12] American Thoracic Society, The tuberculin skin test, Am. Rev. Respir. Dis. 124 (1981) 356.

[13] Y.A. El-Batawi, Manual of Microbial Infection of Man, 4th ed., El-Naser Modern Bookshop, Cairo, Part III, 1981, pp. 85–89.

[14] W. Young, A. Maslansky, M. Lafar, Development of a paper strip test for detection of niacin produced by mycobacteria, Appl. Microbiol. 10 (1970) 939–945.

[15] S. Virtanen, A study of nitrate reduction by mycobacteria, Acta. Tuberc. Scand. Suppl. 48 (1960) 1–119.

[16] I. Smith. What is the health, social, and economic burden of tuberculosis, in: T. Frieden (Ed.) Tomańs tuberculosis case detection, treatment, and monitoring: questions and answers, second ed., WHO, Geneva, 2004, pp. 233–237. WHO/HTM/TB/2004.334.

[17] K.R. Steingart et al, Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review, Lancet Infect. Dis. 6 (2006) 664–674.

[18] J.H. De Waard, J. Robledo. Conventional Diagnostic Methods in Tuberculosis from Basic Science to Patient Care, first ed., Palomino, Leao, Ritacco, 2007, CH.12, pp. 401–418.

[19] S. Levidiotou, D. Papamichae, E. Gessou, S. Golegou, S. Anagnostou, E. Galanakis, C. Papadopoulou, G. Antoniadis, Detection of mycobacteria in clinical specimen using the mycobacteria growth indicator tube (MGIT) and the Lowenstein Jensen medium, Microbiology 154 (1999) 151–155.

[20] A.B. Rivera, T.E. Tupasi, E.R. Grimaldo, R.C. Cardano, V.M. Co, Rapid and improved recovery rate of *Mycobacterium tuberculosis* in mycobacteria growth indicator tube combined with solid Lowenstein Jensen medium, Int. J. Tuberc. Lung Dis. 1 (5) (1997) 454–459.

[21] L. Heifets, T. Linder, T. Sanchez, D. Spencer, J. Brennan, Two liquid medium systems, mycobacteria growth indicator tube and MB Redox Tube, for *Mycobacterium tuberculosis* isolation from sputum specimens, J. Clin. Microbiol. 38 (2000) 1227–1230.

[22] M. Palaci, R. Dietze, D.J. Hadad, C.K. Ribeiro, R.L. Peres, S.A. Vinhas, E.L.N. Maciel, V. Dettoni, L. Horter, W.H. Boom, J.L. Johnson, K.D. Eisenach, Cavitary disease and quantitative sputum bacillary load in cases of pulmonary tuberculosis, J. Clin. Microbiol. 45 (12) (2007) 4064–4066.

[23] G. Rathman, J. Sillah, P.C. Hill, J.F. Murray, R. Adegbola, T. Corrah, C. Lienhardt, K.P. MacAdam, Clinical and radiological presentation of 340 adults with smear-positive tuberculosis in The Gambia, Int. J. Tuberc. Lung Dis. 7 (2003) 942–947.

[24] S. Matsuoka, K. Uchiyama, K. Shima, K. Suzuki, A. Shimura, Y. Sasakic, F. Yamagishi, Relationship between CT finding of pulmonary tuberculosis and the number of acid-fast bacilli on sputum smears, Clin. Imaging 28 (2004) 119–123.

[25] F.M. Perrin, S.L. Batt, N. Woodward, T.D. McHugh, M.C. Lipman, S.H. Gillespie. Cavitation and mycobacterial load in patients with pulmonary tuberculosis, in: Program and Abstracts of the American Thoracic Society International Conference 2006. American Thoracic Society International, San Diego, CA, 2006, p. A505.

[26] K.E. Dooley, R.E. Chaisson, Mutation in the signal-transducing chain of the interferon-gamma receptor and susceptibility to mycobacterial infection, J. Clin. Invest. 101 (2009) 2364–2369.

[27] C.P. Guzman, A.T. Cruz, H.V. Velarde, M.H. Vargas, Progressive age-related changes in pulmonary tuberculosis images and the effect of diabetes, Am. J. Respir. Crit. Care Med. 162 (2000) 1738–1740.