

EFFICACY OF BACTEC TB IN THE RAPID CONFIRMATORY DIAGNOSIS OF MYCOBACTERIAL INFECTIONS

A Lebanese Tertiary Care Center Experience

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ABSTRACT • OBJECTIVES : Rapid detection of *Mycobacterium tuberculosis* (MTB), especially multidrug-resistant strains, is of importance for prompt clinical management and initiation of public health control measures. Culture remains the "gold" standard in the confirmatory laboratory diagnosis of mycobacterial infections. The reliability of the automated radiometric BACTEC 460 TB (BACTEC) system for the rapid detection of mycobacteria in clinical specimens was evaluated and compared to the conventional culture on Lowenstein-Jensen (LJ) medium.

METHODS : All clinical specimens submitted for mycobacterial culture were processed and simultaneously cultured on both BACTEC broth medium and LJ solid medium. Acid-fast bacilli (AFB) smears were also performed on the sediments. Differentiation of mycobacterial isolates as MTB or *Mycobacterium* sp. other than tuberculosis (MOTT) was based on the BACTEC NAP test. All positive culture findings recovered between January 1997 and December 2003 were analyzed in this study.

RESULTS : A total of 3300 specimens were tested of which 355 (10.7%) yielded positive cultures consisting of 233 (65.6%) MTB and 122 (34.4%) MOTT. The percentages of AFB smear-positive were 45% and 49% in clinical specimens yielding MTB & MOTT, respectively. Though several types of specimens were cultured, most isolates (72% of MTB & 91% of MOTT) were recovered from respiratory specimens. Overall, the BACTEC showed significantly higher mycobacteria recovery rate (91%) than LJ (77%).

In terms of times to detection, BACTEC showed significantly shorter detection time of isolates than LJ for the overall (mean 9.6 days for BACTEC vs 22.8 days for LJ) and for each category of AFB smear finding. The detection time is shortened for BACTEC with the increasing grade of smear positivity.

CONCLUSIONS : BACTEC is substantially more sensitive, efficient and rapid than LJ in the laboratory diagnosis of mycobacterial infections. This system also provides rapid differentiation of MTB from MOTT and susceptibility test results on MTB. However, the simultaneous use of BACTEC and LJ is recommended to provide maximum optimal recovery of isolates from clinical specimens. The time-saving in BACTEC provides an excellent facility for physicians in patient management and to public health personnel for prompt initiation of infection control measures.

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RÉSUMÉ • OBJECTIFS : La détection rapide de *Mycobacterium tuberculosis* (MTB), surtout les souches multi-résistantes, est importante pour l'initiation rapide du traitement et la prise de mesures de contrôle en santé publique. La culture reste l'examen de laboratoire de choix pour le diagnostic des infections à mycobactéries. La fiabilité du système radiométrique automatisé BACTEC 460 TB (BACTEC) dans la détection rapide des mycobactéries a été évaluée et comparée à la culture conventionnelle dans le milieu de Lowenstein-Jensen (LJ).

MÉTHODE : Les spécimens soumis pour culture de mycobactéries ont été simultanément analysés sur BACTEC et LJ. La coloration à l'acide-alcool (AFB) a été également pratiquée sur les sédiments. La différenciation des mycobactéries en MTB et autres souches non tuberculeuses (MOTT) a été basée sur le BACTEC NAP test. Toutes les cultures positives entre janvier 1997 et décembre 2003 ont été incluses dans cette étude.

RÉSULTATS : 3300 spécimens ont été testés dont 355 (10,7%) étaient positifs : 233 (65,6%) MTB et 122 (34,4%) MOTT. Les pourcentages de lames d'AFB positives étaient 45% et 49% dans les spécimens cliniques positifs pour MTB et MOTT respectivement. La majorité des cultures positives (72% des MTB et 91% des MOTT) provenaient du tractus respiratoire. BACTEC s'est avéré plus rapide que LJ dans la détection des mycobactéries (moyenne 9,6 jours pour le BACTEC vs 22,8 jours pour LJ) quel que soit le résultat de la coloration AFB. Le temps de détection par BACTEC était inversement proportionnel au grade de positivité de l'AFB. Le pourcentage de positivité des méthodes BACTEC et LJ était de 91% et 77% respectivement.

CONCLUSION : BACTEC est plus sensible, efficace, et rapide que LJ dans le diagnostic des infections à mycobactéries. Ce système permet aussi une différenciation rapide entre MTB et MOTT et la détection d'une résistance aux antibiotiques parmi les souches de MTB. Cependant l'utilisation simultanée de BACTEC et LJ est recommandée pour obtenir des résultats optimaux. La rapidité des résultats est un outil important pour la conduite clinique et pour le personnel de la santé publique afin d'initier rapidement des mesures de contrôle des infections.

INTRODUCTION

Mycobacterial infections especially tuberculosis (TB) entails high morbidity and mortality and constitute a major public health worldwide [1]. Infections with *Mycobacterium tuberculosis* (MTB), especially with the multidrug-resistant strains (MDR-TB), and *Mycobacterium* spp.

other than tuberculosis (MOTT) have been increasingly encountered globally including Lebanon [2-4]. Such a situation poses several clinical challenges and public health threats not only in developing countries but also in developed ones. To subdue TB infections and minimize their spread, several conventional, serodiagnostic, molecular, and other techniques were developed to facilitate and expedite detection, diagnosis, antimicrobial susceptibility testing and treatment [5-8]. In addition, concordant efforts to establish and implement local, regional and global surveillance programs have been emphasized in order to control this worldwide threatening disease.

Despite the above noted diagnostic advances, culture still represents the cornerstone and the "gold" standard for the definitive diagnosis of tuberculosis and other mycobacterioses. Conventional culture using the egg-based Lowenstein-Jensen (LJ) slant or agar-based Middlebrook medium takes a long time and has a low yield. To improve and speed the recovery of culture, a broth-based medium together with a semi-automated radiometric BACTEC 460 TB (BACTEC) system was the first to be introduced in clinical laboratories for the rapid diagnosis of mycobacteria in the late 1970s by Becton Dickinson Microbiology Systems (Cockeysville, Md. USA) [9]. The reliability of this rapid system was proven in subsequent studies [10-12]. The concept of this system is based on metabolism of ^{14}C -labeled palmitic acid, as a growth nutrient, which is converted to $^{14}\text{CO}_2$ by the growing mycobacteria [13]. To date, this system is widely accepted and still considered the reference standard to the other lately introduced nonradiometric alternative automated systems for TB cultivation such as the Mycobacteria Growth Indicator Tube (MGIT; Becton Dickinson), and MB Redox (Biotest AG, Dreieich, Germany), MB/BacT/Alert (Organon Teknika, Durham, NC. USA), and the ESP II (Difco Laboratories, Detroit, Mich. USA) [8].

In Lebanon, studies on the efficacy of culture in the diagnosis of mycobacterial infections are nonexistent, thus, justifying the rationale for this study in presenting the experience of a major tertiary care center in this country. Therefore, this study was undertaken to reveal the mycobacterial culture findings, particularly in comparing the performance of BACTEC TB vs the conventional LJ medium in the laboratory diagnosis of mycobacterial infections at this institution.

MATERIALS AND METHODS

Patient's isolates and study location

The *M. tuberculosis* isolates analyzed in this study were collected from patients specimens investigated at the clinical microbiology section of the American University of Beirut Medical Center (AUBMC) between January 1997 and December 2003. The specimen sources of isolates, each obtained from one patient,

were noted. The AUBMC is a major tertiary care teaching hospital in Lebanon.

Processing of specimens, isolation and identification of mycobacterial isolates

Processing of specimens and identification of isolates were done by using standard methods [13-14]. Briefly, specimens from contaminated sources were decontaminated by the 2% N-acetyl-L-cysteine NaOH method for 15-20 min and neutralized with phosphate buffer (pH 6.8). Smears were made from specimen concentrates after neutralization and stained with Ziehl-Neelsen (see below) for the presence of acid-fast bacilli. Part (0.25 ml) of each decontaminated specimen was inoculated onto one slant of Lowenstein-Jensen (LJ) Medium (Becton Dickinson Microbiology Systems, Cockeysville, MD) and another part (0.5 ml) was also inoculated into Middlebrook 7H12 broth medium (BACTEC 12B medium Becton Dickinson) supplemented with PANTA (polymyxin 50 U/ml, amphotericin B 5 mg/l, nalidixic acid 20 mg/l, trimethoprim 5 mg/l and azlocillin 10 mg/l) and with polyoxyethylene stearate (POES) (Becton-Dickinson). BACTEC vials were routinely primed before inoculation to establish an atmosphere of 5% CO_2 . All media were incubated at 35-37°C for 8 weeks. Both the LJ media and BACTEC vials were examined three times weekly for the first 3 weeks, and once weekly thereafter until 8 weeks. Differentiation of mycobacteria as MTB or MOTT was based on the p-Nitro- β -acetylaminobenzyl hydroxy-propiophenone (NAP) BACTEC test [13].

Acid-fast stain (KINYOUN)

The preparation, performance and interpretation of acid-fast bacilli (AFB) stain was done according to standard procedure [15]. Briefly, smears were prepared by spreading the sample on the slides. They were air dried and heat fixed. The smear was flooded with carbolfuchsin and let stand for 5 min, then rinsed with tap water. Decolorization was done with acid alcohol for 1 to 2 min or until no more dye runs off the slide. Subsequently, the slides were rinsed with tap water and counterstained with methylene blue, by flooding the slides and keeping the stain for 30 sec. Then, the slides were rinsed with tap water, air dried and examined under oil immersion for at least 15-20 minutes prior to calling it as negative (none seen). Acid-fast organisms stain pink-red; the background and other organisms stain blue.

Grading of positive smears was done using the following criteria based on the number of AFB in the smear under oil immersion field (OIF): Negative = No AFB seen in the entire smear (100 fields); Rare = 1-9 AFB/100 fields; Few = 10-99 AFB/100 fields; Moderate = 1-10/OIF; Numerous > 10/OIF [15].

Quality control

Quality control of culture media was done using *Mycobacterium tuberculosis* H37Rv (ATCC 27294) and that for AFB smear was by using *M. tuberculosis* H37Rv and *Escherichia coli* (ATCC 259220).

TABLE I
DISTRIBUTION OF RECOVERED MTB AND MOTT ISOLATES BASED ON CLINICAL SPECIMEN SOURCE

SPECIMEN	Number (%)* of isolates	
	MTB (n = 233)	MOTT (n = 122)
SPUTUM	167 (72)	111 (91)
LYMPH NODES	19 (8)	1 (1)
GASTRIC ASPIRATE	7 (3)	0
CSF	8 (3)	0
ASCITIC FLUID	10 (4)	0
SKIN BX	6 (3)	4 (3)
URINE	2 (1)	4 (3)
OTHERS**	14 (6)	1 (1)

*Decimal numbers were rounded

**Others • For TB : 3 vertebral abscess, 2 paraspinal mass, 1 skin abscess, 1 body fluid, 1 pleural biopsy, 1 fistula swab, 1 synovial fluid, 1 leg skin biopsy, 1 scrotal sac, 1 thyroid aspirate, 1 wound swab • For MOTT : 1 body fluid.

RESULTS

During the study period, 3300 specimens were processed for mycobacterial investigation, yielding positive cultures in 355 (10.75%). Identification of the latter revealed 233 (65.6%) *M. tuberculosis* complex (TB) and 122 (34.4%) MOTT. The percentages of AFB smear positive findings were 45% and 49% in clinical specimens yielding TB and MOTT, respectively.

Table I shows the *source of tested specimens in relation to recovery of TB and MOTT*. Though the isolates originated from several types of specimen sources, most isolates (72% of MTB and 91% of MOTT) were recovered from respiratory specimens (Table I).

Table II presents the *overall comparison between BACTEC and LJ in the recovery of MTB and MOTT*. Simultaneous growth in BACTEC and LJ was observed in 73% of MTB and 58% of MOTT isolates. However, 14% and 17% of MTB and MOTT, respectively, grew only in BACTEC. These were missed by LJ, thus showing the importance of BACTEC. On the other hand, few cultures (9% of MTB and 10% of MOTT) grew only on LJ.

TABLE II
OVERALL COMPARISON BETWEEN BACTEC TB vs LJ IN RECOVERY OF MTB AND MOTT

BACTEC & LJ RECOVERY STATUS*	Number (%) of Isolates	
	MTB (n = 233)	MOTT (n = 122)
Pos. BACTEC - Pos. LJ	170 (73)	71 (58)
Pos. BACTEC - NEG. LJ	32 (14)	21 (17)
NEG. BACTEC - Pos. LJ	21 (9)	12 (10)
Pos. BACTEC - CONT.** LJ	10 (4)	18 (15)

*The overall recovery rate for BACTEC was 322/355 isolates (91%) and for LJ 274/355 (77%).

**Cont = contamination : the overall contamination rate among all (3300) the parallel tested specimens was 3.8% for BACTEC and 11.4% for the LJ.

Overall, the highest recovery rate in culture was obtained from BACTEC [322 of 335 (91%) isolates] compared to LJ [274 of 335 (77%) isolates] (Table II). The superiority of BACTEC to LJ in recovery of mycobacterial isolates was observed regardless of the specimen source.

Table III presents the *overall time (days) to detection of MTB in BACTEC and LJ* requested for AFB staining and yielded positive culture. BACTEC yielded significantly earlier detection of isolates than LJ. The mean time among negative AFB smear in BACTEC vs LJ was 13.4 days vs 26.4 days (p value < 0.000). Furthermore, the time to detection was shorter with the increasing category/grade of smear positivity. Overall, the mean time of detecting MTB in BACTEC was 9.6 days (standard deviation [SD], 8.9 days) compared to 22.8 days (SD, 14.3 days) for LJ (p value < 0.000).

DISCUSSION

In our study, the broth-based semi-automated radiometric BACTEC TB system showed higher recovery rate and shorter time to detection of mycobacteria compared to the solid-based conventional LJ medium in a clinical laboratory at a tertiary care center in this country. Other advantages of this system include, automated reading of positive bottles, rather than by the manual obser-

TABLE III
TIME TO DETECTION OF MTB FROM CLINICAL SPECIMENS IN BACTEC & LJ vs AFB SMEAR CATEGORY

AFB SMEAR CATEGORY	No. of isolates	Mean days ± SD to MTB recovery in		p value*
		BACTEC	LJ	
NEGATIVE	68	13.4 ± 10.1	26.4 ± 14.8	< 0.000
FEW	32	9.9 ± 8.9	20.1 ± 10.6	< 0.000
MODERATE	19	8.6 ± 6.8	20.4 ± 14.3	< 0.005
NUMEROUS	54	5.0 ± 5.2	20.7 ± 15.0	< 0.000
ALL	173	9.6 ± 8.9	22.8 ± 14.3	< 0.000

* Significant differences, p value 0.05.

vations needed for the solid media, thus, decreasing technologist time, as well as the ability to perform identification and susceptibility directly from the broth bottles, thus, decreasing turnaround time of results. On another hand, this system has a higher cost per test compared to LJ, and though negligible and not requiring major attention, the ^{14}C -labelled palmitic acid or released $^{14}\text{CO}_2$ in the bottles warrant some arrangement for storage and disposal. Despite the latter possible concern and the introduction of alternative non radiometric systems, the BACTEC 460 remains the reference method for automated MTB detection and susceptibility methods.

The 10.7% overall isolation rate of acid-fast bacilli in our study is close to the 9.3% average rate (range from 4.8% to 20.5%) reported in the meta-analysis study by Cruciani et al. [16].

The higher recovery rates of BACTEC vs LJ for MTB (91% vs 77%) and MOTT (90% vs 68%) provides a valuable advantage of BACTEC in avoiding false negative culture results. The recovery of MOTT in our study was 34.4% and this rate falls within the 20-80% reported rates globally. The latter reported wide range of MOTT recovery is attributed to the different geographic regions and types of patients investigated. These bacteria are recognized to be acquiring increasing role as pathogens causing pulmonary disease, disseminated disease, or both in immunocompetent and immunocompromised individuals [4]. Though the importance of MOTT is well recognized, the discussion below will be essentially addressing findings pertaining to MTB.

In our study, the 91% recovery rate of MTB in BACTEC is very close to those reported by Cruciani et al. (90%) [16], Anargyros et al. (95%) [17], Manterola et al. (89.9%) [18], and Somoskovi et al. (92.7%) [19]. On the other hand, the 77% recovery rate of MTB in LJ in our study was lower than those reported by Anargyros et al. (87%) [17], Manterola et al. (98.7%) [18] and Somoskovi et al. (81.8%) [19] but was almost similar to that reported by Cruciani et al. (76%) [16]. The latter rate was generated from a meta-analysis study comparing BACTEC-MGIT and BACTEC-TB with or without solid media for detection of mycobacteria.

The rapid detection of mycobacteria in culture is of crucial importance for prompt diagnosis, treatment and control of TB [5, 8]. Therefore, the introduction of BACTEC TB system was warranted in order to shorten the time needed for detection of such organisms [9]. In regards to turnaround times in our study, BACTEC showed significantly shorter mean detection times than for LJ medium. In BACTEC, the overall average detection time of MTB was 9.6 days, which falls between the 7.3 days and 16.6 days reported in other studies [16-21].

Among smear-positive specimens, the mean detection time in BACTEC was shorter compared to smear-negative specimens as shown in our study (7.2 days vs 13.4 days) and as reported by Cruciani et al. (11.6 days vs 18 days) [16], Somoskovi et al. (13.8 days vs 17.7 days) [19], Pfyfer et al. (9.3 days vs 15.6 days) [22], and

Scarparo et al. (11.7 days vs 21.3 days) [23].

In our study as well as in others, the mean detection times of MTB in BACTEC was shorter than for the conventional LJ medium. The average detection time in LJ in our study was 22.8 days. This falls between the 19.4 days and 26.1 days reported in the literature [16-18, 20].

Among the liabilities of processing specimens for culture is the breakthrough contamination of media (presumptively positive medium subsequently found positive for bacteria other than acid-fast bacilli). In our study, the overall contamination rate (exclusive to respiratory specimens) in BACTEC TB was lower than LJ (3.8% vs 11.4%) among the 3300 specimen processed and cultured in parallel. These findings are comparable to what was reported in the literature. The lower contamination rates in BACTEC TB were also reported by other authors even versus the newer nonradiometric version, BACTEC MGIT. For example, the contamination rates of BACTEC TB, BACTEC MGIT and LJ in comparative studies were reported to be 4.4%, 8.6% and 12.8%, respectively, by Cruciani et al. [16], 11.7%, 13.2%, and 14.7%, respectively, by Huang et al. [21] and 3.7%, 9.9% and 17.75%, respectively, by Tortoli et al. [24].

In our evaluation and despite the fact that the overall recovery rates obtained with the BACTEC TB systems are clearly higher than those achieved with solid media, BACTEC missed 9% of MTB and 10% of MOTT isolates. On the other hand 14% of MTB and 17% of MOTT were missed by LJ. Such a finding was also reported by others [16, 24]. Because of these problems in sensitivity and to maximize recovery of mycobacterial isolates, the combined use of one liquid medium and one solid medium was recommended by the Centers for Disease Control and Prevention, and nowadays the use of such a combination is acknowledged worldwide [25].

In conclusion, this study showed significantly higher rates of recovery and a shorter time to detection of mycobacteria in clinical specimens cultured in BACTEC TB compared to the conventional LJ medium for overall mycobacteria as well as for each AFB smear result. Despite this valuable accuracy of BACTEC and its convenient technology, the combination of liquid-based with solid media, remains the "gold standard" for diagnosis of acid-fast bacilli. Moreover, the BACTEC entails other interesting features including the efficient and rapid differentiation of MTB from MOTT and the reliable susceptibility testing on MTB isolates. These favorable features, coupled with an elevated diagnostic accuracy on almost all of the clinically most important *Mycobacterium* species, make the BACTEC system in combination with conventional solid media a valuable system in the laboratory diagnosis of mycobacterial infections.

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التأكد السريع لتشخيص الانتان بالجرثوم الفطري وفعالية الجرثوم في حالة التدرن (السل). تجربة مركز الاعتماء الثلاثي اللبناني.

موجز الموضوع - إن البحث السريع عن الجرثوم الفطري التدرني وخاصة أصل (أرومة) المتعدد المقاومة مهم للبدء السريع في المعالجة واتخاذ الاحتياطات حنظاً للصحة العامة. يبقى الزرع المخبري الطريقة المختارة لتشخيص الانتان بالجرثوم الفطري وقبول جهاز قياس الاشعاع الألي BACTEC 460 TB للبحث السريع عن الجراثيم الفطرية قُيِّمت وقورنت مع الزرع الاصطلاحي في وسط لوشنتاين - جنسين. الطريقة - وُثِّبت النماذج المرسله لزرع الجرثوم الفطري في وقت واحد على لدا - BACTEC والصفائح المحضرة من الراسب لُوِّثت بطريقة كنيون والتفريق بين الجرثوم الفطري التدرني وبقية الأرومات غير التدرنية استندت إلى اختبار BACTEC - NAP وكلّ المزروعات الجرثومية الايجابية بين كانون الثاني (يناير) ١٩٩٧ وكانون الأول (ديسمبر) ٢٠٠٢ أدخلت في هذه الدراسة.

النتائج - اختبر ٢٣٠٠ نموذجا وكان ٢٥٥ منها (٧, ١٠٪) إيجابياً؛ ٢٣٣ (٦, ٦٥٪) جرثوم فطري تدرني و١٢٢ (٤, ٢٤٪) غير تدرني. النسبة المئوية للصفائح الايجابية AFB (جرثوم مقاوم الحمض بالتلون) كانت ٤٥٪ في النماذج السريرية الايجابية للتدرني و٤٩٪ لغير التدرني. إن أغلبية النماذج الإيجابية للتدرن ٧٢٪ وغير التدرن ٩١٪ مأخوذة من المسالك الهوائية. وتبين ان BACTEC هو أسرع من لوشنتاين - جنسين للبحث عن الجرثوم الفطري (وسطياً ٩, ٦ يوماً بواسطة BACTEC مقابل ٢٢, ٨ يوماً للوشنتاين - جنسين ومدة البحث بواسطة BACTEC كانت متناسبة عكساً للدرجة الإيجابية للجراثيم مقاومة الحمض. النسبة الإيجابية المئوية لطريقة BACTEC ٩١٪ وللوشنتاين - جنسين ٧٧٪.

الخلاصة - BACTEC أكثر حساسية وفعالية وأسرع من لوشنتاين - جنسين لتشخيص الانتانات بالجرثوم الفطري وهذه الطريقة تسمح بالتفريق السريع بين الجرثوم الفطري التدرني وغير التدرني ومع ذلك ينصح باستعمال BACTEC ولوشنتاين - جنسين معاً للوصول إلى نتيجة فائقة. إن سرعة النتائج هامة سريرياً لمن يعمل في ميدان الصحة العامة ولتدريبهم على اتخاذ سريعاً وسائل المراقبة الصحية للانتان.