J. Med. Microbiol. — Vol. 47 (1998), 821–827

DIAGNOSTIC MICROBIOLOGY

Clinical evaluation of the Mycobacteria Growth Indicator Tube (MGIT) compared with radiometric (Bactec) and solid media for isolation of *Mycobacterium* species

W. K. CHEW, R. M. LASAITIS, F. A. SCHIO and G. L. GILBERT

Mycobacterium Reference Laboratory, Centre for Infectious Diseases and Microbiology Laboratory Services, Institute for Clinical Pathology and Medical Research, Westmead Hospital, Sydney, New South Wales 2145, Australia

The aim of this study was to evaluate the clinical use of a new culture system for the isolation of mycobacteria. Routine clinical specimens were cultured in the Mycobacteria Growth Indicator Tube, the radiometric Bactec 460 TB system and on Lowenstein Jensen (LJ) medium to compare recovery rates and times for detection of mycobacteria and contamination rates. MGIT was tested for its ability to support the growth of a wide range of mycobacterial species. Acid-fast bacilli (AFB) were detected on direct smears of 76 of 603 clinical specimens and mycobacteria were isolated by at least one method from 109 specimens; 93% of these were detected in the MGIT, 95% in the Bactec 460 TB system and 87% on LJ medium. The MGIT, Bactec and LJ media detected 92%, 97% and 95%, respectively, of 61 *M. tuberculosis* isolates and 94%, 94% and 77% of the 48 isolates belonging to the *M. avium* complex (MAC). The mean detection times in MGIT, Bactec and LJ media for *M. tuberculosis* were 22, 14 and 27 days respectively, and for MAC were 14, 12, and 29 days, respectively. Growth of *M. tuberculosis* was detected in Bactec, within 4 weeks, in 93% of the 61 culture-positive specimens, compared with only 61% in MGIT and 66% on LJ. The number of MAC detected within 4 weeks was similar in Bactec and MGIT, but less in LJ medium. Differences in sensitivity and time to detection of growth between media were greater for specimens in which AFB were not detected on direct smear than those on which AFB were seen. Contamination rates were similar in the three systems (3–4%). MGIT supported the growth of all 28 *Mycobacterium* spp. inoculated. MGIT has significant safety advantages and is less labour intensive than other methods, but the time to detection of *M. tuberculosis*, especially in smear-negative specimens, was longer in MGIT than in Bactec.

Introduction

A presumptive diagnosis of tuberculosis sometimes can be made on the basis of a patient's medical history, clinical and radiological findings and the presence of acid-fast bacilli (AFB) on sputum smear. However, in our experience, acid-fast bacilli are detectable in direct smears of only *c.* 60% of specimens from which mycobacteria are isolated subsequently. Culture is required to confirm the diagnosis of clinically suspected tuberculosis and is the mainstay of diagnosis of clinically atypical tuberculosis or non-tuberculous mycobacterial infection. Nucleic acid amplification techniques can be used for rapid diagnosis of tuberculosis by direct detection of *M. tuberculosis*, and several systems are commercially available [1]. However, culture is still required for identification of non-tuberculous *Mycobacterium* spp. and susceptibility testing of *M. tuberculosis* and other species.

Many studies have demonstrated significantly reduced times for detection of mycobacteria with the use of liquid media rather than solid egg or agar-based media [2]. The Bactec 460 TB system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) is the most commonly used mycobacterial broth culture system and is regarded widely as the gold standard [3]. However, it has several disadvan-

Received 19 Aug. 1997; revised version accepted 17 Dec. 1997.
Corresponding author: Dr W. K. Chew.

tages: it involves the use of radioactive materials (bacterial growth causes release of $^{14}CO_2$ into the head space above the broth, which is sampled by a needle to penetrate the seal and detected by the reader); reading of cultures is relatively labour intensive and associated with a risk of cross-contamination of cultures [4] or needlestick injury; and purchase and maintenance of the instrumentation is expensive. The Mycobacteria Growth Indicator Tube (MGIT, Becton Dickinson Microbiology Systems) is similar to the Bactec 12B medium used in the Bactec 460 TB in that it contains modified Middlebrook 7H9 broth to which oleic acid-albumin-dextrose-catalase (OADC) enrichment and PANTA antibiotic supplement (polymyxin, azlocillin, nalidixic acid, trimethoprim and amphotericin B) are added. However, the detection system is a silicon rubber disk impregnated with ruthenium pentahydrate, a fluorescent indicator whose natural fluorescence is quenched in the presence of oxygen. Bacterial growth utilises oxygen in the medium and the indicator fluoresces. Cultures can be read rapidly, in batches, with a 365-nm UV transilluminator Wood's lamp. However, published experience with the routine use of this system in the clinical laboratory is still limited [5–8].

The present study aimed to compare isolation rates and times for detection of mycobacteria from clinical specimens in the MGIT system with those in the Bactec 460 TB system and on Lowenstein Jensen (LJ) medium. The latter two media are currently used routinely in this Mycobacterium Reference Laboratory, where *c.* 6500 specimens are cultured each year and mycobacteria are isolated from *c.* 300 of these. In addition, the ability of MGIT to support the growth of 28 different mycobacterial species was assessed.

Materials and methods

All procedures involving handling of mycobacterial cultures were performed in a physical containment level 3 laboratory. Clinical specimens were processed in a dedicated laboratory separated from other laboratories.

Selection of specimens

Specimens were selected from those received by the National Mycobacterium Reference Laboratory (NMRL) during a 9-month period (Jan. 1996–Sept. 1996) and included all specimens from normally sterile sites and all specimens in which acid-fast bacilli (AFB) were seen on direct smear. In addition, smear-negative specimens were selected from patients in whom there was a high clinical suspicion of mycobacterial infection. Blood cultures, which are normally inoculated directly into Bactec 13A bottles at the time of collection, were not included.

Specimen and smear preparation

Specimens from non-sterile sites, such as respiratory specimens (sputum and bronchial washings), pus and faeces were decontaminated before inoculation with N-acetyl-L-cysteine-0.5 % w/v, NaOH 2 % w/v, (NALC-NaOH) [9]. An equal volume of NALC-NaOH was added to the specimen and the tube was vortex mixed for 5–20 s, inverted to mix thoroughly and left to stand for 20 min. The reagent–specimen mixture was then diluted with sterile distilled water to 50 ml and centrifuged at 3000 *g* for 15 min. After the supernate was completely decanted, the pellets were resuspended in 2 ml of 0.067 M phosphate buffer, pH 6.8. Smears were prepared from this and stained for the presence of acid-fast bacilli with the Ziehl-Neelsen stain [3].

Urine and other non-sterile fluids were concentrated by centrifugation and the deposit was decontaminated with an equal volume of NALC-NaOH, as for sputum. Smears were not prepared from urine or swabs as acid-fast bacilli are too scanty to be detected in these specimens. Samples from sterile sites (mainly tissues) and normally sterile body fluids were not decontaminated unless other bacteria were likely to be present, as suggested by a coffee colour and purulent appearance of tissue, cloudiness of body fluid or bacteria seen in a Gram-stained smear. Impression smears were prepared from tissue specimens which were then cut into pieces with sterile scissors and homogenised in a mortar and pestle with sterile sand.

Control organisms

Twenty-four reference cultures were obtained from the Trudeau Mycobacterial Culture Collection (TMC) (12301 Parklawn Drive, Drockville, MD 20852, USA), one isolate of *M. thermoresistible* was supplied by State Health Laboratory, Brisbane, Queensland (SHLQ) for quality assurance purposes and three strains (*M. africanum*, *M. bovis* BCG and *M. terrae* complex) had been isolated and identified in this laboratory (NMRL) (Table 1).

All stock cultures had been stored frozen at −70°C before this study. They were subcultured on to LJ media to confirm their viability. Cultures were incubated at 37°C, except for *M. xenopi*, and *M. shimoidei* which were incubated at 40°C, and *M. marinum* and *M. haemophilum* at 30°C. Media were supplemented with haemin 7 μg/ml of broth (FOS™ Bactec culture supplement kit, BBL, Becton Dickinson), for culture of *M. haemophilum.*

Preparation of organisms

Mycobacterial stock cultures were subcultured on to LJ slopes and incubated for 2–3 weeks until growth was visible. Organisms were harvested and transferred to a sterile 7-ml screw-capped bottle containing five glass

Table 1. Organism source and time to detection of growth in MGIT of 28 *Mycobacterium* spp. after inoculation of *c.* 10^4 cfu/ml

Organism	Source and ref. no.	Time to detection (days)	Organism	Source and ref. no.	Time to detection (days)
M. tuberculosis	TMC201	21	*M. africanum*	NMRL	18
M. bovis	TMC	28	BCG	NMRL	24
M. asiaticum	ATCC25276	21	*M. kansasii*	TMC1201	15
M. marinum	ATCC927	5	*M. flavescens*	TMC1541	13
M. gordonae	TAMC1319	9	*M. scrofulaceum*	ATCC19981	9
M. paraffinicum	ATCC12670	14	*M. szulgai*	ATCC35799	9
M. avium	TMC1403	9	*M. intracellulare*	TMC1405	12
M. gastri	TMC1456	10	*M. malmoense*	ATCC29571	21
M. nonchromogenicum	ATCC19530	8	*M. shimoidei*	ATCC27962	21
M. simiae	ATCC25275	5	*M. terrae*	NMRL	8
M. triviale	TMC1453	18	*M. xenopi*	ATCC19250	21
M. haemophilum	ATCC29548	22	*M. abscessus*	ATCC19977	5
M. chelonae	ATCC35752	5	*M. fortuitum*	TMC1530	5*
M. peregrinum	ATCC14467	5	*M. thermoresistibile*	SHLQ	5*

*These species were only acid-fast but not acid alcohol-fast in the Ziehl Neelsen stain.

beads and 1 ml of distilled water, vortex mixed for 3 min and allowed to stand for 20 min. The supernatant fine suspension was transferred to a glass centrifuge tube and adjusted with normal saline to match a 1.0 McFarland standard to produce a suspension equivalent to 10^7 cfu/ml for *M. tuberculosis* complex and 10^8 cfu/ml for non-tuberculous mycobacteria [8]; 5-ml suspensions containing 10^4 cfu/ml were prepared in saline and processed as if they were sputum. The final pellets were resuspended in 2.5 ml of phosphate buffer, pH 6.8, and 0.5 ml of this was inoculated into MGIT.

Media and culture techniques

Approximately 0.2 ml of each sample concentrate was inoculated on to each of two LJ slopes (one with added glycerol 0.75% and one with pyruvate 0.45%). Sample concentrate (0.5 ml) was inoculated into a Bactec 12B vial containing 4 ml of broth supplemented with polymyxin B 50 IU/ml, amphotericin B 5 μg/ml, nalidixic acid 20 μg/ml, trimethoprim 5 μg/ml, azlocillin 5 μg/ml (PANTA supplement, Beckton Dickinson) and polyoxyethylene stearate 0.1 mg/ml (Becton Dickinson). Before inoculation, the atmosphere in the vial was enriched with CO_2, according to the manufacturer's instructions. For the MGIT system, 0.5 ml of sample concentrate was inoculated into a MGIT tube containing 4 ml of broth supplemented with 0.5 ml of MGIT OADC (Becton Dickinson) and polymyxin B 50 IU/ml, amphotericin B, 5 μg/ml, nalidixic acid 20 μg/ml, trimethoprim, 5 μg/ml, azlocillin 10 μg/ml (MGIT PANTA, Becton Dickinson). All media were incubated at 36 ± 1°C. Duplicate cultures of specimens taken from skin or wounds were incubated at 30°C for each of the three methods. If *M. haemophilum* was suspected (specimens from immunocompromised patients), an extra set of all media containing added haemin (Bactec FOS™) was inoculated and incubated at 30°C.

Cultures were inspected three times weekly for growth of mycobacteria for up to 8 weeks for AFB smear-negative and up to 12 weeks for AFB smear-positive samples. Growth of mycobacteria in Bactec 12B vials was detected by the Bactec 460 TB reader to detect a growth index of >50 and in the MGIT system by exposing the tubes to a Wood's lamp to demonstrate fluorescence. When growth was detected, smears were made from the broth after they had been thoroughly vortex mixed. The smears were stained by the Ziehl-Neelsen method [3] and examined for the presence of AFB. The time to detection, expressed in days, i.e., the incubation time at which AFB were found in smears made from media, was determined for all isolates and methods.

Identification of isolates

All presumptive mycobacterial isolates were identified by AccuProbe™ (Gen-Probe, San Diego, CA, USA) or standard biochemical tests [3].

Statistical analysis

The sensitivities of individual culture media were expressed as percentages of the total number of positive cultures in any medium. Differences between sensitivities were compared by χ^2 or Fisher's exact test, as appropriate. Mean times to detection of growth were compared by Student's t test. Differences between the mean times to detection of mycobacterial growth between smear-negative specimens and smear-positive specimens in all media were calculated by the Kruskal-Wallis test for two groups with the computer software EpiInfo 5 [10].

Results

A total of 603 clinical specimens was processed during this study (Table 2). These included 160 respiratory specimens (sputum and bronchoalveolar lavage fluids); acid-fast bacilli were seen in smears of 70 and mycobacteria were isolated from 77. There were 443 non-respiratory specimens, from 32 of which (including

Table 2. Specimen type, microscopy and culture results for 603 clinical specimens

Specimen type	Number of specimens	Number smear-positive	Number culture-positive	Number from which *M. tuberculosis* isolated	Number from which MAC isolated
Sputum	149	68*	74	51	23
BAL	11	2	3	1	2
Serous fluids†	55	1*	0	–	–
Tissue biopsies‡	351	3	29	7	22
Miscellaneous§	37	2	3	2	1
Total	603	76	109	61	48

MAC, *M. avium* complex; BAL, bronchoalveolar lavage.
*One smear-positive specimen each of sputum and pleural fluid was culture-negative in all three media.
†Forty-three serous fluids were pleural fluids.
‡223 tissue biopsies were lymph nodes, 26 were skin and six were bone (one bone was culture-positive)
§This included 2 cerebrospinal fluid; 3 faeces; 1 urine; 6 pus; *M. tuberculosis* was isolated from two specimens of pus and *M. avium* complex from one faecal specimen.

29 tissue specimens) mycobacteria were isolated; acid-fast bacilli were seen on smears of only six. Because of the method of specimen selection, the yield of mycobacteria was much higher than usual for this laboratory (generally, 3–5% of all unselected specimens are culture-positive). Mycobacteria were not isolated in any medium from one-smear-positive sputum specimen and one smear-positive pleural fluid. In both cases previous specimens from these patients had been culture-positive and treatment had been started. The sensitivity of acid-fast stain was 90% (69 of 77) for respiratory specimens and 16% (5 of 32) for non-respiratory specimens, or 68% overall. The specificity of acid-fast stain was 97%.

Sixty-one isolates were identified as *M. tuberculosis* and all 48 non-tuberculous isolates as belonging to MAC. Recovery rates of isolates from each of the three media are shown in Table 3. The difference in sensitivity between Bactec and LJ was statistically significant for all isolates (104 of 109 *versus* 95 of 109 respectively; $p = 0.02$, Fisher's exact test) and for isolates from smear negative specimens (33 of 35 *versus* 25 of 35, respectively; $p = 0.01$, Fisher's exact test), but not for those from smear positive specimens (71 of 74 *versus* 70 of 74, respectively, not significant). The differences in isolation rates between Bactec and MGIT or MGIT and LJ were not significant overall or for any subgroup of isolates. The sensitivities of either combination of liquid plus solid media – Bactec plus LJ or MGIT plus LJ – were similar (98% and 97% respectively).

The mean and median times to detection of mycobacterial growth in each system are shown in Table 4. They were significantly longer ($p = < 0.05$, Kruskal-Wallis test) for cultures of smear-negative specimens than for those of smear-positive specimens in all media; there were wide ranges of times in all groups. Growth of *M. tuberculosis* was detected in Bactec, on average, 8 days and 13 days sooner than in MGIT and LJ, respectively. MGIT detected growth of *M.*

Table 3. Isolation of mycobacterium from clinical specimens in three media

	M. tuberculosis isolated		*M. avium* complex isolated	
Medium	smear-positive specimen (%) (n = 46)	smear-negative specimen (%) (n = 15)	smear-positive specimen (%) (n = 28)	smear-negative specimen (%) (n = 20)
MGIT	43 (93)	13 (87)	27 (96)	18 (90)
Bactec	45 (98)	14 (93)	26 (93)	19* (95)
LJ	46 (100)	12 (80)	24 (86)	13* (65)
LJ/Bactec	46 (100)	14 (93)	28 (100)	19 (95)
LJ/MGIT	46 (100)	15 (100)	27 (96)	18 (90)

*Difference between Bactec and LJ significant ($p \leq 0.02$; Fisher's exact test).

Table 4. Average times for detection* of mycobacterial growth in three media

	M. tuberculosis isolates			*M. avium* complex isolates		
Medium	smear-positive specimens (n = 46)	smear-negative specimens (n = 15)	all specimens (n = 61)	smear-positive specimens (n = 28)	smear-negative specimens (n = 20)	all specimens (n = 48)
MGIT	19/16 (6–54)	32/32 (20–49)	22/19†	12/8 (4–37)	17/16 (5–54)	14/11†
Bactec	12/11 (3–33)	20/18 (6–51)	14/11†	10/6 (2–38)	15/14 (3–32)	12/9†
LJ	25/24 (11–54)	33/35 (20–42)	27/26	26/26 (14–45)	36/38 (14–54)	29/27

*Mean/median time to detection (range) in days.
†Differences compared with LJ significant ($p \leq 0.01$; t-test).

tuberculosis from smear-positive specimens 6 days earlier than LJ, but there was no difference between them in the time to isolation of *M. tuberculosis* from smear-negative specimens. Growth of MAC was also detected more rapidly in both MGIT and Bactec (15 and 17 days sooner, respectively) than on LJ.

Isolates were detected in all systems up to the last (eighth) week of incubation. However, in the first 4 weeks of incubation, 57 (93%) of *M. tuberculosis* isolates from the 61 culture-positive specimens were isolated in Bactec, compared with only 37 (61%) from MGIT and 40 (66%) from LJ ($p < 0.001$) (Fig. 1). The recovery of MAC isolates from the 48 culture-positive specimens in the first 4 weeks was not significantly different in MGIT and Bactec (41, 85% and 39, 81% respectively), but was significantly less in LJ (27, 56%; $p < 0.01$) (Fig. 2).

Contaminants were isolated from 27 specimens (4.2%), including 18 (11%) of 169 respiratory and other potentially contaminated specimens and 9 of 434 specimens (2%) from normally sterile sites. Contamination rates did not differ significantly between the three systems (MGIT 4.1%; Bactec 3%; LJ 4.1%).

MGIT supported the growth of all *Mycobacterium* species in numbers comparable with those found in clinical specimens [11, 12] and growth was detectable after 5–28 days (Table 2).

Discussion

This study has shown that, in common with other broth media, MGIT is generally more sensitive than solid media and can detect mycobacterial growth more rapidly. Overall, the sensitivity of MGIT was comparable with that of Bactec 460 TB, and both were better than LJ, although the difference was significant only between Bactec and LJ. The greatest overall difference between both liquid media and LJ was in the time required for detection of growth. Growth of MAC was detected at least 2 weeks sooner and >80% of these isolates were detected within the first 4 weeks in liquid media compared with only 56% on LJ.

Growth of *M. tuberculosis* was detected nearly 2 weeks sooner in Bactec than LJ from both smear-positive and smear-negative specimens. Of the total isolates of *M. tuberculosis* from all specimens, 93% were detected in Bactec in the first 4 weeks of incubation, only 61% and 66% were detected within 4 weeks by MGIT and LJ, respectively. However, although the time to detection of growth of *M. tuberculosis* from smear-positive specimens was 6 days less in MGIT than LJ, growth from smear-negative specimens took a similar time in both. This is a potentially serious disadvantage of MGIT, as detection of mycobacterial growth from a smear-negative specimen may be the first clue to the diagnosis of tuberculosis.

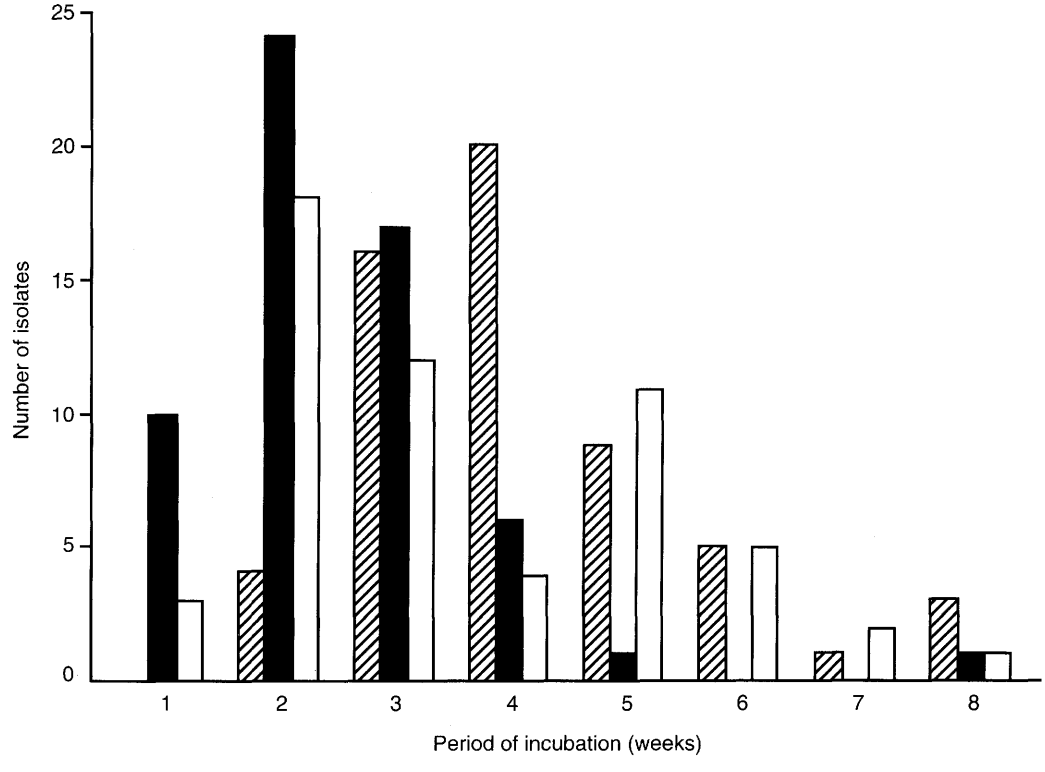

Fig. 1. New isolates of *M. tuberculosis* during incubation for up to 8 weeks by LJ solid culture (▨), Bactec 460 TB (■) and MGIT (□).

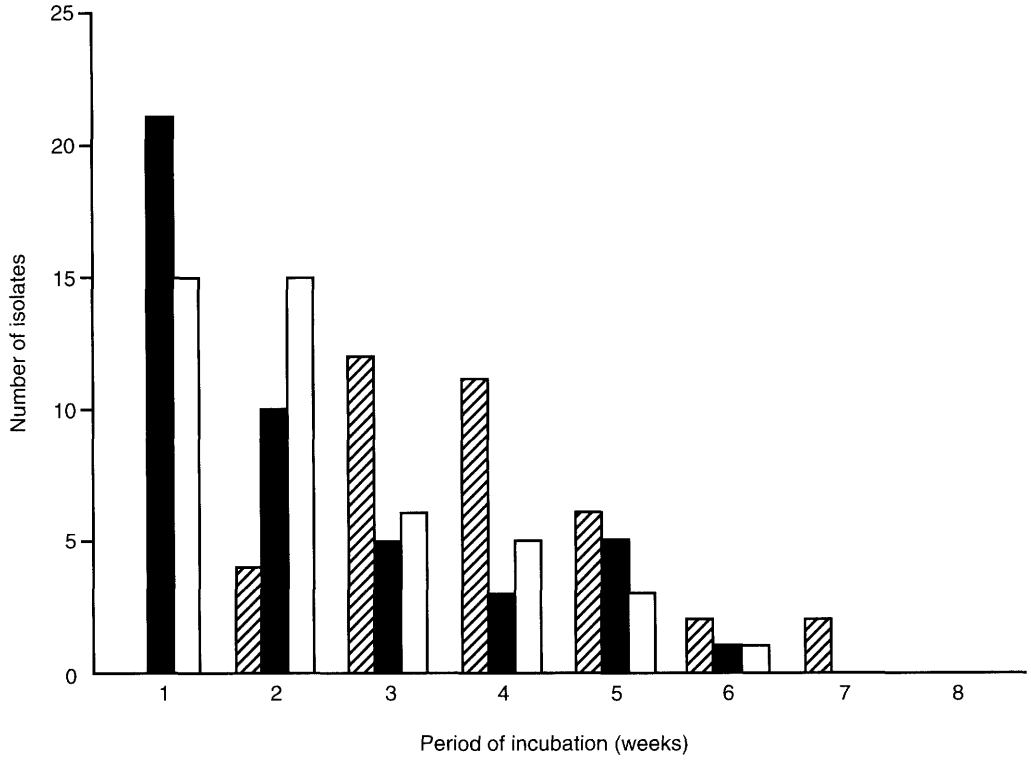

Fig. 2. New isolates of MAC during incubation for up to 8 weeks by LJ solid cultures (▨), Bactec 460 TB (■) and MGIT (□).

Two previous studies have compared MGIT with both Bactec 460 TB and solid media for isolation of mycobacteria from clinical specimens [7, 8]. The present study attempted to avoid the biases reported in these earlier studies caused by differences in inoculum size, frequency of reading cultures or periods of incubation between different media. In this study, all three types of media were inoculated with similar volumes of specimen (0.4 ml for two LJ slopes compared with 0.5 ml for the broths), read at the same frequency (three times a week) and incubated for the same period (8 weeks). This study also differed from the other two in that a greater proportion of specimens cultured were non-respiratory (73% *versus* 21% [7] and 29% [8]) and a greater proportion were smear positive (68% *versus* 52% [7] and 39% [8]). Despite these differences the results of the present study were generally comparable with the previous studies. The sensitivities of all three media were higher in this study than in the other two (93%, 95% and 87% for MGIT, Bactec and LJ, respectively *versus* 85%, 85%, 77% [6] and 76%, 88%, 69% [7]) and there were fewer isolates that grew from only one medium, which probably reflects the higher proportion of smear-positive samples. In common with previous studies, the present study showed that a greater proportion of mycobacteria were isolated in both MGIT and Bactec than on LJ from specimens in which AFB had not been seen on direct smear, indicating lower concentrations of bacteria in these specimens. None of the three media alone detected all culture-positive specimens and, ideally, a combination of liquid and solid media should be used for culture of mycobacteria. Either combination of LJ and Bactec or LJ and MGIT gave similar isolation rates overall.

The ability of MGIT to support the growth of a wide range of other *Mycobacterium* spp. was confirmed in this study with inocula similar to those found in clinical specimens (*c.* $5 \times 10^3 - 10^4$ cfu/ml are required for a positive smear for AFB) [13]. The times taken for detection of mycobacterial growth in MGIT from these inocula were similar to those from clinical specimens.

Falsely positive results can be due to contamination with bacteria other than mycobacteria, fungi or a positive reading (fluorescence of the MGIT or growth index reading above threshold in Bactec system) with no growth on subculture. In this study, contamination rates were similar in all three media. Rates vary in different centres (for example, from 2% to 14% in MGIT in different centres participating in a multicentre study [8]) and with different media (e.g., 14% in MGIT *versus* 6% on solid media for the same specimens [8]). However, it probably depends more on delays in transport and processing of specimens and the method of decontamination than the media used. Both broth media contain a similar cocktail of antibiotics which inhibit most potential contaminants providing that the numbers in the inoculum are limited by rapid processing and effective decontamination of

the specimen. Fluorescence of MGIT tubes in the absence of growth did not occur in the present study, but has been reported by others [7].

MGIT shares the advantages of other liquid media, including Bactec, over solid egg-based media, in that recovery of isolates is faster, overall. However, for smear-negative specimens from which *M. tuberculosis* was isolated, growth was significantly slower in MGIT than in Bactec and similar to that in LJ, which could significantly delay the diagnosis of tuberculosis in some cases. Nevertheless, MGIT overcomes most of the practical disadvantages of the Bactec 460 TB system in that it does not involve radioactive materials, the use of needles or, necessarily, the use of expensive instrumentation. Reading the cultures visually was less time-consuming than reading cultures in the Bactec 460 TB system (saving an estimated 4 h in reading 250 cultures) and detection of growth was easier than on LJ slopes. The Bactec 960 automated continuous reading incubation system for MGIT cultures will soon be available and should be a significant improvement. This is likely to reduce the time to detection of mycobacterial growth and will further reduce labour compared with the Bactec 460 TB system. The choice between MGIT and Bactec 460 TB radiometric system will depend on the requirements of the laboratory and the cost of culture bottles. Despite the somewhat longer detection times, the practical advantages of MGIT make it an attractive alternative for smaller laboratories and, when automated readers become available, may ultimately replace the Bactec 460 TB.

References

1. Bennedsen J, Thomsen VO, Pfyffer GE *et al.* Utility of PCR in diagnosing pulmonary tuberculosis. *J Clin Microbiol* 1996; **34**: 1407–1411.
2. Abe C, Hosojima S, Fukasawa Y *et al.* Comparison of MB-Check, BACTEC, and egg-based media for recovery of mycobacteria. *J Clin Microbiol* 1992; **30**: 878–881.
3. Nolte FS, Metchock B. *Mycobacterium* In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds) Manual of clinical microbiology, 6th edn. Washington DC, ASM Press. 1995: 400–437.
4. Costa R, March F, Garrigo M, Moreno C, Coll P. Demonstration of a cross contamination in the 460TB *Bactec* system by molecular typing of the strains with IS6110 (letter). *Enferm-Infecc-Microbiol-Clin* 1995; **13**: 321–323.
5. Hanna BA, Walters SB, Bonk SJ, Tick LJ. Recovery of mycobacteria from blood in Mycobacteria Growth Indicator Tube and Lowenstein-Jensen slant after lysis-centrifugation. *J Clin Microbiol* 1995; **33**: 3315–3316.
6. Palaci M, Ueki SYM, Sato DN *et al.* Evaluation of Mycobateria Growth Indicator Tube for recovery and drug susceptibility testing of *Mycobacterium tuberculosis* isolates from respiratory specimens. *J Clin Microbiol* 1996; **34**: 762–764.
7. Badak FZ, Kiska DL, Setterquist S, Hartley C, O'Connell MA, Hopfer RL. Comparison of Mycobacteria Growth Indicator Tube with BACTEC 460 for detection and recovery of mycobacteria from clinical specimens. *J Clin Microbiol* 1996; **34**: 2236–2239.
8. Pfyffer GE, Welscher H-M, Kissling P *et al.* Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. *J Clin Microbiol* 1997; **35**: 364–368.
9. Kubica GP, Dye WE, Cohn ML, Middlebrook G. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodium hydroxide for culture of mycobacteria. *Am Rev Respir Dis* 1963; **87**: 775–779.
10. Dean AG, Dean JA, Burton AH, Dicker RC. EpiInfo 5 programme for IBM-compatible microcomputers. Division of Surveillance and epidemiologic studies, Epidemiology program office, CDC; Atlanta, Georgia 30333 and The Global programme on AIDS, WHO, Geneva, Switzerland.
11. Stitt DT, Kodsi SE. A rapid method for the growth and detection of mycobacteria in clinical and stock cultures. Abstract C-115. In: Abstracts of the 94th General Meeting of the American Society for Microbiology. Washington, DC, ASM Press. 1994; 510.
12. BBL (R) MGIT™ products for the detection of mycobacteria. 88-0950-1 Revised: August 1996.
13. David HL. Bacteriology of the mycobacterioses. Atlanta, Centers for Disease Control, PHS, HEW, 1976. (Available from Superintendent of Documents, US Government Printing Office, Washington, DC 20402.)