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Multicenter Comparison of ESP Culture System II with BACTEC 460TB and with Lowenstein-Jensen Medium for Recovery of Mycobacteria from Different Clinical Specimens, Including Blood

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The recently developed ESP Culture System II (AccuMed, Chicago, Ill.) was compared with radiometric BACTEC 460TB (Becton Dickinson, Towson, Md.) and with Lowenstein-Jensen medium for recovery of mycobacteria from over 2,500 clinical specimens both of respiratory and nonrespiratory origin, including blood. The majority of the 219 mycobacterial isolates (129) belonged to the *Mycobacterium tuberculosis* complex, followed by 37 isolates of the *Mycobacterium avium* complex (MAC) and 53 isolates of eight other mycobacterial species. Rates of recovery obtained with BACTEC, ESP, and Lowenstein-Jensen medium were 89, 79, and 64%, respectively, with such differences being statistically significant. Different media and systems appeared to behave differently when the more frequently detected organisms were considered: *M. tuberculosis* complex isolates grew better with BACTEC, and MAC isolates grew better with ESP. An analysis of the combinations of Lowenstein-Jensen medium with BACTEC and with ESP did not reveal significant differences in recovery rates. With regard to the times needed for the detection of positive cultures, they were significantly longer on Lowenstein-Jensen medium (average, 28 days) than with the remaining two systems, between which there was no difference (average, 18 days). We conclude, therefore, that the ESP system, when used in combination with a solid medium, performs as well as the thoroughly validated radiometric BACTEC system and offers the advantages of full automation and absence of radioisotopes.

Tuberculosis still represents, among infectious diseases, the main cause of morbidity and mortality (9), and in recent years the goal of eradication has become more distant both in the third world and in developed countries (1).

A prompt diagnosis of active disease represents the best measure to prevent the spreading of tuberculosis (13) and has become particularly important because of the emergence of hardly treatable forms due to multidrug-resistant *Mycobacterium tuberculosis* (4).

The prevalence of tuberculosis is extraordinarily high among AIDS patients, but nontuberculous mycobacterioses too, mainly the ones due to the *Mycobacterium avium* complex (MAC), may seriously threaten the survival and quality of life of human immunodeficiency virus-infected patients (3).

It has been reported that amplified nucleic acid assays for the detection of the *M. tuberculosis* complex are not highly sensitive (16) and that skilled personnel are required (8). Therefore, they are an adjunct to, and not a substitute for, the conventional procedures of microscopy and culture.

The isolation of mycobacteria in culture remains the most reliable diagnostic tool, and it is the indispensable starting point for identification and susceptibility testing. Solid media still play an irreplaceable role in mycobacterial culture, not only because of their cost-effectiveness but also because in many cases they allow a prompt recognition of contaminations and mixed cultures; they do not, however, aid in the reduction of turnaround times, which is currently considered the primary requirement (13). Liquid media appear to be the best answer to this need, as demonstrated by the radiometric BACTEC system (Becton Dickinson, Towson, Md.), which has been successfully used worldwide for the last 20 years (5, 7). Despite its enviable record, radiometry cannot be considered the optimal solution, mainly because of rules regulating the use of radiolabeled materials which, in many situations, may represent an insuperable hindrance.

The aim of this investigation was to compare the performance of the newly developed, fully automated method for the culture of mycobacteria, the ESP Culture System II (AccuMed, Chicago, Ill.) with that of the BACTEC method and with that of conventional solid media.

(These results were partially presented at the meeting Resistance to Antimicrobial Agents, Monte Carlo, Principality of Monaco, 1997.)

MATERIALS AND METHODS

The investigation was performed in four Italian centers with 2,673 clinical specimens (Table 1) of both respiratory and nonrespiratory origin, including blood (Table 2).

Specimens from nonsterile body sites were processed according to the standard *N*-acetyl-L-cysteine–NaOH digestion-decontamination method (7); after neutralization and centrifugation (3,000 × *g* for 15 min) the pellet was resuspended in 2 ml of distilled water and used for preparation of an acid-fast smear and for inoculation of different culture media. Mechanically homogenized biopsies and fluids from sterile body sites were processed as indicated above but

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TABLE 1. Specimens processed by single centers

Center	No. of samples	% Positive
A	899	5.78
B	900	11.00
C[a]	411	1.70
D[b]	463	8.64
Total	2,673	8.19

[a] Over 50% were blood specimens.
[b] The isolation rate for center D has been corrected by deleting the 24 isolates from the pseudo-outbreak of *M. gordonae.*

without decontamination. Blood samples were collected in both a heparin-containing tube and an Isolator tube (Wampole Laboratories, Cranbury, N.J.) for lysis centrifugation (11). Smears were not prepared from blood.

Media used for the comparison included ESP bottles, BACTEC 12B or 13A vials, and Lowenstein-Jensen (L-J) slants. ESP bottles contain a modified Middlebrook 7H9 medium, which is enriched prior to use with OADC (oleic acid, albumin, dextrose, and catalase) growth supplement (Myco GS) and with Myco PVNA (polymyxin B, vancomycin, nalidixic acid, and amphotericin B). The bottles also contain a compressed sponge submerged in the broth, which provides a growth support platform. BACTEC vials contain radiolabeled Middlebrook 7H12 broth and are also supplemented before inoculation. The 12B vials, which were used for all specimens other than blood, were supplemented with PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin). The 13A vials, used for direct inoculation of whole blood, were supplemented with the provided albumin solution.

Inoculum sizes were as follows: 0.5 to 1 ml for ESP bottles, 0.5 ml for BACTEC 12B vials, 5 ml of unprocessed blood for BACTEC 13A vials, and 4 drops (approximately 0.1 ml) for each of two L-J slants.

ESP bottles were incubated at 35°C in the instrument for 42 days or until a positive signal was obtained.

BACTEC vials were incubated at 36°C and read with the BACTEC 460TB instrument, twice in each of the first 2 weeks and subsequently once a week; the final reading of negatives was made on the 42nd day. Cultures presenting a growth indexes (GI) >10 (>20 for blood specimens) were read daily until the achievement of GI ≥100.

L-J tubes were incubated at 36°C for a maximum of 56 days and inspected weekly.

From colonies grown on slants, from positive ESP bottles, and from BACTEC vials presenting GI ≥100, two smears each were prepared, one with Ziehl-Neelsen acid-fast stain and one with Gram stain; the latter staining was performed for recognition of contaminants. The time to detection of positive cultures was recorded at the moment of microscopic confirmation of the presence of acid-fast bacilli. Cultures which grew other rods or cocci were considered contaminated. The incubation of bottles in which no organism was microscopically detectable was continued but, in the case of a further positive signal accompanied by the absence of visible organisms, the cultures were considered false positives. Bottles positive for mycobacteria were subcultured on solid media, and subsequent colonies were identified with commercial DNA probes (AccuProbe, San Diego, Calif.) (12) or, when the probe results were negative, by conventional biochemical and cultural tests (7) and by high-performance liquid chromatography of cell wall mycolic acids (2).

A specimen was considered to be positive when a mycobacterium was isolated on any of the cultures seeded; recovery rates (sensitivities) were computed as the percentage of specimens scoring positive versus the number of positive specimens tested. The differences between recovery rates were compared by the χ^2 method or Fisher's exact test, when appropriate, while differences between mean detection times were analyzed by Student's *t* test.

TABLE 2. Breakdown of the study samples by kind of specimen

Specimen type	No. of samples	% Positive
Sputum	1,009	11.59
Urine	475	1.05
Bronchial aspirate	315	13.33
Brochoalveolar lavage	221	9.05
Blood	221	2.71
Pleural fluid	137	2.19
Stools	100	16.00
Pus	43	2.32
Biopsy	33	9.09
Ascitic fluid	29	3.45
Gastric juice	17	29.41
Cerebrospinal fluid	11	0
Others	62	0
Total	2,673	8.19

TABLE 3. Mycobacteria isolated

Organism	No. (%) of isolations		
	Total	Respiratory	Nonrespiratory
M. tuberculosis complex	129 (58.90)	115 (64.24)	14 (35.00)
MAC	37 (16.89)	17 (9.50)	20 (50.00)
M. gordonae	32 (14.61)	30 (16.76)	2 (5.00)
M. xenopi	13 (5.93)	13 (7.26)	
M. kansasii	3 (1.37)		3 (7.50)
M. fortuitum	1 (0.46)	1 (0.56)	
M. genavense	1 (0.46)		1 (2.50)
M. lentiflavum	1 (0.46)	1 (0.56)	
M. simiae	1 (0.46)	1 (0.56)	
M. terrae	1 (0.46)	1 (0.56)	
Total	219 (100)	179 (100)	40 (100)

RESULTS

Of 219 mycobacteria isolated, 129 belonged to the *M. tuberculosis* complex (Table 3); the MAC and *Mycobacterium gordonae* were the most frequently represented nontuberculous mycobacteria (MOTT). During the trial a pseudoepidemic of *M. gordonae* (24 isolates) occurred at one of the centers due to a contaminated bronchoscope, thus contributing to the unusually high prevalence of this organism in our survey.

The majority of isolates (179) were obtained from respiratory specimens. Nonrespiratory samples grew 40 isolates, six of which were from blood (Table 3).

Recovery rates of the different culture systems are summarized in Table 4: ESP, BACTEC, and L-J medium detected 79, 89, and 64% of all isolates, respectively. Pairwise comparisons of these differences showed that they were statistically significant, on the basis of the χ^2 test. Equally significant differences emerged from a separate analysis of recovery rates from respiratory samples, whereas differences between the systems for detection from nonrespiratory and blood specimens were not significant. With respect to microscopy, the BACTEC and ESP systems were comparable for smear-positive specimens, while for smear-negative specimens, BACTEC was significantly more sensitive.

Interestingly, a comparison of recovery rates for various organisms (Table 5) showed that the performance of L-J medium was comparable to that of liquid media only for the *M. tuberculosis* complex. Significant differences between the BACTEC and the ESP systems were found. Isolates of the *M. tuberculosis* complex grew better in the BACTEC vials, while isolates of MAC were more readily detected by the ESP system; furthermore, in our study, the ESP system consistently missed the *Mycobacterium xenopi* strains.

Forty three mycobacterial isolates were detected with a single medium only (Table 6): 21 with the BACTEC system, 19 with the ESP system, and 3 with solid media.

An analysis of the combinations of solid and liquid media (Tables 4 and 5) revealed identical recovery rates for BACTEC plus L-J medium and for ESP plus L-J medium; the highest percentage of recovery was obtained by combining BACTEC and ESP.

The mean numbers of days needed for the detection of mycobacteria were practically identical for BACTEC and ESP but significantly greater for solid media (Table 7). Once more, liquid media behaved differently according to whether the *M.*

TABLE 4. Number of isolates obtained from various specimens and recovery rates with different media or their combinations

Sample	No. of isolates obtained (recovery rate [%]) with:					
	ESP	BACTEC	L-J medium	ESP + L-J medium	BACTEC + L-J medium	ESP + BACTEC
All	173 (78.99)	195 (89.04)	141 (64.38)	199 (90.87)	199 (90.87)	216 (98.63)
Respiratory	138 (77.09)	162 (90.50)	116 (64.80)	161 (89.94)	165 (92.18)	177 (98.88)
Nonrespiratory[a]	29 (85.29)	29 (85.29)	23 (67.65)	32 (94.12)	30 (88.23)	33 (97.06)
Blood	6 (100)	4 (66.67)	2 (33.33)	6 (100)	4 (66.66)	6 (100)
Smear positive	102 (89.47)	108 (94.74)	90 (78.95)	110 (96.49)	110 (96.49)	114 (100)
Smear negative	65 (65.66)	83 (83.84)	49 (49.49)	83 (83.84)	85 (85.96)	96 (96.97)

[a] Excluding blood.

tuberculosis complex or MAC was considered, with a slightly faster growth rate of the former with BACTEC and of the latter with ESP.

The contamination rate was 7.86% for ESP, 4% for BACTEC, and 8.90% for L-J cultures. Gram-positive cocci and fungi were the most frequently detected contaminants.

A false-positive signal was obtained with 34 (1.27%) ESP bottles; in such cases microorganisms were not microscopically detectable and did not grow in subcultures.

DISCUSSION

In recent years several culture systems have been developed based upon the use of liquid media and automated instrumentation. The ESP Culture System II is a fully automated method originally developed for blood cultures and subsequently adapted to the detection of mycobacteria in body fluids. The ESP technology is based on the continuous monitoring of pressure changes due to the consumption or production of gas resulting from metabolic activity of microorganisms growing in a liquid medium. Mycobacterial metabolism, characterized by consumption of oxygen, makes these organisms detectable by the reduction of pressure in the headspace of sealed culture bottles.

In a field evaluation of a new culture system like the ESP, the BACTEC system represents the established or "gold standard" for comparison. However, comparison with conventional solid media too is important, because they remain the most widely used methodology worldwide and their complementary role with respect to liquid culture is universally acknowledged.

Salient data emerging from our evaluation indicate good performance of ESP, in spite of a sensitivity lower than that of BACTEC with respiratory and smear-negative specimens. The different formulations of broth used by BACTEC and ESP appear to imply a different, and somehow selective, growth advantage for the different species of mycobacteria. More *M. tuberculosis* complex strains were detected faster with the BACTEC system, whereas more MAC strains were detected faster with the ESP system. Excellent results were obtained by the ESP with blood cultures; however, an overestimate of such positive performance cannot be ruled out due to the almost exclusive presence, in such samples, of MAC, the organism most readily detected by ESP.

Once more the universally acknowledged usefulness of testing with liquid media emerged; in the majority of cases, in fact, at the moment of isolation on solid media, the strains, grown beginning an average of 10 days earlier in BACTEC or ESP bottles, had already been identified with DNA probes and tested for antimicrobial susceptibility.

The combined use of liquid media and traditional solid slants for the recovery of mycobacteria, which has been proved to provide faster detection and significantly higher recovery rates, is currently considered essential in good laboratory practice (10). In the present study the results of the combination of L-J medium with BACTEC or ESP were practically overlapping, both as a whole and when various species were considered separately. The only exception was with MAC isolates, for which the ESP used jointly with slants succeeded in detecting 100% ($P < 0.01$). The best results were obtained from the combined use of BACTEC and ESP, which detected practically all mycobacteria (216 of 219 isolates); the importance of this finding is limited, however, given that the implementation in a single laboratory of both systems is not cost-effective.

Two evaluations concerning the performance of the ESP system have been published to date, one of which (14) was limited to blood samples and was a comparison with Middlebrook agar only. The second evaluation, concerning specimens not substantially differing from ours in both number and type, was a direct comparison with BACTEC and yielded different results (15). The slightly better (though not to a statistically significant degree) performance of the ESP system found in that study conflicts with our data; however, this could be due to the fact that MAC represented 49% of all mycobacteria isolated in that study and only 17% in ours. The optimal growth of these organisms in ESP broth, documented in both studies, may well account for the difference.

The prevention of contamination needs to be improved in the ESP system, as the present rate is clearly over the limit of 5% usually considered acceptable. It is reasonable to expect, however, that the contamination rate with the ESP system will be greater than that with the BACTEC 460TB system due to

TABLE 5. Number of isolates of various mycobacteria and recovery rates with different media or their combinations

Organism	No. of isolates obtained (recovery rate [%]) with:					
	ESP	BACTEC	L-J medium	ESP + L-J medium	BACTEC + L-J medium	ESP + BACTEC
M. tuberculosis complex	110 (85.27)	126 (97.67)	107 (82.94)	124 (96.12)	128 (99.22)	128 (99.22)
MAC	35 (94.59)	28 (75.67)	21 (56.76)	37 (100)	29 (78.38)	36 (97.30)
M. gordonae	24 (75.00)	23 (71.87)	1 (3.12)	24 (75.00)	23 (71.87)	32 (100)
M. xenopi	0 (0)	12 (92.31)	8 (61.53)	8 (61.53)	13 (100)	12 (92.31)
Other MOTT	4 (50.00)	6 (75.00)	4 (50.00)	6 (75.00)	6 (75.00)	8 (100)

TABLE 6. Mycobacteria detected by one system only

Organism	No. of isolates detected by:		
	ESP	BACTEC	L-J medium
M. tuberculosis complex	1	6	1
MAC	8		1
M. gordonae	8	8	
M. xenopi		5	1
M. fortuitum		1	
M. genavense		1	
M. lentiflavum	1		
M. terrae	1		
Total	19	21	3

the use a richer medium, which is needed because of a less-sensitive detection technology.

False-positive signals occurred with the ESP system at a rate of 1.27%. The problem of false-positive signals has not been reported with BACTEC but is counterbalanced by BACTEC's well-known difficulty of microscopic confirmation of the presence of acid-fast organisms when testing is performed on early-positive vials. This results in a delay of positive confirmation until the vial reaches a GI near 100. In contrast, ESP bottles identified as positive almost always contain enough organisms to be immediately microscopically detectable.

Handling of blood cultures in the ESP system is hindered by the lack of special vials. The use of lysis centrifugation processing is more time-consuming and adds risks for the operator compared with the direct inoculation procedure which is possible with BACTEC 13B bottles.

In conclusion, in our multicenter trial the ESP system performed at levels comparable to those of the radiometric BACTEC system. Differences in sensitivity do exist, but they vary with the different species of mycobacteria, and they are obviated when, in accordance with current recommendations (10), either system is used in combination with solid media. The times to detection of positive specimens are comparable to those of the BACTEC system and are therefore the best achievable at present. The ability of each system to detect mycobacterial species most frequently encountered in its area could therefore represent, for single laboratories, an important point to take into account while making a choice.

The ESP instrument, because of the internal incubation of bottles, occupies more space than the BACTEC reader but, in return, no external incubator is needed. One unit, with a capacity of 384 bottles, supports a weekly routine of about 60 specimens.

A comparison of instrument cost is not easy, as it must consider the peak workload of the laboratory, which dictates the number of ESP instruments required. Price variations from country to country severely affect the comparison of running costs; with this proviso, the current list price of ESP bottles (including additives) is in our country, Italy, 60% higher than that of BACTEC 12B vials; the disposal of radioactive waste is not, however, included in the price of the latter.

TABLE 7. Times to detection for various organisms

Organism	Mean time to detection (days) ± SD with:		
	ESP	BACTEC	L-J medium
All mycobacteria	18.07 ± 9.33	17.77 ± 10.17	27.78 ± 11.54
M. tuberculosis complex	19.06 ± 8.70	18.61 ± 9.69	28.56 ± 10.80
MAC	10.26 ± 5.85	11.04 ± 5.20	21.00 ± 12.49
Other MOTT	24.26 ± 9.15	24.39 ± 13.68	44.46 ± 11.59

Despite a nonoptimized software, whose major shortcomings are a limited capacity for storage of data and the lack of any alert for cultures which completed the due incubation period, the full automation of the ESP system substantially reduces the hands-on time in comparison to the semiautomated BACTEC system. The latter requires more maintenance, lacks any program for the registration of cultures and the reporting of results, and needs the vials to be flushed before inoculation and loaded from an external incubator at the moment of reading. Additional features offered by the ESP system include continuous growth monitoring, which minimizes delays in the detection of growth, the absence of radioisotopes and related problems concerning safety and regulatory restrictions, and the elimination of possible cross-contamination, which represents a real risk when an invasive system of growth monitoring is used (6).

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