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Use of the BacT/Alert MB Mycobacterial Blood Culture System for Detection of Mycobacteria in Sterile Body Fluids Other than Blood▽

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The definitive diagnosis of extrapulmonary tuberculosis is made by a positive body fluid culture result. Conventional culture methods require centrifugation or filtration of body fluid (peritoneal, pleural, synovial, or pericardial fluid) in order to improve the sensitivity. The aim of the present study was to evaluate the feasibility of the direct inoculation, at the patient's bedside, of up to 5 ml of uncentrifuged fluid onto BacT/Alert MB culture bottles (bioMérieux, Durham, NC).

Tuberculosis remains one of the major global public health problems (7) and often remains undiagnosed. Because of the low sensitivity of conventional techniques for the recovery of *Mycobacterium tuberculosis* (4), the diagnosis of extrapulmonary tuberculosis, in many cases, is made on the basis of the clinical presentation, the history of contacts, a positive tuberculin test result, and characteristic abnormalities on the chest radiograph. The isolation of mycobacteria from body fluids remains a challenge owing to the low bacterial charge characterizing such samples (1, 2). Conventional culture methods require centrifugation or the filtration of body fluid (peritoneal, pleural, synovial, or pericardial fluid) in order to improve the sensitivity. Moreover, the diagnosis of tuberculous lymphadenopathy is problematic because of the small volumes of needle aspirates. Therefore, it is very important to evaluate the usefulness of new diagnostic methods (3, 5, 6).

The isolation of the etiologic agent is regarded as the "gold standard" for the diagnosis of tuberculosis, and it is universally accepted that liquid culture media are needed for the sensitive detection of mycobacteria, in particular, from samples in which very few bacterial units may be present.

In our laboratory, automated culture of the BacT/Alert MP culture bottles (MP bottles; bioMerieux, Durham, NC) is routinely used for the detection of mycobacteria in clinical samples.

From the beginning of 2006 to June 2008, we processed 96 body fluid specimens (pleural fluid, peritoneal fluid, and biopsy tissue specimens) using both MP bottles and BacT/Alert MB blood culture bottles (MB bottles).

In order to evaluate whether the different yields found by the two methods was effective or not, we compared the results obtained by the routine culture method (with MP bottles) with those obtained by direct inoculation into MB bottles.

MATERIALS AND METHODS

This study involved 96 body fluid specimens (75 pleural fluid, 11 peritoneal fluid, and 10 biopsy tissue specimens) which were collected over a period of 30 months from 96 patients (1 sample from each patient) admitted to the Department of Infectious Diseases at the Campo di Marte Hospital, Lucca, Italy. The final diagnoses for these patients are reported in Table 1.

According to the standard procedure used in our hospital, the pleural and peritoneal fluids were collected in sterile containers and transported, in the shortest period of time, to the microbiology laboratory for processing. After centrifugation, the pellet was treated with an equal volume of NaOH–*N*-acetyl-L-cysteine (NALC-NaOH; final NaOH concentration, 2%) for 15 min at room temperature and neutralized with sterile phosphate buffer (0.067 M, pH 6.8). After centrifugation at $4,200 \times g$ for 15 min, the pellet was resuspended in 1 to 2 ml of sterile distilled water, which was used for the inoculation of MP bottles and Lowenstein-Jensen medium and the performance of PCR by the BD ProbeTec ET *Mycobacterium tuberculosis* complex direct detection assay (Becton Dickinson, Sparks, MD).

Beginning in 2006, we added to the standard procedure the direct inoculation, at the patient's bedside, of up to 5 ml of uncentrifuged fluid into MB bottles supplemented with the MB/BacT enrichment fluid. MB bottles, to which the proper enrichment is added, are designed for the recovery of mycobacteria commonly isolated from blood. The medium supports the growth of other aerobic organisms, including yeast, fungi, and bacteria. A 3- to 5-ml volume of blood can be inoculated directly into the MB bottle.

Aseptically minced and homogenized biopsy tissue specimens were divided into two parts; one part was digested-decontaminated and inoculated in Lowenstein-Jensen medium and MP bottles and processed for PCR, while the other part was directly inoculated into one MB bottle. The inoculated bottles were placed into the instrument, where they were incubated (35°C to 37°C) and continuously monitored for microbial growth.

In order to compare the conventional method with MP bottles and the innovative diagnostic method with MB bottles, we carried out three experiments in which we used *Mycobacterium tuberculosis* strain H37Rv, which was adjusted with saline to the turbidity of a 0.5 McFarland standard (equivalent to 0.6×10^8 bacteria/ml).

In the first test the 0.5 McFarland *M. tuberculosis* suspension was diluted in saline solution, thus obtaining final concentrations of 1.5, 0.75, 0.15, and 0.075 CFU (see Table 3). Two 5-ml aliquots were taken from each of the four dilutions. The first one was directly inoculated into MB bottles; the other one was decontaminated by the standard NALC-NaOH procedure, and its sediment was resuspended to 0.5 ml of H_2O and finally inoculated into the MP bottles.

In the second experiment, the suspensions inoculated into MP bottles were not treated with NALC-NaOH (see Table 4 for the dilutions tested).

The final experiment was conducted in order to obtain more reliable results by reproducing the usual diagnostic conditions, and the *M. tuberculosis* dilutions were prepared in sterile pleural fluid instead of saline solution (see Table 5).

In the three experiments, each dilution was tested in quadruplicate and all the bottles were inoculated for up to 60 days before they were considered negative. The threshold value was defined as the development of mycobacteria in three of

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TABLE 1. Distribution of patients on the basis of the final clinical diagnosis and the type of sample

No. of patients	Final clinical diagnosis	Specimen type
8	Cirrhosis	Peritoneal fluid
3	Hepatic tumor	Peritoneal fluid
2	Cancer	Tissue biopsy
1	Tubercular spondylodiscitis with multiple abscesses	Tissue biopsy
3	Bacterial lymphadenopathy	Lymph node biopsy
1	Nonspecific lymphadenopathy	Lymph node biopsy
3	Superficial tuberculous lymphadenopathy	Lymph node biopsy
5	Empiema	Pleural fluid
22	Parapneumonia	Pleural fluid
10	Lung carcinoma	Pleural fluid
15	Nonspecific pleuritis	Pleural fluid
13	Pleural effusion in cirrhosis	Pleural fluid
6	Tubercular pleuritis	Pleural fluid
4	Pleuropulmonary tuberculosis	Pleural fluid

four replicates. One drop from each saline or pleural fluid dilution and from positive MB or MP bottles was plated on blood agar plates and chocolate agar plates in order to verify the sterility.

The real numbers of CFU present in the bacterial dilutions were double-checked, when possible, by seeding 100 μl on Middlebrook 7H11 agar plates.

RESULTS

The results of the tests with MP bottles and MB bottles and by PCR conducted with positive specimens (11 of 96 clinical samples) are reported in Table 2. The available epidemiological data (collection and hospitalization dates and absence of known contacts between patients) suggest that the mycobacteria isolated represent separate strains.

Two pleural fluid specimens, one PCR positive and the other PCR negative, remained sterile in Lowenstein-Jensen medium and the MP bottles, while *M. tuberculosis* grew in the MB bottles. The average times required for the detection of a

TABLE 2. Comparison of MP and MB bottles for recovery of *M. tuberculosis* in clinical samples

Final diagnosis	Specimen type	Collection date (day/mo/yr)	Time to detection (days): MB bottles	Time to detection (days): MP bottles	PCR result
Pleuropulmonary tuberculosis	Pleural fluid	06/03/06	19	17	Negative
Tubercular pleuritis (HIV[a]-positive patient)	Pleural fluid	26/05/06	25	No growth	Negative
Tubercular pleuritis	Pleural fluid	11/07/06	18	20	Negative
Pleuropulmonary tuberculosis	Pleural fluid	13/02/06	19	31	Negative
Pleuropulmonary tuberculosis	Pleural fluid	22/06/07	30	32	Negative
Tubercular pleuritis (HIV-positive patient)	Pleural fluid	06/10/07	19	21	Positive
Superficial tuberculous lymphadenopathy	Lymph node biopsy	01/09/07	39	30	Positive
Superficial tuberculous lymphadenopathy	Lymph node biopsy	20/03/08	7	9.5	Positive
Tubercular spondylodiscitis with multiple abscesses	Tissue biopsy	16/01/08	20	20	Positive
Superficial tuberculous lymphadenopathy	Lymph node biopsy	29/03/08	6	14	Positive
Pleuropulmonary tuberculosis	Pleural fluid	18/06/08	17	No growth	Positive

[a] HIV, human immunodeficiency virus.

TABLE 3. Time to detection in MB and MP bottles inoculated with *M. tuberculosis* saline dilutions after treatment of samples with NALC-NaOH

Bacillary load in starting solution	No. of CFU/ml[a]	No. of CFU/bottle	Time to detection (days) for: MB bottles: Bottle 1	MB bottles: Bottle 2	MB bottles: Bottle 3	MB bottles: Bottle 4	MB bottles: Avg	MB bottles[b]: Bottle 1	MB bottles[b]: Bottle 2	MB bottles[b]: Bottle 3	MB bottles[b]: Bottle 4
100	0.3	1.5	31.5	36.5	30.2	34.2	33.1	NG[c]	NG	NG	NG
200	0.15	0.75	NG	35.3	44.3	31.0	36.8[d]	NG	NG	NG	NG
400	0.03	0.15	NG	NG	NG	NG		NG	NG	NG	NG
800	0.015	0.075	NG	NG	NG	NG		NG	NG	NG	NG

[a] The numbers of CFU were double-checked on Middlebrook 7H11 agar plates.
[b] All specimens were processed and pretreated with NALC-NaOH, as described in the text.
[c] NG, no growth after 60 days.
[d] One of four bottles showed no growth; the value given is the average of three bottles.

TABLE 4. Time to detection in MB and MP bottles of *M. tuberculosis* saline suspension without pretreatment of the samples with NALC-NaOH

Bacillary load in starting solution[a]	No. of CFU/ml	No. of CFU/ bottle	Time to detection (days) for:									
			MB bottles					MP bottles				
			Bottle 1	Bottle 2	Bottle 3	Bottle 4	Avg	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Bottle 5
100	750[b]	3,750	17	10.7	15	9.5	13.1	13.2	12.3	12.2	12.8	12.6
200	280[b]	1,400	12.7	14.2	28.3	15.5	17.7	15	12.7	15	14.8	14.4
400	210[b]	1,050	17	11.3	18	19.7	16.5	15	15.3	15.7	15.8	15.5
800	120[b]	600	24.7	25.3	21.2	24.7	24.0	17	16.8	18.7	18.4	17.7
1,600	40[b]	200	30.2	23	27.2	33.3	28.4	16.5	18.3	16.7	18	17.4
3,200	15[c]	75	30	31.3	25.8	30.3	29.4	18	19.3	20.8	22.7	20.2
6,400	10[c]	50	26.1	31.7	30.4	32.5	30.2	26.5	25.2	28.1	28.2	27.0
12,800	5[c]	25	26.1	31.7	36.4	32.5	31.9	26.5	29.2	28.1	33.2	29.3
25,600	2.5[c]	12.5	23.8	32.5	34.2	38.2	32.2	NG[d]	30.6	29.8	29.0	29.8[e]
51,200	1[c]	5	37.5	29.6	30.5	33.3	32.7	54.4	NG	51.0	NG	
102,400	0.5[c]	2.5	35.7	30.7	31.0	34.2	32.9	NG	NG	NG	NG	NG
204,800	0.25[c]	1.25	46.0	30.2	41.8	31.7	37.4	NG	NG	NG	NG	NG
409,600	0.125[c]	0.63	47.5	40.0	47.8	46.5	45.5	NG	NG	NG	NG	NG
819,200	0.063[c]	0.31	45.2	32.0	50.2	NG	42.5[e]	NG	NG	NG	NG	NG
1,638,400	0.031[c]	0.16	NG	NG	54	NG		NG	NG	NG	NG	NG
3,276,800	0.016[c]	0.08	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
6,553,600	0.008[c]	0.04	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
13,107,200	0.004[c]	0.02	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

[a] All the samples were centrifuged, but they were not processed with NALC-NaOH.
[b] The number of CFU was double-checked on Middlebrook 7H11 agar plates.
[c] Theoretical number of CFU.
[d] NG, no growth after 60 days.
[e] One of four bottles showed no growth; the value given is the average for three bottles.

positive result were 19.9 days for samples in MB bottles and 21.6 days in samples in MP bottles.

In the first experiment, all MB bottles inoculated with 1.5 CFU and three of four of the bottles inoculated with 0.75 CFU scored positive on average after 33.1 and 36.8 days, respectively. No growth was obtained in any MP bottle (Table 3).

The results of the second experiment (Table 4) showed thresholds of the numbers of CFU per bottle of about 12.5 for MP bottles and 0.31 for MB bottles; with bacterial concentrations greater than 25 CFU/bottle, the average times required for the detection of a positive result were 19.3 days for MP bottles and 23.9 days for MB bottles.

In the third experiment (pleural fluid dilution) (Table 5), the MB and MP bottles (without digestion-decontamination) showed the same sensitivity for the recovery of mycobacteria; the average times required for the detection of a positive result were 14.4 days for MB bottles and 15.6 days for MP bottles. For the samples pretreated with NALC-NaOH and inoculated into MP bottles, the sensitivity was 5,750 CFU/bottle.

DISCUSSION

The definitive diagnosis of extrapulmonary tuberculosis is made by a positive culture result, but unfortunately, the techniques used for the detection of mycobacteria have a number of limitations: the paucibacillary nature of the specimens, the

TABLE 5. Time to detection in MB and MP bottles of *M. tuberculosis* pleural fluid suspension with and without pretreatment of the samples with NALC-NaOH

Bacillary load in starting solution[a]	No. of CFU/ml	No. of CFU/bottle	Time to detection (days) for:														
			MB bottles										MP bottles[c]				
			Bottle 1	Bottle 2	Bottle 3	Bottle 4	Bottle 5	Bottle 6	Bottle 7	Bottle 8	Bottle 9	Avg	Bottle 1	Bottle 2	Bottle 3	Bottle 4	Avg
100	1,150[b]	5,750	10.5	10.8	10.5	10.6	10.6	11.7	12.5	12.7	11.0	12.0	22.3	19.8	20.3	20.6	20.8
500	265[b]	1,325	12.5	11.8	10.1	11.5	11.5	14	14	14.2	13.5	13.9	22.3	NG[d]	NG	24.1	
1,000	100[b]	500	13.3	13.3	13.8	12.8	13.3	14.7	15.3	14.7	15.0	14.9	NG	NG	26.8	NG	
2,000	35[b]	175	13.8	14.5	14	14.1	14.1	15.8	16.3	16.4	16.6	16.3	NG	NG	NG	NG	NG
4,000	25	125	14	15.3	14.3	14.9	14.6	16	15.7	16.5	16.1	16.1	NG	NG	NG	NG	NG
8,000	20	100	14.7	15.7	15.2	15.0	15.3	14.7	17	16.4	16.4	16.1	NG	NG	NG	NG	NG
16,000	10	50	16.4	15.3	16.3	16.4	16.1	17.2	18.3	17.3	17.5	17.6	NG	NG	NG	NG	NG
32,000	5	25	20	18.7	20.5	19.9	19.8	17.8	18	18.5	18.5	18.2	NG	NG	NG	NG	NG

[a] All the samples were centrifuged, but they were not processed with NALC-NaOH.
[b] The number of CFU was double-checked on Middlebrook 7H11 agar plates.
[c] The specimens were processed and pretreated with NALC-NaOH, as described in the text.
[d] NG, no growth after 60 days.

concentration of organisms (which can be low, despite centrifugation or filtration), the presence of a clot in some samples, the presence of inhibitors that undermine the performance of the PCR, and delays in the transport of the specimen to the microbiology laboratory.

We demonstrated in the first experiment that decontamination of the culture should be avoided. The second experiment showed that the method with the MB bottles is more sensitive than the conventional method with MP bottles, although the time required for the detection of positive cultures with MB bottles is slightly longer. Finally, we showed in third experiment, which was carried out with pleural fluid, that the time required for the detection of positive cultures is slightly longer for MP bottles.

The data obtained from studies with clinical and experimental samples suggest that the use of MB bottles allows higher rates of recovery than the conventional method with MP bottles, while the detection times were comparable. Moreover, the use of MB bottles eliminates the problems linked to the collection and transport of specimens, does not allow the coagulation of the sample, and does not require further technical manipulations. In conclusion, we suggest that MB bottles can be used without decontamination-digestion as a routine method to improve the accuracy and reliability of the diagnosis of extrapulmonary tuberculosis.

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