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**J. M. Manterola · C. G. Thornton · E. Padilla
J. Lonca · I. Corea · E. Martínez · V. Ausina**

# Comparison of the Sodium Dodecyl Sulfate-Sodium Hydroxide Specimen Processing Method with the $C_{18}$-Carboxypropylbetaine Specimen Processing Method Using the MB/BacT Liquid Culture System

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**Abstract** The ability of physicians to diagnose tuberculosis is impacted by the use of smear and culture techniques combined with specimen processing methods. The objective of this study was to evaluate the effects of specimen processing on smear and culture sensitivity by comparing the specimen processing method that uses $C_{18}$-carboxypropylbetaine with the method that combines sodium dodecyl sulfate and sodium hydroxide. A total of 1,201 specimens were entered into this study. Specimens were split approximately equally such that one-half of each specimen was processed with sodium dodecyl sulfate-sodium hydroxide, while the other half was processed with $C_{18}$-carboxypropylbetaine. All sediments were subjected to acid-fast staining and then analyzed using the MB/BacT liquid culture system (bioMérieux, France) and solid media. The sensitivity of smear following processing with sodium dodecyl sulfate-sodium hydroxide and $C_{18}$-carboxypropylbetaine was 61.2% and 58.6% ($P$>0.05), respectively, while the specificities were identical (99.7%). The sensitivity of culture was 84.2% and 96.1% ($P$<0.05), respectively. The time to detection in the MB/BacT liquid culture system was 13.2±5.6 and 15.0±8.8 days ($P$>0.05), respectively, and 20.0±7.6 and 15.7±8.9 days ($P$<0.05), respectively, on solid media. The contamination rates in the MB/BacT system were 0.8% and 8.7%, respectively, whereas the contamination rates on solid media were 2.6% and 4.3%, respectively. $C_{18}$-carboxypropylbetaine specimen processing was less labor-intensive than sodium dodecyl sulfate-sodium hydroxide processing and improved the ability of laboratory staff to detect the presence of mycobacteria by culture.

J. M. Manterola · E. Padilla · J. Lonca · I. Corea
E. Martínez · V. Ausina (✉)
Servicio de Microbiología,
Hospital Universitario Germans Trias i Pujol,
Carretera de Canyet s/n, 08916 Badalona, Spain
e-mail: vausina@ns.hugtip.scs.es
Tel.: +34-93-4978894, Fax: +34-93-4978895

J. M. Manterola · V. Ausina
Departamento de Genética y Microbiología,
Facultad de Medicina,
Universidad Autónoma de Barcelona,
Barcelona, Spain

C. G. Thornton
Integrated Research Technology, LLC,
Baltimore, Maryland, USA

## Introduction

The tuberculosis pandemic continues unabated. The World Health Organization estimates that by 2005 approximately 10.3 million people will become infected with *Mycobacterium tuberculosis* each year [1]. Early and accurate diagnosis remains the key to curtailing its spread. The effects of specimen processing on the ability to detect the presence of mycobacteria in clinical specimens are well documented [2, 3]. First, the buoyant nature of this class of organisms contributes to compromising the quality of clinical specimens (e.g., bacilli are poured off with the supernatant fraction following centrifugation) [4, 5]. Second, specimen processing methods may directly interfere with the diagnostic technique itself: For example, the reagents used to process clinical specimens inhibit nucleic acid amplification [6, 7, 8], and the problem of killing mycobacteria during specimen processing is well known [2]. Yajko et al. [7] report that approximately 90% of input *Mycobacterium tuberculosis* bacilli were killed in spiked samples following processing with the method recommended by the Centers for Disease Control and Prevention [2] [the N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) method].

There have been several reports describing the use of a specimen processing technique that utilizes the zwitterionic detergent $C_{18}$-carboxypropylbetaine (CB-18) [9, 10, 11, 12, 13]. While CB-18 specimen processing has

been reported to enhance smear and culture sensitivity as well as reduce the time-to-positive, this method has only been evaluated against NALC-NaOH specimen processing using the Bactec 460 TB liquid culture system (bioMérieux, France) [10]. The MB/BacT liquid culture system (bioMérieux) was introduced as an automated liquid culture system for use in the clinical laboratory. The MB/BacT system has been shown to have sensitivity equal to that of other liquid culture systems [14, 15], but the lack of radioactive waste and the aspect of automated detection provide an attractive alternative to comparative liquid culture systems.

The purpose of the present study was to compare the CB-18 specimen processing method with the specimen processing method that combines sodium dodecyl sulfate (SDS) with NaOH (SDS-NaOH) using the MB/BacT liquid culture system.

## Materials and Methods

### Clinical Specimens

Between March 1999 and December 1999 a total of 1,201 respiratory and nonrespiratory specimens were collected from the clinical microbiology laboratory of the Hospital Universitari Germans Trias i Pujol, Barcelona, Spain. Specimens were selected randomly for entry into this study, the only criteria being sufficient specimen volume or mass: only specimens with greater than 4 ml or 2 g were selected for processing. Upon entry into the study, specimens were split approximately equally by cutting. Respiratory specimens and other liquid specimens were separated by pouring from the specimen collection cup into two 50-ml conical centrifuge tubes. Tissue and fecal specimens were also sterilely cut and separated into two 50-ml conical centrifuge tubes. Because of the nature of clinical specimens and the difficulty associated with splitting, and, in an effort to normalize the effects of splitting on study results, that portion removed first and processed with each processing method was alternated on a daily basis.

Specimens included 836 sputa, 305 bronchial-washings, 29 tissues, and 31 other specimen types (17 exudates, 7 spermatic fluids, 4 urines, and 3 feces).

### Decontamination Procedures

Two different decontamination methods were compared in this study. The specimen processing method used by the clinical microbiology laboratory of the Hospital Universitari Germans Trias i Pujol was a modified version of the SDS-NaOH procedure originally described by Tacquet and Tison [16]. Briefly, to the 50-ml conical tube containing 2–3 ml of the specimen were added two volumes of 1% NaOH with 3% SDS. Specimens were subjected to vortexing for 1 min and then centrifuged at 3,000×*g* for 20 min at 15°C. Specimens were decanted and neutralized by adding sterile 1.43% orthophosphoric acid containing 0.006% bromocresol purple until neutralization occurred. Specimens were subjected to a second centrifugation step at 3,000×*g* for 20 min at 15°C, decanted, and the resulting sediment subjected to acid-fast staining (see below). Prior to culture, sediments were resuspended again by the addition of 30 ml of sterile water and subjected to a third centrifugation step at 3,000×*g* for 20 min at 15°C. Specimens were decanted, and 1 ml of 0.067 M phosphate buffered saline (pH 6.8) was added to all sediments and the pellets resuspended. Two tubes of Löwenstein-Jensen (L-J) medium were each inoculated with 200 µl of sediment, and one MB/BacT culture vial (bioMérieux, France) supplemented with Mycobacteria Antibiotic Supplement (MAS, a commercially available mixture of polymyxin B, azlocillin, nalidixic acid, trimethoprim, amphotericin B, and vancomycin) (bioMérieux) was inoculated with 500 µl of sediment.

The SDS-NaOH specimen processing method was compared with two different versions of CB-18 processing. The CB-18-based procedures were modified versions of that originally described [10]. In the first phase (phase 1), 10 ml of the buffered CB-18 (1 mM CB-18, 50 mM Tris-HCl, 12.5 mM citrate pH 7.6, 1.5 mM NaCl, 0.5% NALC) was initially added to specimens and this mixture subjected to vortexing at room temperature for 1 min to facilitate liquefaction of specimens. Subsequently, the same buffered CB-18 solution was added to each specimen to a final volume of 40 ml, mixed and then immediately subjected to centrifugation at 3,000×*g* for 20 min at 25°C. Specimens were decanted and resuspended in 1 ml of the previously described lytic enzyme resuspension buffer [11] that contained lysozyme, zymolyase, *Cytophaga* extract, and *Trichoderma* extract (LZCT) and 0.15% lecithin in 50 mM Tris-HCl, 12.5 mM citrate pH 7.6, 3 mM NaCl, 0.5% NALC. Sediments were incubated in LZCT for 20 min at 37°C. A portion of the digested sediments was subjected to acid-fast staining using fluorochromes (see below). A 200 µl portion of all processed sediments was then inoculated onto a single 7H11-selective plate (LZCT-treated specimens cannot be used to inoculate L-J media because the lytic enzyme liquefies this inspissated media [Lytic Decon product literature, Integrated Research Technology, LLC]), and a 500-µl portion was used to inoculate an MB/BacT vial supplemented with MAS.

Interim study results suggested that the concentration of CB-18 in MB/BacT culture bottles might be interfering with the sensitivity of liquid culture (a concern raised in previous reports [10, 11]). Consequently, the CB-18 procedure was modified in such a way as to ensure that the concentration of CB-18 in the MB/BacT culture bottle was as low as possible. Modifications in the second phase (phase 2) of the study were as follows: First, a 1 ml aliquot of 12.5 mM CB-18, 100 mM Tris-HCl, 25 mM citrate pH 7.6, 3 mM NaCl, and 0.5% NALC was added directly to each specimen. Specimens were thoroughly mixed and then incubated at 37°C for 30 min. After the incubation period, Tris-citrate buffer (50 mM Tris-HCl, 12.5 mM citrate pH 7.6, 1.5 mM NaCl) was added to all specimens to a final volume of 40 ml. Specimens were thoroughly mixed and then subjected to centrifugation, LZCT treatment, and analysis as described above with the exception that lecithin was omitted from the LZCT resuspension buffer (this dilution-based version of CB-18 processing was designed to ensure that the CB-18 concentration during incubation was below critical levels, thereby obviating the need for lecithin [11]).

### Microscopy

Smear analysis was performed using auramine-rhodamine stains according to recommended procedures and graded as suggested by the Centers for Disease Control and Prevention [2]. All positive slides were confirmed using the Ziehl-Neelsen technique per recommended procedures [2].

### Culture

The L-J slants were incubated at 35–37°C for 2 months in a humidified atmosphere and inspected twice weekly. The 7H11 plates were incubated at 35–37°C for 2 months in a 5% $CO_2$ atmosphere and read twice weekly under an optical microscope at 100-fold magnification. The MB/BacT bottles were incubated at 35–37°C for 2 months or until they were considered positive by the BacT/Alert incubator. After 8 weeks all MB/BacT bottles considered negative were subcultured onto an L-J slant by transferring 200 µl of culture media. These L-J slants were incubated as indicated above.

### Identification of Mycobacterial Isolates

Mycobacterial isolates were identified by standard biochemical procedures [2, 3], gas-liquid and thin-layer chromatography [17] and specific DNA probes (AccuProbe; Gen-Probe, USA) [18].

### Statistical Analysis

Statistical comparisons were performed using chi-squared analysis. Analyses of the time-to-positive were carried out using the two-tailed Student's *t* test.

## Results

The effects of specimen processing on smear sensitivity and specificity, culture sensitivity, time-to-positive, and contamination were evaluated using split specimens. A total of 1,201 specimens were analyzed in this study. While the same SDS-NaOH procedure was used throughout this study, two different versions of the CB-18 method were evaluated. There were only several notable differences between the two different versions of CB-18 processing. These differences are highlighted where indicated.

From this group of specimens, a total of 97 (8.1%) were positive following acid-fast staining, regardless of the processing method or culture result. A total of 154 acid-fast bacteria (AFB) were isolated from 152 (12.7%) specimens by culture, regardless of the processing method or culture media (2 specimens produced multiple isolates). Respiratory specimens produced 144 AFB-positive culture results, while nonrespiratory specimens produced 8 AFB-positive culture results. Of the 154 isolates, 124 were identified as *Mycobacterium tuberculosis* complex bacilli, and 30 were identified as nontuberculous mycobacteria (NTM). Evaluation of those patients from whom NTM-positive specimens were obtained suggested that all 30 NTM isolates were clinically significant (e.g., these patients were either in a high-risk group [elderly], immune compromised [AIDS], or had a prolonged respiratory infection). Included in these 30 NTM isolates were two *Nocardia asteroides* isolates and one *Tsukamurella* species isolate (since all 3 were considered clinically significant, for the purposes of discussion they were categorized with the NTM mycobacteria. The remaining NTM isolates included nine *Mycobacterium kansasii*, five *Mycobacterium xenopi*, four *Mycobacterium chelonae*, two *Mycobacterium fortuitum*, two *Mycobacterium gordonae*, two *Mycobacterium mucogenicum*, one *Mycobacterium avium* complex, one chromogenic rapidly growing *Mycobacterium*, and one unknown *Mycobacterium*. For the purposes of brevity these NTM mycobacteria were treated as a single group in all analyses.

Of the two specimens that produced multiple isolates, the first produced *Mycobacterium xenopi* following processing by the SDS-NaOH procedure and *Tsukamurella* species following CB-18 processing. The second specimen yielded *Mycobacterium tuberculosis* following CB-18 processing and *Mycobacterium fortuitum* following SDS-NaOH processing. The former specimen was treated as NTM positive by both methods, and no distinction was made in specimen-based analyses. The latter specimen, however, was considered culture positive for *Mycobacterium tuberculosis* by the CB-18 method, but culture negative by the SDS-NaOH method, since the *Mycobacterium tuberculosis* result was considered the more clinically significant result.

Laboratory cross-contamination originating from comingling of specimens during processing was evaluated by comparing culture-positive specimens and dates of processing. Only two positive specimens could not be ruled out as having resulted from laboratory cross-contamination.

### Smear Sensitivity and Specificity

Of the 97 specimens that were identified by acid-fast staining as harboring AFB, only 3 could not be associated with a positive culture result (Table 1). All three specimens were smear positive following processing by both processing methods. In two instances the specimen source was fecal material, and in the third instance the patient was on antituberculosis therapy. While the results from the feces may have been false positives, the smear result from the patient on therapy was considered legitimate. Regardless, the specificities of smear analysis following processing by SDS-NaOH and CB-18 were identical (99.7%). The increase in smear sensitivity among all culture-positive specimens ($n$=152) was 4.4% higher following SDS-NaOH processing; however, this result was not statistically significant ($P$>0.05).

**Table 1** Comparison of smear results obtained by the decontamination methods

| Group | Total no. of AFB-positive specimens | No. of specimens with positive smear result | | | Smear sensitivity by processing method (%) | | |
|---|---|---|---|---|---|---|---|
| | | SDS only | CB-18 only | Both methods | SDS | CB-18 | *P* value |
| All AFB | 152 | 5 | 1 | 88 | 61.2 | 58.6 | >0.05 |
| MTBC | 124 | 5 | 1 | 83 | 71.0 | 67.7 | >0.05 |
| NTM | 28 | 0 | 0 | 5 | 17.9 | 17.9 | – |
| | | | | | Smear specificity (%) | | |
| Not culture positive | 3 | 0 | 0 | 3 | 99.7 | 99.7 | – |

AFB, acid-fast bacilli; MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria; SDS, sodium dodecyl sulfate

**Table 2** Evaluation of smear values and smear sensitivities by method and mycobacterial group

| | Phase 1: no incubation (*n*=600) | | | Phase 2: 30 min incubation (*n*=601) | | |
|---|---|---|---|---|---|---|
| | All | NTM | MTBC | All | NTM | MTBC* |
| Identical smear values | 16 | 2 | 14 | 27 | 2 | 25 |
| CB-18 smear value >NaOH smear value | 5 | 0 | 5 | 2 | 0 | 2 |
| NaOH smear value >CB-18 smear value | 33 | 1 | 32 | 5 | 0 | 5 |
| CB-18 smear pos. and NaOH smear neg. | 1 | 0 | 1 | 0 | 0 | 0 |
| NaOH smear pos. and CB-18 smear neg. | 4 | 0 | 4 | 1 | 0 | 1 |
| Total smear pos. | 59 | 3 | 56 | 35 | 2 | 33 |

NTM, nontuberculous mycobacteria; MTBC, *Mycobacterium tuberculosis* complex

**Table 3** Comparison of culture results by decontamination method and smear result

| Group | Smear category | Total no. of cultures positive for AFB | No. of specimens with positive culture result by decontamination method[a] | | | Sensitivity of culture by processing method (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | SDS only | CB-18 only | Both methods | SDS | CB-18 | *P* value |
| All AFB | Positive | 94 | 0 | 0 | 94 | 100 | 100 | – |
| | Negative | 58 | 6 | 24 | 28 | 58.6 | 89.7 | <0.05 |
| | Total | 152 | 6 | 24 | 122 | 84.2 | 96.1 | <0.05 |
| MTBC | Positive | 89 | 0 | 0 | 89 | 100 | 100 | – |
| | Negative | 35 | 4 | 11 | 20 | 68.6 | 88.6 | >0.05 |
| | Total | 124 | 4 | 11 | 109 | 91.1 | 96.8 | >0.05 |
| NTM | Positive | 5 | 0 | 0 | 5 | 100 | 100 | – |
| | Negative | 23 | 2 | 13 | 8 | 43.5 | 91.3 | <0.05 |
| | Total | 28 | 2 | 13 | 13 | 53.6 | 92.9 | <0.05 |

AFB, acid-fast bacilli; MTBC,*Mycobacterium tuberculosis* complex; NTB, nontuberculous mycobacteria; SDS, sodium dodecyl sulfate

[a] Subculture of negative MB/BacT culture bottles to L-J slants produced 8 culture-positive results from SDS-NaOH-processed specimens and 21 culture-positive results from CB-18-processed specimens. The SDS-NaOH-positive subcultures included 2 *Mycobacterium tuberculosis* and 6*Mycobacterium xenopi* isolates. The CB-18-positive subcultures included 8 *Mycobacterium tuberculosis*, 11*Mycobacterium xenopi*, and 2 *Mycobacterium kansasii* isolates. In all instances in which*Mycobacterium tuberculosis* and *Mycobacterium kansasii* isolates were subcultured from the MB/BacT bottle, the corresponding culture on solid media from the primary sediment was positive, regardless of the processing method. Of the 6 *Mycobacterium xenopi* isolates recovered from MB/BacT subcultures that had been processed by SDS-NaOH, in only 1 instance was the corresponding L-J slant positive; the other 5 subcultures were new positive results. Of the 11 *Mycobacterium xenopi* isolates recovered from MB/BacT subcultures that had been processed by CB-18, in 4 instances the corresponding 7H11 selective slant was positive; the other 7 subcultures were new positive results. Only 1 *Mycobacterium tuberculosis* and 3 *Mycobacterium xenopi* isolates were subculture positive using the CB-18 processing protocol of phase 2

## Smear Values

One of the differences observed between the two different versions of CB-18 processing was seen in the resulting smear values (Table 2). In the first half of the study (i.e., phase 1), a diluted solution of CB-18 was initially added in a small volume to facilitate dispersion of the specimen. This was followed immediately with a large volume of the same solution and then subjected to centrifugation (i.e., with no incubation period). In the second half of the study, a concentrated solution of CB-18 was added in a small volume, incubated for 30 min, and then diluted prior to centrifugation. In the first half of the study, 55.9% of the smear-positive specimens had higher smear values following SDS-NaOH processing, whereas in the latter half of the study this percentage dropped to 14.3%. In phase 2 of the study, the largest group of smear-positive specimens had identical smear values (77.1%) by both processing methods. While the percentage of 4+ smear-positive specimens was not dramatically different between the first and second halves of the study following SDS-NaOH processing, there was a marked increase in the number of 4+ smear results following CB-18 processing in the second half of the study. For example, in the first half of the study, 72.4% of the smear-positive specimens were reported as 4+ following SDS-NaOH processing, but only 21.8% of the smear-positive specimens were classified as 4+ following CB-18 processing. Alternatively, in the second half of the study, 68.6% of the smear-positive specimens were reported as 4+ following SDS-NaOH processing, and 64.7% were classified as 4+ following CB-18 processing.

## Culture Sensitivity

Overall, a statistically significant increase in culture sensitivity of 14.1% was achieved when specimens were processed with CB-18. This increase was due exclusively to an increase in the isolation of AFB from smear-negative specimens (Table 3). These results were segregated to evaluate the differences in culture sensitivity relative

**Table 4** Summary of time-to-positive (ttp) results for different mycobacterial groups analyzed by culture method and processing method (mean±SD)

| Group | Total no. of culture-positive specimens | Time to detection by decontamination method and culture method | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SDS-NaOH | | | | CB-18 | | | |
| | | L-J | | MB/BacT | | 7H11 selective | | MB/BacT | |
| | | No. | Avg. ttp (days) | No. | Avg. ttp (days) | No. | Avg. ttp (days) | No. | Avg. ttp (days) |
| All AFB | 154 | 121 | 20.0±7.6 | 121 | 13.2±5.6 | 116 | 15.7±8.9 | 127 | 15.0±8.8 |
| MTBC | 124 | 109 | 19.3±5.8 | 108 | 12.8±4.8 | 100 | 15.9±8.5 | 108 | 15.3±8.3 |
| NTM | 30 | 12 | 26.2±15.7 | 13 | 16.2±9.9 | 16 | 14.9±11.3 | 19 | 13.3±11.3 |

AFB, acid-fast bacilli; L-J, Löwenstein-Jensen; MTBC, *Mycobacterium tuberculosis* complex; NTM, nontuberculous mycobacteria; SDS, sodium dodecyl sulfate

**Table 5** Independent comparison of culture sensitivity values for each processing method

| Method | Smear result | Culture result[a] (*n*) | | | | Sensitivity (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | Liquid & solid media | Liquid medium only | Solid medium only | Total positive for AFB | Liquid medium | Solid medium | *P* value |
| SDS-NaOH | Positive | 90 | 2 | 1 | 93 | 98.9 | 97.8 | >0.05 |
| | Negative | 23 | 6 | 6 | 35 | 80.6 | 83.3 | >0.05 |
| | Total | 113 | 8 | 7 | 128 | 93.8 | 93.8 | >0.05 |
| CB-18 | Positive | 68 | 9 | 12 | 89 | 86.5 | 89.9 | >0.05 |
| | Negative | 29 | 21 | 7 | 57 | 87.7 | 63.2 | <0.05 |
| | Total | 97 | 30 | 19 | 146 | 87.0 | 79.5 | >0.05 |

AFB, acid-fast bacilli
[a] Both processing methods used the MB/BacT liquid culture system, but SDS-NaOH-processed sediments were inoculated onto L-J slants, whereas CB-18-processed sediments were inoculated onto 7H11 selective slants

to the isolation of *Mycobacterium tuberculosis* and NTM bacilli. When specimens were processed with CB-18, increases in culture sensitivity of 6.3% and 73.3% were observed among *Mycobacterium tuberculosis* and NTM isolates, respectively. The increase among NTM-positive specimens was the only difference considered statistically significant. Again, in both instances the increases in culture sensitivity were due exclusively to increases in isolation from smear-negative specimens. Evaluation of the two versions of CB-18 processing in this analysis did not offer any revelations (i.e., distribution of CB-18-positive specimens between the two phases did not generate any significant findings) with the exception of the subculture results: Subculture of negative MB/BacT culture bottles produced 8 culture-positive results from SDS-NaOH processed specimens and 21 culture-positive results from CB-18 processed specimens (see Table 3 footnote). Only 4 of the 21 CB-18-subculture-positive results were from phase 2 of the study.

## Time-to-Positive

Whereas processing specimens with CB-18 increased culture sensitivity, an analysis of the unpaired time-to-positive results in the MB/BacT liquid culture system revealed that, in the aggregate (i.e., among all positive specimens), processing with SDS-NaOH reduced the average time-to-positive by 1.8 days, but this difference was not significant ($P>0.05$) (Table 4). In contrast, the average time-to-positive among *Mycobacterium tuberculosis* complex bacilli was reduced by 2.5 days ($P<0.05$) following SDS-NaOH processing, whereas the average time-to-processing among NTM bacilli was increased by 2.9 days ($P>0.05$) following SDS-NaOH processing.

The aggregate, unpaired time-to-positive results on solid media were in contrast to the results in MB/BacT: the average time-to-processing was reduced by 4.3 days ($P<0.05$) among all AFB following CB-18 processing, and reductions in the average time-to-processing of 3.4 days ($P<0.05$) and 11.3 days ($P<0.05$) were observed among *Mycobacterium tuberculosis* and NTM-positive specimens, respectively, following CB-18 processing.

## Liquid versus Solid Culture Media

The culture results were further analyzed to ascertain the sensitivity of the MB/BacT system relative to the sensitivity on solid media following processing by the two different methods (L-J medium following SDS-NaOH processing, and 7H11-selective medium following CB-18 processing). In this analysis the results of each processing method were considered independently.

The sensitivity of liquid and solid media was identical following SDS-NaOH processing (Table 5); however,

**Table 6** Comparison of CB-18 culture sensitivities for the two different processing phases

| CB-18 phase | Smear result | Culture result (*n*) | | | | Sensitivity (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | Liquid & solid media | Liquid medium only | Solid medium only | Total positive for AFB | Liquid medium | Solid medium | *P* value |
| 1 | Positive | 36 | 8 | 11[a] | 55 | 80.0 | 85.5 | >0.05 |
| | Negative | 8 | 10 | 5 | 23 | 78.3 | 56.5 | >0.05 |
| | Total | 44 | 18 | 16 | 78 | 79.5 | 76.9 | >0.05 |
| 2 | Positive | 32 | 1 | 1[b] | 34 | 97.1 | 97.1 | >0.05 |
| | Negative | 21 | 11 | 2 | 34 | 94.1 | 67.6 | <0.05 |
| | Total | 53 | 12 | 3 | 68 | 95.6 | 82.4 | <0.05 |

AFB, acid-fast bacilli
[a] Of these 11 specimens, 9 yielded *Mycobacterium tuberculosis* and 2 yielded *Mycobacterium kansasii*. Eight of these 11 were from patients on antituberculous therapy, and 9 produced a positive result upon subculturing to L-J (7 of the 9 yielded*Mycobacterium tuberculosis*, and 2 yielded*Mycobacterium kansasii*). Six of the 8 patients diagnosed with tuberculosis and on antituberculous therapy were positive by subculturing
[b] This patient's specimen was positive for *Mycobacterium tuberculosis*; the patient was not on antituberculous therapy, and subculturing of the MB/BacT bottle produced *Candida*

the difference in sensitivity among smear-negative specimens following processing with CB-18 was significantly different: the increase in culture sensitivity of the MB/BacT system relative to the corresponding solid media result was 38.8% ($P<0.05$). The highest total number of AFB isolations in the study was achieved with the MB/BacT system following processing with CB-18 ($n=127$ [82.5% overall]); however, both SDS-NaOH and CB-18 processing resulted in the same total number of positive *Mycobacterium tuberculosis* results in the MB/BacT system (Table 4; $n=108$).

The analysis shown in Table 6 evaluated the difference in liquid versus solid media sensitivity in relation to the two different CB-18 protocols used. The occurrence of 11 specimens in phase 1 that were smear positive, but positive on solid media-only (i.e., MB/BacT negative), was the driving force behind modifying the CB-18 processing protocol and implementing phase 2 of this study. Of the 11 smear-positive MB/BacT-negative specimens in phase 1, only 1 was related to contamination. Previous reports suggested that, if the concentration of CB-18 in liquid culture was above 15–20 µg/ml, then it could interfere with the isolation of *Mycobacterium tuberculosis* [10, 11]. If 500 µl of undiluted supernatant (1 mM CB-18=383 µg/ml) were placed in an MB/BacT culture bottle with a final volume of 11 ml, then the maximum CB-18 concentration would be approximately 17 µg/ml. Any dilution of phase 1 sediments would drop the final concentration of CB-18 further (sediments that were resuspended with 1 ml of LZCT); however, the ability to effectively dilute sediments was dependent on the nature of the specimen [11]. The dilution-based protocol of phase 2 was designed to ensure that the concentration of CB-18 would be below 5 µg/ml, regardless of the nature of the specimen. In phase 2, only one smear-positive specimen was positive on 7H11 selective media but negative in MB/BacT media (Table 6). The highest overall culture sensitivity was achieved with the MB/BacT system in phase 2, a difference that was statistically significant relative to solid media.

Culture Contamination

One of the most significant differences between the two processing methods was in culture contamination rates. In the MB/BacT liquid culture system, the contamination rate following CB-18 processing was 8.7%, compared with a rate of only 0.8% following SDS-NaOH processing. On solid media, the contamination rates were 4.3% and 2.6%, respectively, but the SDS-NaOH method used L-J slants whereas the CB-18 method used 7H11 selective plates.

Regardless, contamination did not impact culture sensitivity results. For example, among all AFB-positive specimens ($n=152$), none of the L-J slants were contaminated following SDS-NaOH processing, whereas only 1 of these 152 specimens presented with contamination in the MB/BacT culture. Similarly, among all AFB-positive specimens, only three of the 7H11 selective plates were lost to contamination following CB-18 processing, and four of the MB/BacT cultures presented with contamination; however, AFB were eventually recovered from two of these four liquid cultures.

## Discussion

The goal of the present study was to evaluate the CB-18 specimen processing method for use with the MB/BacT liquid culture system. The method that had been validated in the clinical microbiology laboratory of the Hospital Universitari Germans Trias i Pujol at the time of this study was the SDS-NaOH method. The results of the study reported here were consistent with previous findings [10] wherein specimen processing was shown to impact the dynamics of several diagnostic parameters. Effects were seen on smear results, culture sensitivity, time-to-positive, and contamination rates.

In contrast to a previously published study [10], there was no improvement in overall smear sensitivity following CB-18 processing. However, in that study, CB-18

processing included an incubation step and was compared with the NALC-NaOH method, and attempts to control contamination involved the use of ceftazidime, not lytic enzymes [11]. This difference in study results might be explained by the following: either (a) the lytic enzyme formulation (i.e., LZCT) interfered with smear analysis, or (b) the efficacy of CB-18 processing is time dependent. The lytic enzyme formulation used in this study has been described as increasing the background during smear analysis (Lytic Decon product literature, Integrated Research Technology, LLC). This background appears as a mesh-like particulate that complicates reading of smears. Future evaluations of the CB-18 method should provide for removal of that portion of the sediment to be analyzed by smear prior to subsequent decontamination with lytic enzymes.

The second explanation for these smear results is reflected in the shift in smear values when the incubation step was included in the second phase of this study. In the initial study describing the CB-18 procedure, an incubation step of 90 min was used prior to centrifugation: under these conditions, an increase in smear sensitivity of 58% ($P<0.05$) was reported [10]. In the first phase of the study described herein, there was no incubation step, whereas in the second phase a 30 min incubation step was incorporated. While there was no difference in the overall smear sensitivity between these two protocols, there was clearly a shift in comparative smear values. An in vitro study comparing processing methods concluded that exposure of bacilli to CB-18 could be minimized to the time of the centrifugation step (i.e., no incubation step was required) [13]. However, these same authors have hypothesized that the action of CB-18 is dependent on a physiological mechanism (i.e., the active uptake of CB-18 forms lipoidal bodies, which alters the buoyant density of the bacilli) [9, 10, 11, 12, 13]. The results herein suggest that there is a minimum exposure time required to take advantage of CB-18 processing. Therefore, future use of the CB-18 protocol in processing specimens should include a longer incubation step (e.g., 90 min) to enhance recovery.

In addition to inclusion of the incubation step, another significant finding of this study is that there is a preferred CB-18 processing algorithm. In phase 1 of this study, the sensitivity of the MB/BacT system was only 80% among smear-positive specimens. Stone et al. [19] have shown that the sensitivity of the Bactec 12B liquid culture system among smear-positive specimens exceeds 99%: There were 11 smear-positive, MB/BacT-negative, and 7H11-positive specimens using the CB-18 protocol of phase 1. A previous report has shown that concentrations of CB-18 in excess of 15–20 µg/ml in the Bactec 12B vial during incubation adversely affect the recovery of *Mycobacterium tuberculosis* [11]. In the companion report, the sensitivity of Bactec 12B liquid culture among tuberculous mycobacteria was 12.1% lower following CB-18 processing [10]. In this study, the sensitivity of MB/BacT liquid culture among tuberculous mycobacteria following CB-18 processing was 6.3% higher. In neither instance were these results statistically significant, but the trend in the former instance was disconcerting, as was the sensitivity of MB/BacT in phase 1 among smear-positive specimens.

There are two primary differences between the Bactec 12B and the MB/BacT culture systems. The first is the media. These systems use a 7H12-based media and a supplemented 7H9-based media, respectively. The second difference is the final volume during incubation. When following recommended procedures, the final Bactec 12B volume would be 4.6 ml. The final MB/BacT volume would be 11 ml. As previously discussed, inoculating Bactec 12B bottles with undiluted sediments following processing with the CB-18 generates a final CB-18 concentration in excess of 35 µg/ml [11]. As described above, even though CB-18-processed sediments were resuspended with LZCT, the final concentration of CB-18 in MB/BacT bottles might exceed 15 µg/ml in some instances. Processing with the CB-18 protocol of phase 2 sets the final CB-18 concentration below 5 µg/ml. In phase 2 of this study, the sensitivity of the MB/BacT system exceeded 97% among smear-positive specimens, and there was a significant reduction in the number of subculture-positive specimens. Therefore, a dilution-based protocol is the preferred method.

The CB-18 specimen processing method was less labor-intensive than the SDS-NaOH specimen processing method, but it did not impact smear sensitivity in this study. Increasing the incubation time in the presence of CB-18 and removing aliquots before exposure to the lytic enzyme decontamination step should improve the smear sensitivity. CB-18 processing did provide a higher overall culture sensitivity. This increase was due primarily to greater recovery of NTM, but the increase in the recovery of tuberculous mycobacteria did contribute positively to the enhancement in culture sensitivity. A dilution-based protocol should further increase the recovery of *Mycobacterium tuberculosis* complex bacilli. The dilution protocol described in this study calls for a small volume of buffered CB-18 (e.g., 1 ml). The ability to liquefy specimens is adversely affected by such a small volume. A modified version of the dilution protocol that provides a lower concentration of CB-18 in a larger volume (e.g., 10 ml) is currently being evaluated to examine the trade-off between liquefaction of specimens and subsequent dilution of CB-18 prior to culture. The greatest disadvantage with the CB-18-lytic enzyme procedure is the contamination rate; however, this rate was not unacceptably high, as was the case in previous studies wherein ceftazidime was used in an attempt to control contamination [10].

Diagnostic procedures in mycobacteriology based on analyses of culture bring with them complications associated with contamination and, therefore, the need to process/decontaminate specimens prior to such analyses. The use of sodium hydroxide as originally reported by Petroff [20] in 1915 has changed very little in 85 years [2, 3]. The unfavorable aspects associated with culture have always been (a) the negative impacts of specimen

processing on mycobacterial viability (i.e., sensitivity), (b) lack of ease-of-use, (c) risk of high contamination rate, and (d) expense. When using a dilution-based protocol with an incubation step, the CB-18 processing method results in improved performance in each of these areas, but based on a novel hypothesis, the CB-18 method is the only procedure to compensate for the innate buoyancy of these bacteria and provide enhanced recovery during specimen processing [13]. This last advantage may be the most important, especially as it relates to the diagnosis of tuberculosis. Behr et al. [21] suggested that approximately 22% of all new cases of tuberculosis in San Francisco were the result of transmission from smear-negative patients with active disease. The greatest increase in culture sensitivity following CB-18 specimen processing was among smear-negative specimens. While this increase was due primarily to the recovery of NTM, recovery of tuberculous mycobacteria contributed positively to this outcome as well.

As it relates to nucleic acid amplification, Catanzaro et al. [22] have reviewed the clinical data submitted to the U.S. Food and Drug Administration for two commercial amplification tests for diagnosis of tuberculosis. The sensitivity of these tests among culture-positive and smear-negative specimens was approximately 50%. Improved recovery of bacilli will enhance amplification sensitivity among this group of specimens and facilitate diagnosis of infectious, smear-negative patients. A follow-up study using CB-18 processing with a polymerase chain reaction test is in progress.

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