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Isolation of Mycobacteria from Undecontaminated Specimens with Selective 7H10 Medium

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Media containing antimicrobial agents have been formulated for use as an adjunct to the standard media in an effort to reduce contamination and improve isolation of mycobacteria from clinical specimens. Selective 7H10 (S7H10) was developed for use in the isolation of mycobacteria from undecontaminated material. During a 33-month period, 10,782 clinical specimens were cultured in parallel on S7H10 without decontamination and on 7H11 after treatment with 2% sodium hydroxide–*N*-acetyl-L-cysteine. Results of this study show the overall contamination rate to be threefold lower on S7H10 than on 7H11 (304 versus 1,000). The number of specimens negative on NaOH-treated, 7H11-cultured specimens and contaminated on S7H10 was 282, whereas that negative on S7H10 but contaminated on NaOH-7H11 was 923. There were 6 positive cultures missed due to contamination on S7H10, compared with 61 on 7H11. Positive cultures on S7H10 outnumbered those on 7H11 by 106. This evaluation of S7H10 shows that it can be used with undecontaminated specimens in conjunction with standard methods and media for isolation of mycobacteria from clinical specimens.

Most specimens submitted to microbiology laboratories for isolation of mycobacteria are treated before culturing with alkaline reagents to reduce the number of bacteria and fungi that might overgrow the more slowly growing acid-fast organisms. Many mycobacteria are also killed by these alkaline decontaminating agents (4). An adjunct to decontamination is the use of selective culture media (1, 2, 4), allowing milder specimen processing procedures. Selective 7H10 (S7H10) was developed for isolation of mycobacteria from undecontaminated clinical specimens (4) to preserve the greatest number of acid-fast organisms.

This paper reports the mycobacterial culture results in a study of the use of S7H10 for undecontaminated specimens and those of 7H11 for the same specimens after alkaline decontamination.

MATERIALS AND METHODS

Media. 7H11 agar and S7H10 with added asparagine (0.25%) were prepared as described previously (7). Final antimicrobial concentrations in the selective media were 200 U of polymyxin B sulfate per ml (Sigma Chemical Co., St. Louis, Mo.), 10 μg of amphotericin B per ml (E. R. Squibb & Sons, Inc., New York, N.Y.), 100 μg of carbenicillin per ml (Beecham Laboratories, Bristol, Tenn.), and 15 μg of trimethoprim per ml (Sigma). The media were poured into 100- by 15-mm plastic petri dishes. They were stored in the dark, the 7H11 at ambient room temperature and the S7H10 at 5 to 10°C. Both were used within 4 weeks of preparation. Mycobactosel-7H10 was prepared from commercial agar base (BBL Microbiology Systems, Cockeysville, Md.).

Specimens. In preliminary studies, only sputum specimens from tuberculous patients were used. In the extended study, specimens of all types (sputum, gastric lavage, bronchial washing, tracheal aspirate, abscess material, urines) were assessed. In both studies, specimens, other than urines, were liquified by treatment with an equal volume of 0.5% *N*-acetyl-L-cysteine (NALC) (Sigma) in 0.1 M aqueous sodium citrate, pH 8.1, mixed for 10 to 15 s, allowed to stand for 15 min, and centrifuged at 1,500 × *g* for 30 min. (Since completion of this work, evidence has been presented emphasizing the need to increase centrifuge speed at least to 3,800 × *g* to enhance recovery of mycobacteria [6].) The supernatants were discarded, and the sediments were resuspended in 5.0 ml of sterile, distilled water. Approximately 0.3 ml of each was inoculated on the selective medium. Urine specimens were processed by direct centrifugation; the sediments were resuspended in 5.0 ml of sterile distilled water and inoculated on the selective medium. The remaining portions of all specimens were then decontaminated by addition of an equal volume of 2 or 4% NaOH-NALC digestion mixture. After 15 min of exposure to the NaOH-NALC, 40 ml of phosphate buffer (M/15, pH 6.8) was added, and the tubes were recentrifuged. The sediments were resuspended in 4.7 ml of phosphate buffer and 0.3 ml of each was inoculated on 7H11. Care was taken in adjusting the volumes of resuspended sediments so that colony count comparisons were valid. Plates were placed in individual poly-

ethylene plastic bags, incubated at 36°C in a 5 to 10% CO_2 atmosphere, and examined weekly for 4 weeks and again at 6 and 8 weeks for contamination and growth of mycobacteria. Isolation plates were considered contaminated if the area inoculated was more than 50% obscured by growth of contaminating organisms.

Mycobacterial strains. Mycobacteria were identified as to species by using standard methods (8). Organisms identified as *M. avium-intracellular* complex were sent to a reference laboratory for serovar determinations.

RESULTS

Table 1 shows the results of the preliminary study comparing isolation of mycobacteria and contamination rates of undecontaminated sputum specimens plated on S7H10 and the same specimens treated with either 2 or 4% NaOH-NALC before plating on selective and nonselective media. The contamination rate of undecontaminated specimens on S7H10 compared favorably with that on other media used for NaOH-treated specimens. The frequency of isolation of mycobacteria from undecontaminated specimens was better than that of treated specimens plated on Mycobactosel-7H10 and somewhat less than that on nonselective media. The number of colonies isolated on S7H10 frequently exceeded those found on the other media after NaOH treatment. In addition, growth could often be seen on the S7H10 plates after only 1 week of incubation. Therefore, it was decided to evaluate further the use of S7H10 in a larger trial for processing all types of clinical specimens. Mycobactosel-7H10 was not included in the larger study.

During a 33-month period, November 1974 to July 1977, 10,872 clinical specimens were cultured in parallel on S7H10 without decontamination and on 7H11 after treatment with 2% NaOH-NALC (final concentration). Culture results on S7H10 and 7H11 are shown in Table 2. The contamination rate is threefold lower on S7H10 than on 7H11 (304 versus 1,000). The number of specimens negative on NaOH-treated, 7H11-cultured specimens and contaminated on S7H10 was 282, whereas that negative on S7H11 but contaminated on NaOH-7H11 was 923. The number of positive specimens missed due to contamination was 10 times as great on 7H11 as on S7H10 (61 versus 6), and the number of positive cultures on S7H10 exceeded that for 7H11 (564 versus 458).

TABLE 1. *Isolation of mycobacteria and contamination comparison 636 sputum specimens*

Decontamination process	Culture medium	Positive cultures	Contaminated cultures	Negative cultures
None	S7H10	44	2	590
1% NaOH[a]-NALC	7H11	48	18	570
	Mycobactosel-7H10	33	4	599
2% NaOH[a]-NALC	7H11	48	7	581

[a] Final concentration.

TABLE 2. *Comparison of culture results of 10,872 specimens*[a]

Decontaminated (7H11)	Undecontaminated (S7H10)		
	Contaminated	Negative	Positive
Contaminated	16	923	61
Negative	282	9,041	91
Positive	6	40	412

[a] After 8 weeks of incubation.

Table 3 is a quantitative evaluation of the performance of S7H10 and 7H11 media after 4 weeks of incubation, when most positive cultures had occurred. As noted in the preliminary study, a greater number of colonies was found more often on S7H10 than on 7H11. There were more positive cultures on S7H10 when 7H11 was negative or contaminated than there were on 7H11 when S7H10 was negative or contaminated. The difference between the two media was not as apparent when the number of colonies isolated was greater than 100. Far fewer cultures were equally positive when the number of colonies was less than 100.

Table 4 summarizes the mycobacterial species isolated on S7H10 and the number of isolates of each. The list includes species reported to be inhibited by the selective medium (3, 4). With the exceptions noted, the same strains were also isolated on 7H11.

Table 5 indicates the types of contaminants found with the two processes used. The S7H10 appears to be effective in reducing all types of contaminants, especially fungi. The percent total contaminations for 10,872 specimens was 9.2% on 7H11 compared with 2.8% on S7H10.

DISCUSSION

The stated purpose for development of S7H10 medium was "to allow counting of the number of viable bacilli in sputum without the use of decontamination procedures which kill a portion of the bacilli present" (4). Since the publication of the original work (4), two additional reports of the use of the selective medium have been published. In one, S7H11 with modification of the carbenicillin concentration was used for heavily contaminated specimens (sputa) after NaOH-NALC treatment and for specimens less

TABLE 3. *Quantitative evaluation of positive cultures[a] (undecontaminated [S7H10] versus decontaminated [7H11])*

Colonies per plate	Culture medium positive						
	Both			S7H10		7H11	
	Equal	S7H10 greater	7H11 greater	7H11 Neg.[b]	7H11 Cont.[b]	S7H10 Neg.	S7H10 Cont.[b]
>99	213	26	6	2	11	1	0
21-99	36	52	7	11	14	3	3
1-20	9	37	14	61	10	29	3

[a] After 4 weeks of incubation.
[b] Cont., Contaminated; Neg., negative.

TABLE 4. *Primary isolation of mycobacteria on S7H10 and 7H11 media*

Species isolated	No. of isolates
M. tuberculosis	
Susceptible	354
Resistant[a]	
INH	23
SM	1
INH/RMP	6
INH/EMB	12
INH/SM/PAS	1
INH/SM/EMB/RMP	13
INH/SM/ETA/RMP	22
INH/SM/PAS/EMB/RMP[b]	5
Total	437
M. bovis[b]	1
M. kansasii[c]	37
M. avium-intracellulare	
Serovar 1	10
Serovar 9	1
Serovar 19	20
Serovar unclassified	32
M. simiae	20
M. gordonae	4
M. fortuitum[b]	2

[a] INH, Isoniazid; SM, streptomycin; RMP, rifampin; EMB, ethambutol; PAS, sodium-*p*-aminosalicylate; ETA, ethionamide.
[b] Primary isolation on S7H10 only.
[c] Twenty-five isolated on S7H10 only.

TABLE 5. *Specimen contaminants*

Organisms	No. of contaminants on:	
	S7H10	7H11
Pseudomonas sp.	3	26
Bacillus sp.	27	117
Alcaligenes sp.	47	143
Other bacteria	132	193
Fungi	95	521
Total	304	1,000

likely to be contaminated (spinal fluids, etc.) without decontamination (3). In the other, the medium was used for isolation of mycobacteria from tissue specimens (5). Both reports indicated improved isolation of mycobacteria and reduced contamination.

Our preliminary evaluation, initiated to compare the performance of S7H10 without decontamination with that of nonselective (7H11) and selective (Mycobactosel-7H10) media after decontamination, suggested the following: (i) S7H10 (without prior decontamination) has potential usefulness in the laboratory, (ii) the number of colonies was also greater when specimens were not treated with NaOH, and (iii) Mycobactosel-7H10 was too inhibitory to mycobacteria for further use.

The subsequent 33-month trial involving all clinical specimens received gives good evidence for the usefulness of S7H10. Contamination is one of the greatest problems for the microbiology laboratory processing specimens for isolation of mycobacteria. The use of S7H10 allowed 51 more positive culture reports and 641 additional negative culture reports which would have been lost due to contamination if 7H11 had been the only medium used. The use of S7H10 in the diagnostic laboratory can be recommended based on the reduction of contamination. The isolation of 51 more positive cultures from specimens untreated with NaOH were observed through use of S7H10. This is an additional reason to recommend the use of the selective medium.

Drug-susceptible and -resistant strains of *Mycobacterium tuberculosis* were isolated on S7H10. One highly resistant strain from strongly smear-positive sputum specimens was isolated in large numbers only on the selective medium, after 6 weeks of incubation. The one isolate of *Mycobacterium bovis* was recovered only on S7H10. With both of these strains and with the

Mycobacterium kansasii strains isolated only on S7H10, it is felt that the NaOH decontamination was responsible for the negative culture results on 7H11 medium. The inhibition of growth of some nontuberculous mycobacteria reported by Mitchison et al. (4) was not observed in this study even though we maintained the carbenicillin concentration at 100 μg/ml rather than reducing it to 50 μg/ml as recommended by McClatchy et al. (3). The reason for this discrepancy may lie in the fact that our isolates were not previously exposed to alkali digestion, a process that might render the remaining organisms more susceptible to the drugs found in S7H10. Primary isolates and subcultures from S7H10 have been used for many of the mycobacterial identification tests with good correlation of results from other media.

Many clinical and hospital laboratories do not have capability for processing specimens for mycobacteria and must rely on a reference laboratory for this service. Inoculation of a portion of the undecontaminated, homogenized specimens on S7H11 might help minimize culture loss due to deterioration of specimens in transit.

Although the antimicrobial agents in S7H10 are effective in controlling contaminating microorganisms, the medium should not be used as a replacement for good aseptic techniques. We are in agreement with others (3, 5) that S7H10 or S7H11 should not be the only medium used for isolation of mycobacteria and recommend that it be used in conjunction with other nonselective isolation media. The evidence that we have presented indicates that it can be used for culturing undecontaminated clinic specimens. We are now evaluating the use of S7H10 for direct susceptibility testing of undecontaminated specimens.

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