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Comparison of Three Culture Media for Isolation of *Mycobacterium tuberculosis*: a 6-Year Study

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Among 2,648 specimens positive on culture for *Mycobacterium tuberculosis* over a 6-year period, 82% grew on Lowenstein-Jensen medium (LJ), 79% on American Trudeau Society (ATS), and 56% on Middlebrook 7H10 (7H10). When these commercial culture media were compared in regard to the number of acid-fast bacilli seen on the original smears, LJ cultures were found to have the highest isolation rates for each smear category, and 7H10 had the lowest rates. Comparing the media from the aspect of number of mycobacterial colonies produced, LJ and ATS had the highest average colony counts, followed by 7H10. These findings were relatively constant over the 6-year period. One possible reason for the low positive rate of 7H10 was the lack of CO_2 enrichment.

Despite the obvious importance of optimal culture methods in diagnosing infections of *Mycobacterium tuberculosis*, relatively few studies comparing various culture media have been reported, and these have produced somewhat variable results. This investigation covers 6 years (1967 to 1972) of daily screening for *M. tuberculosis* and deals with a comparison of the growth on three commercial media, Middlebrook 7H10 agar-base medium (7H10), Lowenstein-Jensen (LJ), and American Trudeau Society (ATS), utilizing the NaOH-NaCl concentration method.

MATERIALS AND METHODS

Specimens. Sputum, urine, body fluids, gastric washings, and miscellaneous tissues were submitted for processing and examination. Spinal fluids were inoculated directly onto the surface of the slant and a smear was made directly from the specimen. A few drops to 1 ml of sterile saline was added to the sputum to help free it from the specimen container. Approximately 25 ml of the sputum was used for the digestion and concentration procedure. Urines, body fluids, and washings of various types were concentrated by centrifugation, reducing the volume to 25 ml.

Digestion and concentration of sputum. The method of concentration used continuously throughout this study was NaOH-NaCl, in which 4% NaOH was the digestant and phenol red indicator in 1 N HCl was the neutralizer. Equal volumes of 4% NaOH and specimen were mixed together in a 50-ml centrifuge tube. The mixture was homogenized and digested for 15 min. After digestion the specimen was centrifuged for 15 min at 3,000 rpm. The liquid was decanted from the sediment, and 25 ml of saline and 2 or 3 drops of phenol red indicator in 1 N HCl were added. If the reaction was too alkaline, 1 N HCl was added to neutralize the mixture. The specimen was again centrifuged, and all but 2 to 3 ml of the liquid was decanted. The sediment was resuspended, the media was inoculated, and smears were made (2).

Reporting and microscope examination. Slides were stained by the modified Kinyoun method (2). Acid-fast smears were categorized by the numbers of bacilli seen: negative, rare (3 to 9 bacilli per smear), few (10 or more per smear), and numerous (10 or more per field).

Cultivation. LJ and ATS tubed slants and 7H10 supplement were purchased from BBL, Division of Bioquest. The powdered 7H10 medium was obtained from Difco Laboratories. The medium was reconstituted, autoclaved, and cooled to 54 C, and supplement was added and tubed in the dark. Tubed media were permitted to cool, completely protected from light by a towel. The cooled slants were stored in a refrigerator until needed, at which time they were brought to room temperature before inoculation. A supply was kept refrigerated for only 30 days. The inoculated tubes of LJ, ATS, and 7H10 agar slants were incubated at 35 to 37 C in a horizontal position for 24 to 48 h, or until the first examination for growth, to allow the inoculum to spread and become fixed onto the media surfaces. Incubation was continued for at least 8 weeks, with weekly examination, before discarding as negative. Screw caps were kept loose, but the media were never allowed to dry out.

Identification. Any colonies appearing on the inoculated slants were transferred to other tubes of media and observed for growth in light and in dark at 25 and 37 C. Appropriate chemical tests, according to the direction of Sommers and Russell (11) were followed

for identification of the various species of mycobacteria.

RESULTS

Of the 25,181 specimens cultured (Table 1), there were 2,648 (11%) positive for *M. tuberculosis*, including 2,560 positive sputum specimens and 88 positive nonsputum specimens. Table 2 showed the positive cultures of *M. tuberculosis* in LJ, ATS, and 7H10 in different combinations. The total number of positive cultures were: LJ, 1,938; ATS, 1,662; and 7H10, 1,091. The contamination rates were LJ, 11%; ATS, 24%, and 7H10, 28%. When the culture media were compared from the aspect of individual specimens which were positive by only one of the three media, however, it was found that in many cases only the LJ (24%), ATS (17%), or 7H10 (11%) produced a positive culture. Figure 1 shows the percentage of positive cultures of *M. tuberculosis* over the 6-year period. This exhibited a fairly constant relationship between the highest isolation rate for LJ, a somewhat lower rate for ATS, and a still lower rate for 7H10. The difference between LJ and ATS was not significant, but compared with 7H10 the statistical significance was $P < 0.05$. In 1970 ATS had a slightly higher positive rate than LJ, and 7H10 showed a similar deviation in 1972. The reason for these variations was unclear.

When the media were compared in regard to the number of acid-fast bacilli seen on the original smear (Table 3), LJ cultures were found to have the highest rate of *M. tuberculosis* isolation for each of the smear categories and 7H10 had the lowest rate of isolation for each category. An exception was ATS, which showed slightly more positive cultures in the numerous category. The percentage of positive cultures was higher when more bacilli were encountered on the smear. This held true for all three media.

TABLE 1. *Specimen source and positive culture rate for M. tuberculosis*[a]

Specimen	No. examined	No. positive
Sputum	25063	2560
Nonsputum:	118	88
Urine	36	26
Bronchial washing	19	17
Gastric juice	19	15
Pleural fluid	8	7
Cerebrospinal fluid	4	3
Peritoneal fluid	2	1
Lung	15	6
Lymph node	6	6
Unknown source	3	2
Liver	2	1
Kidney	1	1
Spleen	1	1
Bone marrow	1	1
Foot	1	1

[a] Total positive: 11%.

TABLE 2. *Specimens positive for M. tuberculosis with three culture media*[a]

Media			No. positive cultures by medium combination
LJ+	ATS+	7H10+	535
LJ+	ATS+	7H10−	643
LJ+	ATS−	7H10−	572
LJ+	ATS−	7H10+	188
LJ−	ATS+	7H10+	142
LJ−	ATS+	7H10−	342
LJ−	ATS−	7H10+	226

[a] Total positive cultures by any medium combination = 2,648. Total LJ+ = 1,938; total ATS+ = 1,662; total 7H10+ = 1,091.

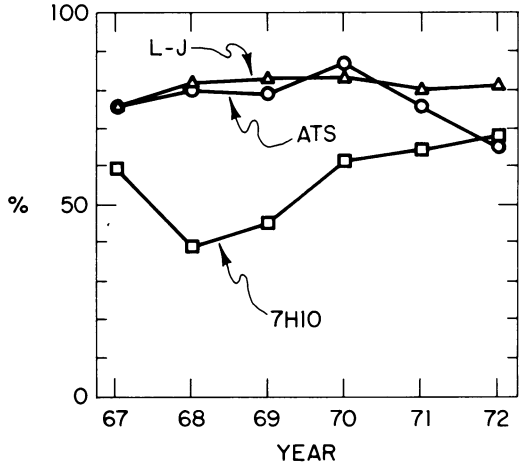

FIG. 1. *Percentage of positive cultures of M. tuberculosis on three media over 6-year period.*

TABLE 3. *Percentage of positive cultures of M. tuberculosis on three media compared with number of acid-fast bacilli on smear*

Media	Percentage of positive cultures				
	Numerous[a]	Few	Rare	Negative	Avg
LJ	91	84	86	75	82
ATS	92	82	80	72	79
7H10	84	74	61	29	56

[a] Numerous, 10 or more bacilli per field; few, 10 or more bacilli per smear; rare, 3 to 9 bacilli per smear; negative, no bacilli.

Comparing the media from the aspect of number of mycobacterial colonies produced (Table 4), LJ and ATS were found to have the highest average colony count, followed by 7H10. This relationship held for each of the 6 years and for each category of number of acid-fast bacilli seen on the original smears; the number of colonies produced on LJ and ATS were never significantly different, but the number on 7H10 was always considerably less. For each of the three media, the number of acid-fast bacilli counted on the smears corresponded well to the number of colonies produced.

DISCUSSION

In comparative studies, LJ appeared to be the most satisfactory single medium of the three investigated. The difference between positive isolations on LJ and ATS medium, however, was not statistically significant. These findings are consistent with the previous reports made by Cummings (1) and Hughes et al. (3). 7H10 agar-base medium was previously described by Middlebrook and Cohn (8) as having a culture sensitivity approximately equal to that of LJ when comparing the number of specimens positive for *M. tuberculosis*. They also reported that the 7H10 produced more colonies per culture than did LJ. However, in this laboratory, studies showed that the 7H10 medium was less effective and produced fewer colonies than did LJ and ATS. In 1959, Jefferies et al. (4) studied the culture result of *M. tuberculosis* by LJ, ATS, and 7H9 using Zephiran in the digestant and concluded that 7H9 was less effective and in some respects more difficult to use. 7H10 was an improved formulation of 7H9 and had malachite green added. Our finding was in contradistinction to previous reports which indicated the superiority of the 7H10 in the culture of *M. tuberculosis* (6–8). This 6-year study did not include CO_2 enrichment of the culture atmosphere, and this may have caused some reduction in the effectiveness of the media, especially 7H10 (12).

TABLE 4. *Average number of M. tuberculosis colonies on three media compared with number of acid-fast bacilli by smear*

Media	No. of *M. tuberculosis* colonies				
	Numerous[a]	Few	Rare	Negative	Avg
LJ	252	133	51	28	116
ATS	266	133	55	25	114
7H10	161	85	35	18	70
Avg	226	117	47	24	104

[a] Numerous, 10 or more bacilli per field; few, 10 or more bacilli per smear; rare, 3 to 9 bacilli per smear; negative, no bacilli.

The use of NaOH-NaCl digestant, as recommended by Kubica et al. (6), would not necessarily make 7H10 look better; as a less toxic digestant, it might improve isolations on all media. It might also increase contamination, in which case technicians should be prepared to increase the NaOH concentration to effectively reduce contamination. Comparative studies of these three media, utilizing NaOH-NaCl digestion-decontamination and CO_2 enrichment, will be performed after sufficient data have been obtained.

Although the overall positive culture rate of 7H10 was low in this study, the superiority of one culture method over another was less apparent when specimens were considered individually; in the 6-year period, 226 (11%) of the specimens grew *M. tuberculosis* only on the 7H10 media. Furthermore, the previously reported faster growth of *M. tuberculosis* on 7H10 has been reconfirmed in this laboratory. Thus, from this study, it was determined that there was no one ideal medium for the isolation of *M. tuberculosis* from clinical specimens. The use of two culture slants, LJ and ATS, with large areas for inoculation, appears to be the most acceptable routine practice. The value of 7H10 cannot be underestimated, however, and if at all possible 7H10 should be routinely included to increase the speed and rate of isolation of *M. tuberculosis*.

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