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Comparison of Four Culture Media for the Isolation of *Mycobacterium Tuberculosis*: a 2-Year Study

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Four media, Lowenstein-Jensen, Middlebrook, Petragnani, and ribonucleic acid, were tested for comparative ability to detect *Mycobacterium tuberculosis*. Specimens used included sputum, urine, tissue, and gastric washings. Three types of comparison were used: (i) comparison derived from randomized specimens; (ii) comparison of cultures from newly diagnosed cases that had received no prior therapy; and (iii) comparison of cultures from specimens whose initial direct smears were negative. Overall, ribonucleic acid medium performed best, but the differences among the four media were small.

Although it is of paramount importance that only optimal isolation methods be used for specimens suspect of containing *Mycobacterium tuberculosis*, few studies comparing the qualities of the various culture media have been reported. Some of the reports have given variable and sometimes even conflicting results.

Our investigations covered a period of 2 years, 1972 to 1973, during which time specimens from various parts of Nova Scotia were screened for *M. tuberculosis*. A comparison was made of isolation rates on four types of media; three are prepared in this institution, and the fourth, 7H10, was obtained from commercial sources. During this time 1,175 specimens were found to be positive for *M. tuberculosis*; 81% grew on Lowenstein-Jensen (LJ) medium; 74% grew on Middlebrook (7H10) medium; 78% grew on Petragnani (P) medium; and 87% grew on ribonucleic acid (RNA) medium (2, 9).

On the basis of these data, no medium was surpassingly superior, but RNA medium appeared to perform best.

MATERIALS AND METHODS

Specimens. Sputa, urine, tissue, and gastric washings were submitted from various institutions, medical practitioners, and sanitoria.

Digestion and concentration of sputum. The method of digestion and decontamination adopted throughout these studies was the *N*-acetyl-L-cysteine–sodium hydroxide combination (12). Equal volumes of each specimen and the above solution were allowed to react for 15 min, followed by the addition of a phosphate buffer, pH 7.2. The mixture was then centrifuged and decanted. The sediment was resuspended in about 0.5 ml of buffer, and 1.0 ml of 0.2% ox albumin was added. The resuspended sediment was then used to seed the culture media and prepare slides. In some cases a few milliliters of sterile distilled water was added to thick, tenacious sputum in order to dislodge it from the container. The volumes of the sputa submitted varied greatly, ranging from a few milliliters to as high as 25 ml.

Cerebrospinal fluids were inoculated directly onto the surface of the slant without prior treatment, and a smear was also prepared directly. Urines, body fluids, and gastric washings were concentrated by centrifugation, reducing the volume to approximately 20 ml before processing.

Microscopic examination of slides. The methods of Manfield (7) and Somlo et al. (11) were used. The slides were examined by fluorescent microscopy, using a Zeiss RA microscope. The instrument was equipped with a Osram-Halogen Bellaphot 12-V 100-W lamp with a BG12 exciter filter fitted. All slides were searched with a 25/0.454 objective and confirmed with a 63/0.90 objective.

Slides were stained first with Auramine O mixture followed by decolorizing and counter-staining with potassium permanganate solution.

Cultivation. The 7H10 supplement and dehydrated media were obtained from BBL (Middlebrook-Cohen 7H10 agar base no. 11422) and Difco (O.A.D.C. enrichment no. 0722-64), respectively. LJ, P, and RNA media were all prepared in the Media Preparation Laboratory of this institute from standard formulas. The media were each dispensed into disposable, previously sterilized pharmaceutical bottles (Boston-Squares) and stored in a refrigerator until required. Refrigerated supplies of media were never more than 14 days old. The inoculated tubes of LJ, P, 7H10, and RNA media were incubated at 35 to 37 C in a horizontal position in order to allow the inoculum to spread evenly over the surface of the medium and become fixed to the surface. Incubation was continued in a walk-in incubator for a period of up to 12 weeks. During this period inspections were made at 3, 6, 8, or 12 weeks.

Cultures showing evidence of growth at any time during this period were checked for morphology by making a smear and staining by the Ziehl-Neelson procedure. All acid-fast organisms were biochemi-

cally tested for niacin and nitrate reduction. Confirmed organisms were then set up for sensitivity patterns.

RESULTS

Of the 24,670 specimens cultured, 1,175 (4.8%) were positive for *M. tuberculosis*. Included in this number were 1,000 positive sputa, 51 gastric lavages, and 123 miscellaneous specimens made up of urines, swabs from lesions, and tissue and biological fluids. One cerebrospinal fluid was positive (Table 1).

When positive cultures were compared (Table 2), it was found that RNA medium gave the highest number of positive isolations (1,021), with 67% of this number becoming positive within the first 3 weeks of incubation and a further 30 and 3% becoming positive after an extended 6 and 8 weeks of incubation, respectively. 7H10 medium gave the lowest number of isolations (869), with 62% of this number growing during the first 3 weeks of incubation and a further 29 and 9% growing after an extended period of 6 and 8 weeks, respectively. LJ and P media were not significantly different in their recovery rates, the numbers of positive isolations being 950 and 915, respectively. In both cases 71% became positive within the first 3 weeks of culturing, with a further 26 and 3% becoming positive after 6 and 8 weeks of extended incubation, respectively.

A concurrent study was also made to compare cultures derived from newly diagnosed cases of tuberculosis (Table 3). These cases had received no previous chemotherapy. During this period, 176 such cases were examined. When cultures obtained from newly diagnosed cases were compared, RNA medium gave the highest number of isolations (147), with 77% becoming positive after 3 weeks of incubation and a further 22 and 1% becoming positive after 6 and 8 weeks of incubation, respectively (Table 3). It is noted that both the LJ and P media yielded 82 and 83%, respectively, of their positive cultures within the first 3 weeks of incubation.

When media were compared in cases where the original direct smear was negative (Table 4), LJ medium was found to give the highest numbers of positive cultures (122); RNA medium was not significantly lower (119). It is noted that both LJ and P media yielded 44% of their positive cultures within the first 3 weeks. 7H10 medium appeared to give the lowest numbers of positive cultures (107), yielding 33% of its positive cultures within the first 3 weeks of incubation.

TABLE 1. *Results obtained with various specimens*

Specimen	No. examined	No. positive
Sputa, gastric lavages	19,654	1,051
Urines, swabs, tissue	4,722	123
Cerebrospinal fluid	294	1

TABLE 2. *Comparison of positive cultures*

Medium	Total no. of positive isolations	% of total recovery after:		
		3 Weeks	6 Weeks	8 Weeks
LJ	950	71 (676)[a]	26 (249)	3 (25)
7H10	869	62 (543)	29 (249)	9 (77)
P	915	71 (649)	26 (236)	3 (30)
RNA	1,021	67 (687)	30 (302)	3 (32)

[a] Numbers in parentheses indicate number of positive cultures isolated.

DISCUSSION

In these comparative studies it would appear that the 7H10 agar base medium, described by Middlebrook and Cohen (8), was not of comparable sensitivity to LJ medium for the isolation of *M. tuberculosis*. Our findings do not agree with those of previous reports that indicate the superiority of 7H10 for the isolation of *M. tuber-*

TABLE 3. *Cultures obtained from newly diagnosed cases of tuberculosis*

Medium	Total no. positive	% Positive after:		
		3 Weeks	6 Weeks	8 Weeks
LJ	137	82 (112)[a]	17 (23)	1 (2)
7H10	127	68 (86)	27 (35)	5 (6)
P	120	83 (100)	14 (17)	2 (3)
RNA	147	77 (113)	22 (33)	1 (1)

[a] Numbers in parentheses indicate total number of positive cultures isolated.

TABLE 4. *Cultures obtained from specimens giving negative original smears*

Medium	Total no. positive	% Positive after:		
		3 Weeks	6 Weeks	8 Weeks
LJ	112	44 (54)[a]	53 (65)	3 (3)
7H10	107	33 (35)	59 (63)	8 (9)
P	112	44 (49)	46 (51)	10 (12)
RNA	119	39 (47)	55 (65)	6 (7)

[a] Numbers in parentheses indicate total number of positive cultures isolated.

culosis (4, 5). Our findings tend to agree with those of Liu et al. (6) and Cummings (1). However, at no time during these studies was the CO_2 enrichment of the atmosphere included in the protocol. This may be of considerable importance in limiting the sensitivity of this medium (10, 13).

This study was divided into three catagories of comparisons. (i) A comparison of cultures derived from randomized specimens received at this Department. RNA and LJ media gave the highest numbers of positive results and 7H10 gave the lowest. (ii) A comparison of cultures derived from newly diagnosed cases that had not received previous therapy. It was felt that perhaps chemotherapy may have altered the nature of the organism in such a way as to change its affinity for certain culture media; however, our results suggest that chemotherapy played little or no role in this direction since RNA medium still produced the highest number of positive cultures, with LJ medium maintaining its position in second place. (iii) A comparison of cultures derived from specimens whose initial direct smears were negative. Results of this study may give valuable information since positive cultures obtained from these sources would have been obtained from specimens containing a minimal number of organisms. Results indicated that LJ and RNA media produced the greatest numbers of positives. The difference between results obtained by these two media was not statistically significant. These were followed in turn by P medium with 7H10 giving a somewhat lower recovery rate. It is noted that in this series a greater length of time was required for the development of observable colonies, which of course would be expected. In all cases a higher percentage of positives developed after 6 weeks of incubation.

Petragnani medium (9) was included for historical comparison. It is interesting to note that although it is now over 50 years since Petragnani first described this medium, in our hands it was still an efficient medium for the isolation of *M. tuberculosis*. It would seem to us that over the years very little significant improvement in the efficiency of isolation of this organism has been realized. The significant gains achieved seem to have been made in ease of media preparation.

Although the overall positive culture rate of 7H10 was low in this study, this medium is strongly recommended for the development of sensitivity patterns, since it is capable of giving accurate reproduction. For the routine culture of *M. tuberculosis* it would appear that the use of LJ and RNA media would be the most satisfactory for routine practice. The use of P medium would also be quite acceptable, except that it is quite time-consuming to prepare.

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