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Comparison of plain egg medium with Lowenstein-Jensen medium in the isolation of M. tuberculosis from sputum.

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The isolation of tubercle bacilli from sputum using a plain egg (PE) medium and the conventional Lowenstein-Jensen (L-J) medium has been investigated on 703 specimens. The isolation of positive cultures and the grades of positivity were similar with the two media. There was an indication that the growth on the PE medium was faster than on L-J medium. The incidence of contamination was similar and low (4.0 per cent on PE and 3.8 per cent on L-J medium). Being cheaper and simpler than L-J medium, the PE medium is ideally suited for the routine culturing of tubercle bacilli.

Lowenstein-Jensen (L-J) medium is the most commonly used egg-based medium for the primary isolation of tubercle bacilli and also for drug-susceptibility and identification tests. This medium contains 1-asparagine and magnesium citrate, both of which are not readily available in India. Further, 1-asparagine is a very expensive chemical ingredient.

Recently, Marks[1] reported a plain egg (PE) medium useful for drug sensitivity and identification tests. This medium avoids the use of 1-asparagine and magnesium citrate and works out to be thus cheaper than L-J medium. A preliminary comparison of this medium with L-J medium on a small number of specimens at this Centre (unpublished observations) showed the two media to be equally satisfactory in the isolation of tubercle bacilli from sputum. Therefore, a full-scale controlled study on a large number of specimens was conducted, the results of which are reported here.

Material and Methods

Media : L-J medium, without potato starch, was prepared as described by Cruickshank[2].

The PE medium was prepared by adding 600 ml of autoclaved (120°C/ 15 min) basal salt solution (containing KH_2PO_4, 0.4 per cent; $MgSO_4$. 7H,O, 0.04 per cent; glycerol, 2 per cent and malachite green, 20 ml of 2 per cent aqueous solution) to 1 litre of egg fluid and mixing the two solutions thoroughly.

Both the media were distributed in approximately 6 ml amounts in universal

containers and inspissated at 85°C for 50 min. The slopes were stored overnight at room temperature, reheated for 30 min and stored in the cold (4°-10°C) until used.

Sputum specimens : This investigation was based on sputum specimens received in the laboratory for routine bacteriological examination. Smear-positive and smear-negative specimens were chosen in the proportion of 1 : 2 to ensure adequate numbers for separate analyses of the two groups. A total of 703 specimens was obtained from 480 patients.

Microscopy : Sputum smears were examined by fluorescence microscopy[3] using x 15 eye-pieces and x 10 objective. The bacillary morphology was confirmed with x 40 objective. A smear was' reported as positive if it contained a minimum of 4 acid-fast bacilli of typical morphology. Positive smears were graded as + if less than 6 bacilli were seen per field, as ++ if 6-100 bacilli were present per field and as +++ if more than 100 bacilli were observed per field (all gradations were done using the x 40 objective).

Culture procedures : Sputa were decontaminated and homogenised by the modified Petroff technique[4]. Two L-J slopes and two slopes of PE medium per specimen were inoculated with a loopful (5 mm internal diameter) of the deposit by the same person. The order of inoculation of the four slopes was randomised. The inoculated slopes were incubated at 37°C for 8-9 weeks.

Reading of cultures : All pairs of slopes (two pairs per specimen) set up in a week were rearranged in a random order, so that, for any individual pair the reader was unaware of the medium used (the two media were identical in appearance) or the identity of the specimen. The slopes were examined weekly for 8 weeks for growth of tubercle bacilli or for presence of contamination. Growth resembling *M. tuberculosis* was graded, when first seen, as +++ if it was confluent, ++ if there were more than 100 discrete colonies and as + if there were 20-100 colonies; the actual number of colonies was reported if this was less than 20. A culture was reported as contaminated only if both slopes were contaminated.

All cultures over the entire period were read by one reader (PV) and their morphology independently checked by another (RP).

Identification tests : All isolates of *M. tuberculosis* were sub-cultured on L-J medium and the niacin production test[5] was performed to confirm their identity as *M. tuberculosis.*

Results

Smears : Of the 703 specimens. 237 were smear-positive. Of these, 154 were reported as +, 66 as ++ and 17 as +++.

Culture positivity : Table I gives the culture results with the PE and L-J media, separately for the smear-positive and smear-negative specimens.

Considering the 237 smear-positive specimens, 204 (86.1 per cent) yielded positive cultures on PE medium, and 203 (85.7 per cent) on L-J medium; the proportions of cultures reported as contaminated were 3.8 per cent and 4.2 per cent, respectively. Among the 466 smear-negative specimens, 20 (4.3 per cent) with PE and 16 (3.4 per cent) with L-J medium

Table I. Comparison of plain egg (PE) medium and Lowenstein-Jensen (L-J) medium in culturing *M. tuberculosis* from sputum specimens

Culture result	Smear-positive		Smear-negative	
	PE (%)	L-J (%)	PE (%)	L-J (%)
Positive	86·1	85·7	4·3	3·4
Negative	10·1	10·1	91·6	92·9
Contaminated	3·8	4·2	4·1	3·7
Total specimens	237		466	

were positive on culture, giving similar findings. The contamination rates were also similar, namely 4.1 per cent and 3.7 per cent respectively.

Considering all the 703 specimens (results not tabulated), 224 (31.9 per cent) were culture positive by the PE medium, compared with 219 (31.2 per cent) by the L-J medium. Two hundred and fourteen specimens (30.3 per cent) were positive by both media. Ten specimens were culture positive only on the PE medium, compared with 5 specimens positive only on L-J medium. This difference was not statistically significant. Thus, the two media were of equal efficiency in respect of proportions of positive cultures.

Contamination : Considering all the 703 specimens, contaminants (on both slopes) were grown on PE medium from 28 (4.0 per cent) and on L-J medium from 27 (3.8 per cent) specimens. Eighteen specimens (2.6 per cent) yielded contaminants on both the media. Ten specimens were contaminated only on PE medium compared with 9 contaminated on L-J medium alone.

Considering the incidence of single slope contaminations, 89 PE slopes were contaminated compared with 76 L-J slopes. This difference was not statistically significant. In 18 specimens, one slope each of both media was contaminated. Thus, the risk of contamination was similar with the two media.

Degree of positivity **:** The correlation between the degrees of growth on the two media from the 229 specimens yielding growth of *M. tuberculosis* on either or both media are presented in Table II. Grades of growth on the two media were identical in 151 specimens culture positive on both media. Forty five specimens yielded heavier growth on the PE medum compared with the L-J medium, whereas 28 specimens had the reverse results (P-0.06). Thus, growth on the PE medium was at least as heavy as that on the L-J medium.

Speed of growth : Table III presents the cumulative figures for cultures positive on each medium according to the week of growth. Thus, growth on the PE medium was obtained from 20.5 per cent

Table II. Correlation between degrees of growth on plain egg (PE) medium and Lowenstein-Jensen (L-J) medium among specimens positive by either or both media

L-J medium	PE medium						
	Negative	1-19	1+	2+	3+	Contaminated	Total
Negative		4	2	1	–		7
1-19	2	15	4	3	–		24
1+		2	16	12	–		30
2+	1	1	3	78	19	1	103
3+	–	–		19	42	1	62
Contaminated	–	1	–	1	1		3
Total	3	23	25	114	62	2	229

Table III. Cumulative figures for positive cultures at different weeks with plain egg (PE) medium and Lowenstein-Jensen (L-J) medium among specimens positive by either or both media

Week of growth	PE medium		L-J medium	
	No.	%	No.	%
1	47	20.5	45	19.6
2	157	68.6	142	62.0
3	205	89.5	194	84.7
4	217	94.8	211	92.1
5 - 8	224	97.8	219	95.6
Total positive	229		229	

by 1 week, 68.6 per cent by 2nd week, 89.5 per cent by 3rd week, 94.8 per cent by 4th week and 97-8 per cent by 8 weeks. The corresponding proportions with L-J medium were 19.6 per cent, 62.0 per cent, 84.7 per cent, 92.1 per cent and 95.6 per cent, respectively. The difference in the speed of growth was statistically significant (Wilcoxon Test, P=0.001). The mean week of growth of positive cultures was 2.23 for the PE medium and 2.33 for L-J medium.

Thus, the proportions of positive cultures and the grades of positivity were similar with the two media. There was

some evidence that the growth was faster with the PE medium.

Isoniazid-resistant strains : Isoniazid sensitivity tests were performed on 97 of the cultures and 43 were found to be resistant (minimal inhibitory concentration 1 µg/ml or more). Analyses (not tabulated) indicated that the degree and the speed of growth of these cultures were similar on the two media.

Discussion

Results have been presented of a comparison of the isolation of tubercle bacilli from sputum using the conventional Lowenstein-Jensen (L-J) medium and a plain egg (PE) medium. The PE medium avoids the use of 1-asparagine and magnesium citrate which are expensive and not readily available in India and so is cheaper and simpler than L-J medium.

The rates of isolation of *M. tuberculosis* were similar with the two media in smear-positive as well as smear-negative specimens. The incidence of contamination was also similar and low.

Although the media gave similar proportions of cultures reported as positive, negative or contaminated, there was an indication that the PE medium gave at least as high a grade of positivity as on L-J medium, and in a shorter time, both factors being highly desirable in a medium used for culturing strains of tubercle bacilli which are slow growing and are fastidious in their requirements of growth-promoting factors. This medium has also been successfully employed for performing drug susceptibility tests after incorporating the drugs before inspissation[1]. As such, this medium is ideally suited for routine bacteriology of tuberculosis.

References

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