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A comparison between minerals-modified glutamate medium and lauryl tryptose lactose broth for the enumeration of *Escherichia coli* and coliform organisms in water by the multiple tube method

By a joint Committee* of the Public Health Laboratory Service and the Standing Committee of Analysts†

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SUMMARY

In a multi-laboratory trial, minerals-modified glutamate medium (MMGM) was compared with lauryl tryptose lactose broth (LTLB) in the multiple tube method for the enumeration of coliform organisms, including *Escherichia coli*, in water. Samples of raw and chlorinated waters yielded a total of 2313 positive tube-reactions with MMGM and 2174 with LTLB. These were interpreted either as *E. coli*; other coliform organisms; or as false positive reactions. The results at first reading (18 or 24 h) and at 48 h have been analysed statistically in terms of (i) most probable numbers of coliform organisms; (ii) positive reactions and their interpretation; and (iii) whether or not the sample yielded any *E. coli* or other coliform organisms. All three analyses indicated the same trends. For the detection of *E. coli* in raw waters LTLB was better than MMGM at 18–24 h, but MMGM was better at 48 h with waters containing small numbers of coliform organisms; for raw waters with greater numbers of organisms, both media performed equally well. Analysis of a subset of samples read at both 18 and 24 h indicated that the superiority of LTLB over MMGM with raw waters disappeared by 24 h. For chlorinated waters, LTLB yielded more positive gas reactions at 18–24 h, but fewer of these were *E. coli* than with MMGM; at 48 h MMGM was clearly better than LTLB for total coliform organisms including *E. coli* – especially if the numbers were small. MMGM therefore remains the medium of choice for the detection of *E. coli* as an indicator of faecal contamination of chlorinated drinking water supplies. It is also better for the detection of small numbers of *E. coli* in other waters.

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INTRODUCTION

For many years surface-active substances have been used as selective inhibitors in media for the multiple tube test for the isolation and enumeration of coliform organisms in water. In Britain, the usual surfactants were bile salts as in MacConkey broth – or more recently Teepol broth (Jameson & Emberley, 1956). These were used almost exclusively in the multiple tube test for these organisms until Gray (1964) developed an improved formate lactose glutamate medium (IFLG). This chemically defined medium, which was based on earlier work (Folpmers, 1948; PHLS, 1958; Gray, 1959) and designed exclusively for the isolation of coliform organisms and *E. coli* in water, was found in trials to be better than either MacConkey or Teepol broth (PHLS, 1968), and it was therefore recommended for routine use (Report, 1969). Subsequently, a minerals-modified version of this glutamate medium was formulated for commercial production, and the superiority of this over MacConkey and Teepol broth, especially with regard to the isolation of *E. coli*, was again confirmed in another trial (PHLS, 1969). Since then, Minerals-Modified Glutamate Medium (MMGM) has to a considerable extent replaced other media in Britain for this purpose. In contrast, in America and in some countries in Europe, lauryl tryptose (lauryl sulphate lactose) broth (LTLB), a nutrient medium containing a chemically defined surfactant, has been widely used and is currently recommended in *Standard Methods for the Examination of Water and Wastewater* (1975). Up to the present time, however, no direct comparison between minerals-modified glutamate medium and lauryl tryptose lactose broth has been reported. The results of such a multi-laboratory investigation are described in this paper.

MATERIALS AND METHODS

Primary isolation media

For this investigation, arrangements were made with Oxoid for supply of a single batch each of dehydrated minerals-modified glutamate medium (CM 289) and lauryl tryptose lactose broth (lauryl tryptose broth CM 451). Appropriate amounts were distributed to each participating laboratory where, for use, they were prepared as double- and single-strength media according to the manufacturer's instructions as follows:

Minerals-modified glutamate medium (MMGM)

Double-strength medium was prepared by dissolving 35·4 g powder in 1 l distilled or de-ionized water containing 5 g ammonium chloride. It was then dispensed in 10 ml volumes in test tubes (150 mm × 19 mm) each containing an inverted inner (Durham) tube and sterilized at 116 °C for 10 min in the autoclave or alternatively by heating in a steamer for 30 min on three successive days. The final pH was approximately 6·7.

Single-strength medium was prepared similarly by dissolving 17·7 g powder in 1 l water containing 2·5 g ammonium chloride. It was dispensed in 5 ml volumes in test tubes (150 mm × 16 mm) and sterilized as above.

Lauryl tryptose lactose broth (*LTLB*)

Double-strength medium was prepared by dissolving 71·2 g powder in 1 l distilled water and dispensing in the same way as for double-strength MMGM. It was sterilized in the autoclave at 121 °C for 15 min. The final pH was 6·8 ± 0·2.

Single-strength medium was prepared similarly by dissolving 35·6 g powder in 1 l distilled water. It was distributed in tubes as for single-strength MMGM and autoclaved at 121 °C for 15 min.

Confirmatory media

Lauryl tryptose mannitol broth (*LTMB*)

A single batch of dehydrated LTMB, specially prepared by Oxoid, was distributed in suitable amounts to each laboratory. For use, 35·6 g powder was dissolved in 1 l distilled or de-ionized water, distributed in 5 ml volumes in test tubes (150 mm × 16 mm) containing inverted Durham tubes, and autoclaved at 121 °C for 15 min. As with LTLB, the final pH was 6·8 ± 0·2.

Tryptone water (*TW*)

This was prepared from Oxoid dehydrated medium (CM 87 or 88) in stock in each laboratory according to the manufacturer's instructions.

Lactose peptone water (*LPW*)

This was prepared from normal stocks held in each laboratory as described in Report (1969).

Confirmatory tests

Each primary tube giving a presumptive positive reaction was subcultured to (i) tubes of LTMB and TW for incubation at 44 °C for 24 h, (ii) plates of MacConkey medium and nutrient agar, and (iii) if necessary, to a tube of LPW for incubation at 37 °C. After incubation, the amount of gas, if any, produced in LTMB was noted, and indole tests were performed on both LTMB and TW cultures as described in Method 2 of Cowan & Steel (1974); if the reaction for indole was very faint, it was further checked by extraction with xylol or ether. Attempts were also made to exclude false positive reactions caused by aeromonads by performing oxidase tests on selected colonies by Method 2 of Cowan & Steel (1974); if negative, the results of all tests were accepted. If the oxidase test was positive, typical coliform colonies were selected from the MacConkey plate for repeat subcultures as for primary tubes; if the oxidase test was still positive, the primary tube reading was regarded as a false positive result.

Interpretation of test results

(1) The results were interpreted as confirming the presence of *E. coli* in tubes if all the following criteria were satisfied: gas and indole was produced in LTMB and

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indole in TW at 44 °C; there were typical lactose fermenting colonies on MacConkey agar; and the oxidase test, if done, was negative.

(2) If there were any discrepant results in (1), laboratories were asked to subculture typical colonies from the MacConkey plate to LPW for gas production at 37 °C. The primary result was then interpreted as probable or possible *E. coli* (?*E. coli*) if the following criteria were satisfied: gas was produced in LPW at 37 °C, and from LTMB at 44 °C; and if one of the indole tests was positive.

(3) The results were interpreted as coliform organisms other than *E. coli* if gas was produced in LPW at 37 °C, and either (*a*) all tests at 44 °C were negative, or (*b*) if gas was produced in LTMB at 44 °C, but both indole tests were negative, or (*c*) if gas production at 44 °C could not be demonstrated, but one or both indole tests were positive.

(4) The result was regarded as a false positive reaction if (*a*) the colonies on MacConkey agar were not typical, or (*b*) gas was not produced in LPW at 37 °C or (*c*) the oxidase test was positive.

(5) In some instances, laboratories omitted to test LPW at 37 °C, but reported positive reactions for some of the other tests. These were included in the ?*E. coli* group since they were almost certainly not false positive results.

(6) There were only three other results which were anomalous out of a total of 4487 presumptive positive tube reactions. In addition, in only eight instances did it appear likely that an organism fermented mannitol but not lactose.

Samples of water

During this investigation a total of 295 samples were taken for examination from a wide range of surface waters from contaminated sources known to contain coliform organisms, including *E. coli*. They were collected in sterile Winchester quart (2·5 l) bottles and taken to the laboratory on the same day. Because stressed or damaged organisms are useful in accentuating differences in resuscitation and growth-promoting properties between different media (PHLS, 1968), 126 of the samples were treated experimentally in laboratories by the modified marginal chlorination method described below. Whenever possible, a preliminary examination was made on receipt of the raw water so that the approximate numbers of coliform organisms present were available on the day of chlorination, thus enabling appropriate adjustments to be made – either in further dilution or in the duration of chlorination. In some instances samples of water were taken at treatment works during chlorination in Winchester bottles containing 2 ml of a 3% solution of sodium thiosulphate, sufficient to neutralize all free and combined chlorine.

Each water sample, whether raw or chlorinated, was inoculated into 5-tube sets of both MMGM and LTLB as follows: double-strength medium – 5 × 10 ml sets with 10 ml water; single-strength medium – 5 × 5 ml sets with 5 ml and 1·0 ml water, and 1·0 ml tenfold dilutions of the sample in quarter-strength Ringer's solution. All tubes were incubated at 37 °C for 48 h and examined at 18–24 and 48 h for acid and gas production with MMGM, and for gas formation only in LTLB. The results were recorded at each reading; any tubes showing presumptive positive reactions were separated for confirmatory tests.

Marginal chlorination of water samples

In each laboratory, Winchester quart bottles with samples of polluted water – likely to contain at least 100 coliform organisms per 100 ml – were cooled by keeping them at 4 °C overnight. On the following day, the water was passed through Whatman No. 1 or similar filter papers to remove gross particulate matter, and 4·0 ml of a solution of ammonium chloride (0·38 %) added to 2 l of the filtrate in a clean flask or bottle to give an ammonium nitrogen concentration of about 1 mg/l. Twenty ml of a solution of hypochlorite containing approximately 100 mg/l available chlorine (e.g. 1·0 ml 'Milton' added to 100 ml sterile distilled water) was then added to give a final concentration of available chlorine of about 1 mg/l, and the flask left to stand for up to 1 h. To reduce the rate of chlorination during this time, the flask was kept as cool as possible – either in the refrigerator or by surrounding it with ice cubes. If this was not possible, the time was halved as the rate of disinfection is approximately twice as fast at room temperature as at 4 °C. The actual duration of chlorination varied with the nature of the water, but was chosen by trial and error so that the final sample was likely to give some positive and some negative test reactions within 48 h: usually a period of 10–20 min was satisfactory, after which the chlorine was neutralized by the addition of 1·0 ml of a solution of sodium thiosulphate (3 %) sterilized by autoclaving. Alternatively, some laboratories found it more convenient to distribute the refrigerated sample into several flasks or bottles each containing 500 ml. These samples were chlorinated as before but with proportionate amounts of reagents. Chlorination was then allowed to proceed for different times in each flask before neutralization with sodium thiosulphate. When this approach was used, the content of each flask was regarded as a separate 'sample' for the purposes of statistical analysis.

RESULTS

A total of 295 water samples (169 raw and 126 chlorinated) were examined by twelve laboratories. These samples yielded a total of 2313 positive presumptive reactions in tubes of MMGM and 2174 similar reactions with LTLB. Positive reactions were either confirmed as *E. coli* or interpreted as probably *E. coli* (?*E. coli*), other coliform organisms, or as false positive reactions as previously defined.

The results recorded at the first reading (18 or 24 h) and at 48 h have been analysed in three ways: (i) in terms of most probable numbers of coliform organisms; (ii) in terms of total positive tube-reactions and their interpretation; and (iii) in terms of whether the water sample yielded any evidence or not of the presence of *E. coli* or of other coliform organisms.

Analysis of most probable numbers of coliform organisms

The most probable numbers (MPN) of coliform organisms were taken in the usual way from standard probability tables (Report, 1969) to give presumptive counts at the first reading (usually 18 h, but 24 h for some laboratories) and at 48 h, and then, by excluding false positive results, the confirmed count at 48 h.

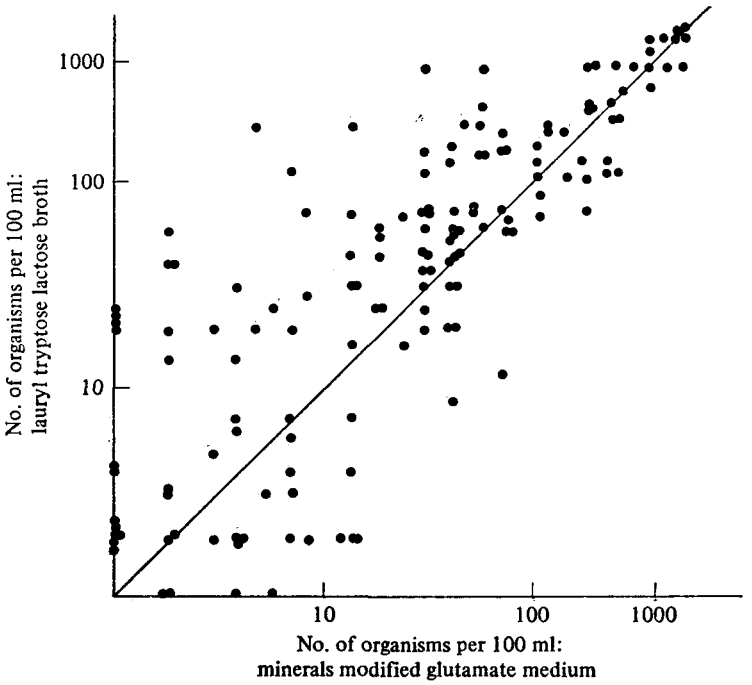

Fig. 1. Raw water samples. Presumptive coliform counts at 18/24 h for all 12 laboratories.

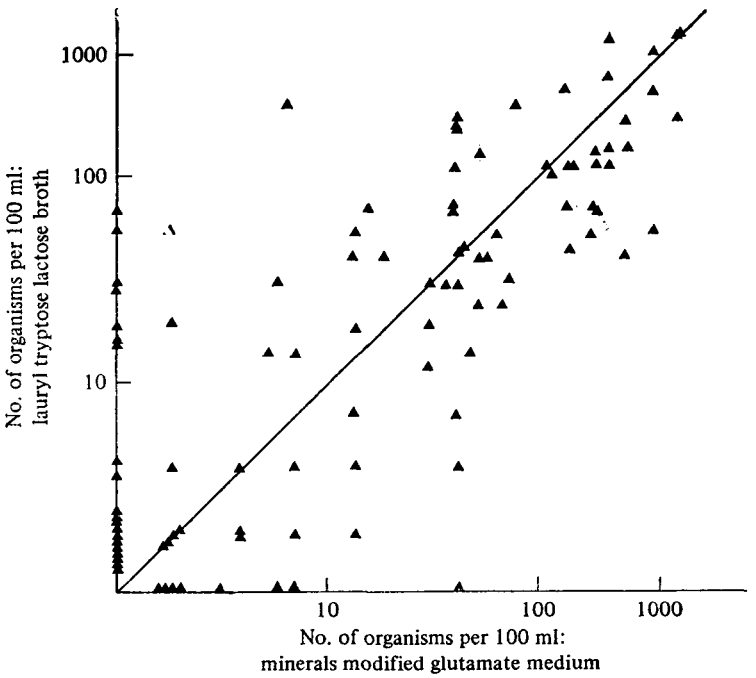

Fig. 2. Chlorinated water samples. Presumptive coliform counts at 18/24 h for all 12 laboratories.

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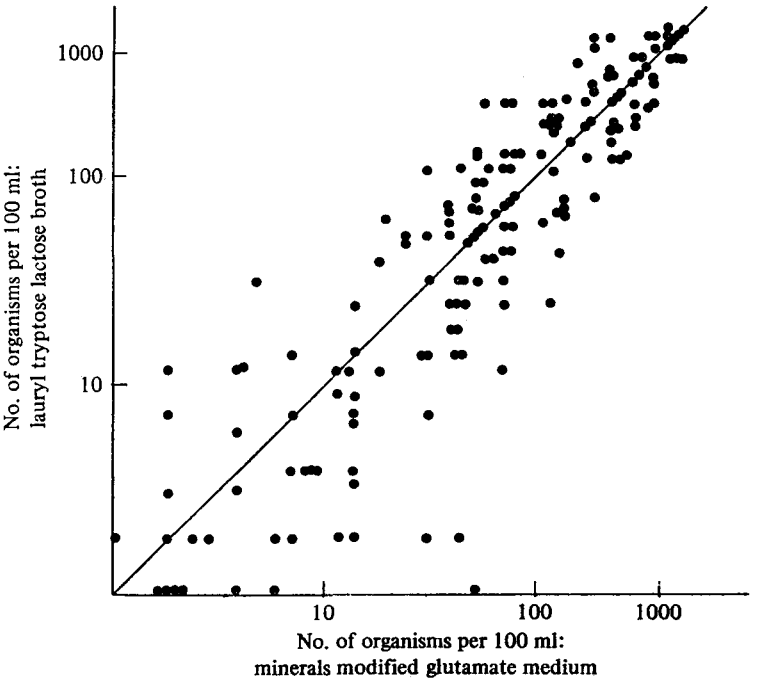

Fig. 3. Raw water samples. Presumptive coliform counts at 48 h for all 12 laboratories.

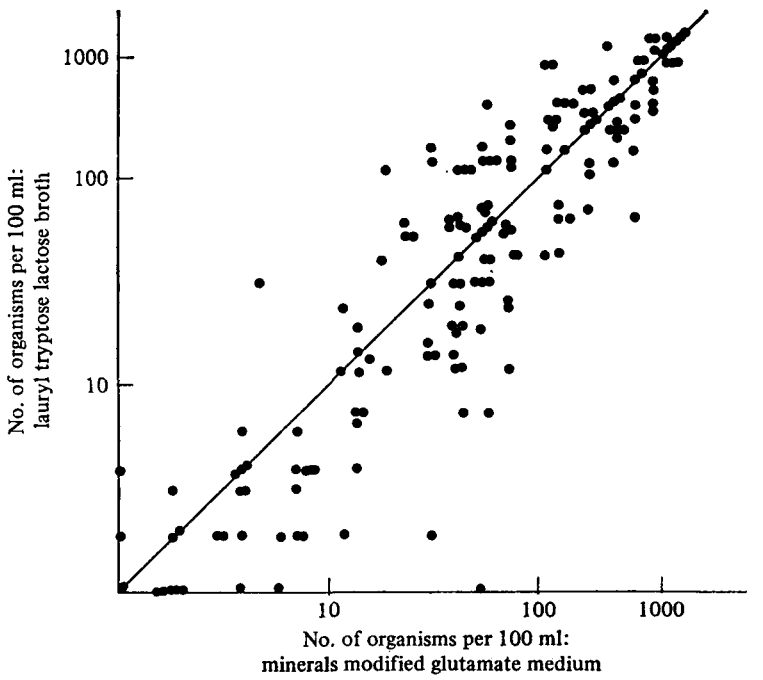

Fig. 4. Raw water samples. Confirmed coliform counts at 48 h for all 12 laboratories.

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The most probable numbers of presumptive coliform organisms and of *E. coli* obtained with the two media are shown in Figs. 1–6. In these graphs, logarithmic scales have been used, but with 'Nil' readings plotted on the axes. The diagonal lines represent samples giving equal MPN counts with both media; points falling below this line indicate samples giving higher MPN counts with MMGM than with LTLB, and points above the line those samples giving higher MPN counts with LTLB.

Figs. 1 and 2 show presumptive MPN counts at 18–24 h with MMGM plotted against LTLB for samples of raw and chlorinated water respectively. Samples which gave negative results with both media at first reading have been excluded from these graphs. For raw waters (Fig. 1), MMGM gave a higher count with 45 and LTLB with 88 samples; in 18 samples the counts with each medium were identical; and 18 samples which gave negative results have been omitted from the graph. For chlorinated waters (Fig. 2) MMGM gave a higher count with 46 and LTLB with 45 samples; with 11 samples the counts in both media were identical; and 24 samples were exluded because of negative results with both media.

The MPN results at 48 h are shown in Figs. 3–6. For raw waters, the presumptive coliform count (Fig. 3) was higher with MMGM for 77 samples, and with LTLB for 65 samples; identical results were given by 27 samples. For the confirmed coliform count (Fig. 4) MMGM gave the higher count with 85 and LTLB with 54 samples; identical results were given by 30 samples. In both graphs, the two media gave similar results for the more contaminated water samples, but for samples with counts lower than about 50 organisms per 100 ml, MMGM was superior.

For chlorinated waters, the presumptive coliform count (Fig. 5) was higher with MMGM for 83 samples, and with LTLB for 31 samples; the results were identical with 12 samples. For the confirmed coliform count (Fig. 6), MMGM gave the higher count for 90 and LTLB for 28 samples; the results were identical with 8 samples. In both graphs, MMGM appears to yield higher counts than LTLB throughout the whole range, but especially when the organisms were present in small numbers.

The MPN results at 48 h were also analysed for each individual laboratory. With chlorinated waters, all laboratories found that more samples gave a higher coliform count with MMGM than with LTLB compared with the number of samples giving higher counts with LTLB than with MMGM. With raw waters, five of the 12 participating laboratories found that more samples gave a higher presumptive coliform count with LTLB than with MMGM; for one of these laboratories this difference was statistically significant, although the difference became insignificant when confirmed rather than presumptive counts were used. These five laboratories, however, had tested mostly water samples with large numbers of coliform organisms; all laboratories testing water samples with small numbers of these organisms agreed that MMGM tended to give the higher counts.

In summary, Figs. 1–6 show that for the most probable numbers of coliform organisms, the comparison between the two media is more in favour of MMGM if the water is chlorinated rather than raw; if results are considered at 48 h rather than at 18 h; and if confirmed rather than presumptive counts are used. At

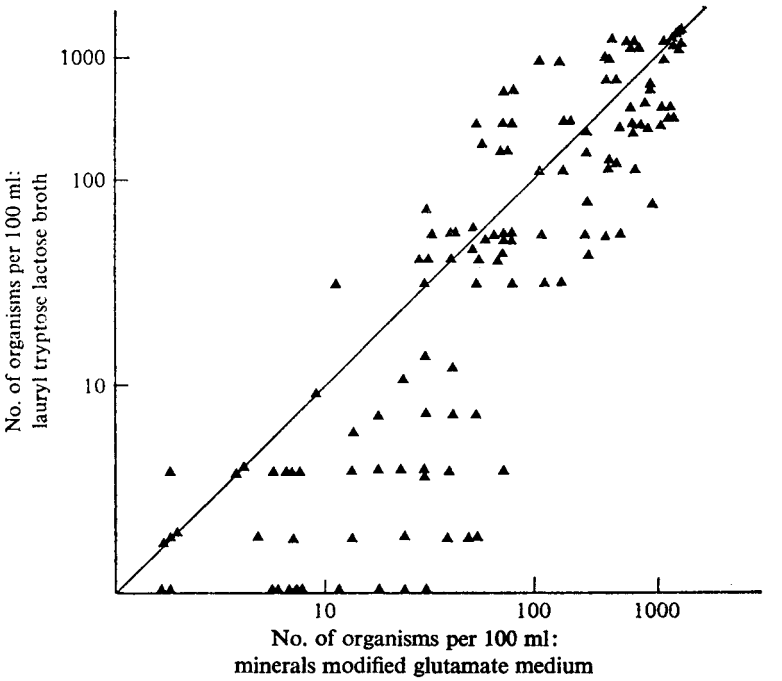

Fig. 5. Chlorinated water samples. Presumptive coliform counts at 48 h for all 12 laboratories.

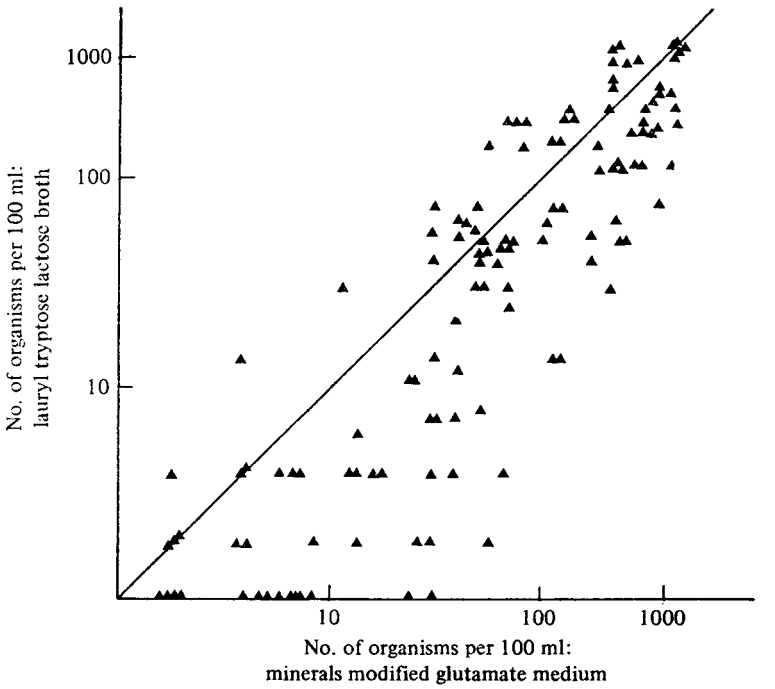

Fig. 6. Chlorinated water samples. Confirmed coliform counts at 48 h for all 12 laboratories.

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Table 1. *Positive tube reactions and their interpretation: all samples*

Type of water	Medium	*E. coli*	?*E. coli*	Coliform organisms other than *E. coli*	False positive reactions	Total no. of tubes positive
Raw						
18/24 h	MMGM	686	151	56	9 (1%)	902
	LTLB	768	117	142	33 (3%)	1060
48 h	MMGM	862	178	203	56 (4%)	1299
	LTLB	817	125	277	85 (7%)	1304
Chlorinated						
18/24 h	MMGM	447	16	19	5 (1%)	487
	LTLB	431	11	77	23 (4%)	542
48 h	MMGM	765	34	168	47 (5%)	1014
	LTLB	571	20	210	69 (8%)	870

18–24 h, LTLB gave higher counts more often than MMGM only with raw waters; but with chlorinated waters, the numbers with both media were similar. With both raw and chlorinated waters, however, the presence of coliform organisms at the first reading in samples was detected more often with LTLB when MMGM was negative than vice versa. At 48 h, MMGM tended to yield higher MPN counts than LTLB with raw water samples which contained fewer than about 50 coliforms per 100 ml, although there was little difference between the media with higher MPN counts. With chlorinated waters, MMGM was superior throughout the whole range of MPN results, but more so with samples containing small numbers of coliform organisms. When the presumptive and confirmed results are compared, there is some evidence that LTLB gave more false–positive reactions than did MMGM. At 48 h there were fewer negative results with MMGM than with LTLB. In addition, there was good agreement between the results from each individual laboratory especially about the superiority of MMGM for small numbers of coliform organisms in raw waters and with all chlorinated waters.

Analysis of total positive tube reactions

This method was used in previous trials (PHLS, 1968, 1969). It gives more weight to results from samples of heavily contaminated water since these yield more positive reactions. This method of analysis has been repeated here for comparison.

Table 1 shows numbers of tubes with positive presumptive reactions and their interpretation at first reading and at 48 h. For raw waters, LTLB tended to give more positive results than MMGM at first reading and about the same number at 48 h. However, at 48 h LTLB gave significantly more false positive reactions as well as coliform organisms other than *E. coli*, and also significantly fewer tubes with *E. coli* or ?*E. coli* than MMGM. With chlorinated waters, LTLB still gave a higher number of total positive reactions at first reading, although a significantly smaller proportion of these were found to contain *E. coli* (431 of 542: 80%) compared with MMGM (447 of 487: 92%). By 48 h, however, MMGM gave many more

Table 2. *Positive tube reactions and their interpretation: samples read three times*

Type of water	Medium	*E. coli*	?*E. coli*	Coliforms other than *E. coli*	False positive reactions	Total no. of tubes positive
Raw						
18 h	MMGM	258	—	10	—	268
	LTLB	292	—	24	8 (2%)	324
24 h	MMGM	305	—	21	1 (0·3%)	327
	LTLB	303	—	35	11 (3%)	349
48 h	MMGM	325	—	41	9 (2%)	375
	LTLB	307	—	47	14 (4%)	368
Chlorinated						
18 h	MMGM	208	—	13	—	221
	LTLB	169	1	50	9 (4%)	229
24 h	MMGM	268	4	30	1 (0·3%)	303
	LTLB	209	5	69	12 (4%)	295
48 h	MMGM	388	9	111	27 (5%)	535
	LTLB	277	7	122	33 (8%)	439

positive reactions than LTLB and, as with the first reading, significantly more of these were *E. coli* ($P \leqslant 0{\cdot}0001$).

With both media and for both raw and chlorinated waters, most tubes found to contain *E. coli* were positive at the time of first reading (18–24 h) whereas other coliform and false positive results were more likely at 48 h. With both media, positive reactions were slower with chlorinated than with raw waters. Thus with MMGM only 487 (58%) of the final 1014 tubes were positive at the first reading. At 48 h MMGM is better at detecting the presence of *E. coli*, but the results at first reading tended to favour LTLB. It was therefore of interest to know whether the timing of the first reading made any difference to the results, and during the trial laboratories were therefore asked to read tubes at both 18 and 24 h. Table 2 shows the results for this subset of samples read at 18, 24 and 48 h. The 48 h results for these are similar to those in Table 1, thus indicating that this subset of water samples was representative of the whole trial. Table 2 shows that there is considerable improvement in the performance of MMGM at 24 h compared with 18 h. With raw waters, of the total of 375 tubes positive after 48 h, 268 (71%) of them occurred at 18 h and 327 (87%) at 24 h – at which time both media gave similar results for *E. coli* (305 and 303 respectively). With chlorinated waters, of 535 tubes positive after 48 h, 221 (41%) occurred at 18 h and 303 (57%) at 24 h – when MMGM tubes containing *E. coli* considerably exceeded those for LTLB (268 compared with 209 respectively).

In summary, the positive reactions indicate that MMGM gave fewer tubes with *E. coli* than LTLB at 18 h with raw waters, but more with chlorinated waters. At 24 h MMGM gave similar numbers of tubes with *E. coli* with raw waters and more with chlorinated waters. By 48 h MMGM yielded more *E. coli* tubes than LTLB

Table 3. *Interpretation of positive tube reactions from samples of raw water*

Content of water sample		LTLB: All tubes negative	LTLB: False positive tube reactions only	LTLB: Confirmed *E. coli*	LTLB: Possible *E. coli* or other coliform organisms only	Total number of samples
(*a*) *First reading* (18/24 h)						
MMGM	All tubes negative	16	—	10	2	28
	False positive tube reactions only	—	—	—	1	1
	Confirmed *E. coli*	5	—	113	5	123
	Possible *E. coli* or other coliform organisms only	—	—	11	6	17
Total number of samples		21	—	134	14	169
(*b*) *Final reading* (48 h)						
MMGM	All tubes negative	—	—	—	1	1
	False positive tube reactions only	—	1	—	1	2
	Confirmed *E. coli*	4	—	129	11	144
	Possible *E. coli* or other coliform organisms only	3	1	10	8	22
Total number of samples		7	2	139	21	169

with all waters. LTLB tended to give more false positive tubes and more coliforms other than *E. coli*.

Analysis of water samples by interpretation of their coliform content

For raw water, Table 3 shows that at first reading LTLB yielded *E. coli* in 134 of 169 samples and other coliform organisms only in 14 further samples, whereas MMGM yielded *E. coli* in 123 samples and other coliform organisms only in 17 samples. These differences between the two media are not statistically significant. At 48 h, MMGM had overtaken LTLB and yielded *E. coli* in 144 samples compared with 139 for LTLB; again the difference is not statistically significant. At 48 h, MMGM detected *E. coli* or other coliforms in 8 samples which were negative or gave only false positive reactions with LTLB; in contrast coliform organisms were isolated in LTLB in two samples which gave negative or only false positive results with MMGM. These differences in false negative rates between the two media are not quite significant ($P = 0{\cdot}052$). Both media gave the same interpretation for 138 of the 169 (82 %) water supplies.

With chlorinated water at first reading (Table 4), coliform organisms – mostly *E. coli* – were found in 91 out of 126 samples with LTLB compared with only 74 samples with MMGM ($P < 0{\cdot}01$). By 48 h MMGM always detected coliform organisms in samples positive with LTLB whereas LTLB gave negative or false positive results in 14 samples where coliforms were detected with MMGM ($P < 0{\cdot}0001$).

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Table 4. *Interpretation of positive tube reactions from samples of chlorinated water*

Content of water sample		LTLB: All tubes negative	LTLB: False positive tube reactions only	LTLB: Confirmed *E. coli*	LTLB: Possible *E. coli* or other coliform organisms	Total number of samples
(*a*) *First reading* (18/24 *h*)						
MMGM	All tubes negative	27	2	15	8	52
	False positive tube reactions only	—	—	—	—	—
	Confirmed *E. coli*	5	—	65	1	71
	Possible *E. coli* or other coliform organisms only	1	—	1	1	3
Total number of samples		33	2	81	10	126
(*b*) *Final reading* (48 *h*)						
MMGM	All tubes negative	—	—	—	—	—
	False positive tube reactions only	—	—	—	—	—
	Confirmed *E. coli*	5	3	91	14	113
	Possible *E. coli* or other coliform organisms only	6	—	2	5	13
Total number of samples		11	3	93	19	126

Three samples yielded false positive reactions with LTLB, but none with MMGM. The two media gave the same interpretation for 96 (76 %) of the 126 samples.

In summary, there was thus fairly good agreement between the two media in the final interpretation of the coliform content of the samples – 82 % with raw and 76 % with chlorinated waters. The differences between the media were similar but possibly greater with chlorinated than with raw waters. At first reading (usually 18 h), LTLB detected coliform organisms in more water samples, but by 48 h MMGM had overtaken LTLB. At 48 h, failure to detect coliform organisms by one medium but not the other – false negative results – was rare with MMGM but much more frequent with LTLB.

DISCUSSION

With the multiple tube test, previous comparisons of glutamic acid and glutamate-based media in Britain have always shown them to be superior to those with bile salts or Teepol in routine use for the isolation of coliform organisms and *E. coli* from water; this was particularly so in relation to the 48 h tube results (PHLS, 1968, 1969). However, there has not been any previous comparison of glutamate media with lauryl sulphate tryptose lactose broth (LTLB) – a medium currently widely used in America and in some countries elsewhere. The work de-

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scribed in this paper concerns a multi-laboratory trial of these media and it confirms the superiority of minerals-modified glutamate medium (MMGM), especially at 48 h.

In this investigation, importance was placed on the distinction between raw and chlorinated waters. With chlorinated water, MMGM proved equal to or better than LTLB after 18–24 h incubation and much superior at 48 h for the total coliform MPN count and the *E. coli* count, especially when the total numbers were less than about 50 organisms per 100 ml. With raw waters, the MPN results after 18 h incubation tended to favour LTLB, but after incubation for 24 h and 48 h, MMGM was clearly superior with waters containing less than 50 coliform organisms per 100 ml. There was little difference between the two media when the coliform numbers were greater. MMGM gave fewer false positive tube reactions than LTLB although the total number of false positive results made little difference to the final interpretation. With regard to water samples, MMGM gave fewer false negative results.

In each individual laboratory MMGM was found to be superior to LTLB for chlorinated waters at 48 h; with raw waters containing few coliform organisms, all laboratories agreed that MMGM was also superior. For raw waters with higher counts, there was no significant difference.

From the overall results of this trial, it would appear that MMGM gives marginally better results than LTLB, and this glutamate medium is therefore again recommended for the routine examination of water supplies. LTLB can be recommended, however, as an alternative, especially if rapid results are essential. Further work to speed the production of acid and gas in MMGM so that the majority of positive results are obtained within 18–24 h would be a great advantage.

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