

A comparison of confirmatory media for coliform organisms and *Escherichia coli* in water

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(Received 23 February 1981)

SUMMARY

Gas production by coliform organisms and *Escherichia coli* from lauryl tryptose lactose broth (LTLB) was compared with that from brilliant green (lactose) bile broth (BGB). These media were compared with lauryl tryptose mannitol broth (LTMB) with and without added tryptophan for both gas and indole production. At 37 °C, LTLB and BGB were both satisfactory for gas production, but at 44 °C, LTLB gave fewer false-negative results and was thus significantly less inhibitory than BGB. However when LTLB and LTMB were compared as single-tube confirmatory media, LTLB give a high proportion of false-negative reactions in the indole test at 44 °C. The substitution of mannitol for lactose and the addition of tryptophan yielded a satisfactory medium for both confirmation of gas production and the demonstration of indole at 44 °C.

INTRODUCTION

In a previous collaborative trial (PHLS, 1968), brilliant green bile broth (BGB) and lactose ricinoleate broth (LRB) were compared as confirmatory media for coliform organisms in the examination of water by the multiple tube (MPN) technique. Both media were satisfactory, but LRB was recommended as first choice because it gave fewer false-negative results at both 37 and 44 °C. However, because of practical difficulties such as turbidity and poor gas-formation, LRB has since fallen out of favour and its manufacture has been discontinued. While single-tube confirmatory media have shown considerable promise (PHLS/SCA, 1980*b*) there is still a need for a medium to show gas formation from lactose at

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37 and 44 °C, which, with the addition of a tube of tryptone water (TW) at 44 °C for indole formation, can be used to confirm the presence of coliform organisms and *E. coli* in water samples.

In the membrane filtration method for the detection of coliform organisms in water sodium lauryl sulphate was recommended as a replacement for Teepol 610 in 0.4 % enriched Teepol broth (PHLS/SCA, 1980c). This substance is chemically defined and can be obtained in pure form, and it has been shown to be a suitable substitute for bile salts in bacteriological media for water examination (PHLS/SCA, 1980a, b).

This paper reports a comparison between standard lauryl tryptose (lactose) broth (LTLB) and BGB for detecting gas formation by coliform organisms at 37 °C, and, in combination with a tube of TW, for detecting gas and indole formation by *E. coli* at 44 °C. LTLB was further compared with lauryl tryptose mannitol broth (LTMB) as a single-tube confirmatory medium for *E. coli*, and the effect on the indole reaction of adding tryptophan to LTMB was also studied.

MATERIALS AND METHODS

Media

The following media were used: (1) Minerals modified glutamate medium (MMGM), Oxoid CM 289. (2) Brilliant green bile broth (BGB), Oxoid CM 31 from a single batch, except for some of the early work, when different batches were used. (3) Lauryl tryptose (lactose) broth (LTLB), CM 451, and Lauryl tryptose mannitol broth (LTMB), both from the same batches of Oxoid dehydrated media as used in a previous trial for the enumeration of coliform organisms and *E. coli* in water (PHLS/SCA, 1980b). (4) Lactose (1 %) peptone water (LPW), mannitol (1 %) peptone water (MPW), tryptone water (TW) and MacConkey agar were prepared in each participating laboratory as described in Report (1969).

Methods

Samples of raw waters known to contain coliform organisms were examined by the multiple tube method with MMGM. The tubes were examined at 24 and 48 h. At the 24 h reading, all tubes showing gas formation (any amount) were subcultured to two tubes each of BGB and LTLB, to one tube each of LTMB and TW, and on to a MacConkey agar plate. At 48 h any further tubes which showed gas formation sufficient to fill the concavity of the Durham tubes were similarly subcultured to the same media.

Tests at 37 °C

One of each pair of tubes of BGB and LTLB was incubated at 37 °C for 48 h, and a record kept of those showing gas formation. If all the tubes gave a positive result, no further tests were done, but if both were negative or if there was a discrepancy, the tests were repeated in LPW by subculture of typical colonies from the MacConkey plate.

Tests at 44 °C

The other tubes of BGB and LTLB, and the tubes of LTMB and TW were incubated at 44 °C for 24 h. Gas production was first recorded and then a few drops

of Kovac's reagent were added to the tubes of TW, LTLB and LTMB: a detectable pink colour was regarded as evidence of indole formation. Discrepancies in gas formation between the media were investigated in the same way as those at 37 °C by repeating the tests in LPW from representative colonies on the MacConkey plate. Any anomalous results for indole formation were investigated by repeating the tests in TW, if necessary at both 37 and 44 °C.

RESULTS

Nine laboratories took part in the trial, yielding a total of 1955 positive tube results.

Gas production at 37 °C

The gas results at 37 °C are summarized in Table 1. The presence of coliform organisms in tubes showing positive reactions was considered to be confirmed if gas was produced in both media at 37 °C; and coliform organisms were considered to be absent if all tests were negative. If there was any discrepancy, LPW was considered to give the correct result.

Table 1 shows that LTLB gave 1866 positive gas reactions of which 10 (0.5%) were incorrect, whereas BGB gave 1851 positive results all of which were correct. This difference in false-positive rates is significant ($P < 0.01$); however, most of this difference was accounted for by the results from one laboratory. LTLB gave 89 negative results, 15 (17%) of which should have been positive as judged by the LPW results. Similarly, BGB gave 104 negative results, 20 (19%) of which should have been positive: this difference in false-negative rates is not statistically significant. All but three of these false negative results involved tubes found to contain coliform organisms other than *E. coli*, as judged by the test results at 44 °C.

Four laboratories graded their results according to the amount of gas produced and found that LTLB gave more gas than BGB significantly more often than *vice versa*.

Gas production at 44 °C

The gas results at 44 °C are summarized and related to the subsequent confirmation of the presence or absence of *E. coli* in Table 2. The presence of *E. coli* in tubes showing positive reactions was considered to be confirmed if all tests at 44 °C, including indole production, were positive and *E. coli* was considered to be absent if all tests were negative. If there were discrepancies, reference was made to the LPW gas and TW indole results: in such instances, the presence of *E. coli* was regarded as confirmed if both were positive. *E. coli* was considered to be absent if gas was not produced in LPW or if all indole tests (TW, LTMB and LTLB, if done) were negative. If LPW was not used, it was assumed that gas would have been produced in this medium if all other media gave positive gas results. There were only seven tubes where results were incomplete or anomalous.

Table 2 shows that LTLB gave 1445 positive gas reactions of which 47 (3%) could not be confirmed as due to *E. coli*, and BGB gave 1418 positive results of which 32 (2%) were similarly incorrect. This difference in false positive rates is not significant. LTLB gave 500 negative results of which 6 (1%) should have been positive; and BGB gave 537 negative results of which 24 (4%) should have been positive. This difference in false negative results is significant ($P < 0.01$). There

Table 1. *Gas production at 37 °C in lauryl tryptose lactose broth and brilliant green bile broth*

Medium	Result	Interpretation		Total no. of tubes tested
		Coliform organism present	Coliform organism absent	
LTLB	+	1856	10	1866
	-	15	74	89
Total		1871	84	1955
BGB	+	1851	0	1851
	-	20	84	104
Total		1871	84	1955

+, Gas produced; -, gas not produced.

Table 2. *Gas production at 44 °C and interpretation of the result in lauryl tryptose lactose broth and brilliant green bile broth*

Medium	Result	Interpretation			Total no. of tubes tested
		<i>E. coli</i> present	Doubtful	<i>E. coli</i> absent	
LTLB	+	1403	5	47	1455
	-	6	2	492	500
Total		1409	7	539	1955
BGB	+	1385	1	32	1418
	-	24	6	507	537
Total		1409	7	539	1955

+, Gas produced; -, gas not produced.

Table 3. *Results of gas production in test media and indole formation in tryptone water at 44 °C*

Test and result		Interpretation			Total no. of tubes tested	
		<i>E. coli</i> present	Doubtful	<i>E. coli</i> absent		
LTLB gas	TW indole					
	+	+	1395	5	1	1401
	+	-	8	.	46	54
	-	+	6	2	10	18
	-	-	.	.	482	482
Total		1409	7	539	1955	
BGB gas	TW indole					
	+	+	1377	1	.	1378
	+	-	8	.	32	40
	-	+	24	6	11	41
	-	-	.	.	496	496
Total		1409	7	539	1955	

Table 4. Gas and indole results at 44 °C from LTMB (8 laboratories) and LTLB (3 laboratories)

Medium	Test and Result		Interpretation			Total no. of tubes tested
	gas	indole	<i>E. coli</i> present	Doubtful	<i>E. coli</i> absent	
LTMB	+	+	1121	1	1	1123
	+	-	111	.	38	149
	-	+	.	2	3	5
	-	-	.	2	432	434
Total			1232	5	474	1711
LTLB	+	+	123	.	1	124
	+	-	244	2	21	267
	-	+	.	.	10	10
	-	-	.	.	190	190
Total			367	2	222	591

was good agreement between laboratories as to which medium gave fewer incorrect results, although laboratories testing only small numbers of tubes obtained error rates of nil for both media.

Gas production in LTLB and BGB and indole formation in TW at 44 °C

These results are shown in Table 3. Of 1401 results with LTLB which were positive for both gas and indole production, only one was judged incorrect according to the criteria used, and none of the 1378 results with BGB was incorrect. None of the results which were negative for both gas and indole production was due to false negative reactions. Of the total of 1955 tubes examined, there were 72 with LTLB and 81 with BGB in which either the gas reaction or the indole result, but not both, was negative, but this difference is not significant. Where the indole reaction was positive but gas was not produced, the interpretation was more likely to be *E. coli* with BGB than with LTLB because BGB gave more false-negative results.

Gas and indole production from LTMB and LTLB at 44 °C

All nine laboratories examined LTMB at 44 °C for the production of both gas and indole, as described previously (PHLS/SCA, 1980*b*). Similar findings were obtained in that there were very few incorrect results when the gas and indole reactions were either both positive or both negative (Table 4). However, one laboratory obtained a much higher proportion of tubes yielding positive gas but negative indole reactions, and these results are shown separately (Table 5). Four laboratories also examined LTLB for indole production. Their results were consistently better with LTMB than with LTLB. The results from three of these laboratories are shown in Table 4. LTMB gave negative indole results in a total of 583 (i.e. 149 and 434) tubes of which 111 (19%) were found to contain *E. coli*; similarly LTLB gave a total of 457 (i.e. 267 and 190) negative indole results, 244 (53%) of which were from tubes containing *E. coli*. This difference in false negative rates is highly significant ($P \ll 0.0001$). In Table 5, the results from the laboratory excluded from Table 4 also show more false negative indole results with LTLB than with LTMB.

Table 5. Gas and indole results at 44 °C from LTMB and LTLB from one laboratory excluded from Table 4

Medium	Test and result		Interpretation			Total no. of tubes tested
	gas	indole	<i>E. coli</i> present	Doubtful	<i>E. coli</i> absent	
LTMB	+	+	82	.	.	82
	+	-	94	2	18	114
	-	+	.	.	1	1
	-	-	1	.	46	47
Total			177	2	65	244
LTLB	+	+	37	1	.	38
	+	-	138	1	16	155
	-	+	.	.	1	1
	-	-	2	.	47	49
Total			177	2	64	243

Table 6. LTMB indole results at 44 °C with and without added tryptophan from six laboratories

Indole result	With added tryptophan		Total	
	+	-		
Without added tryptophan	+	301	9	310
	-	116	149	265
Total		417	158	575

Addition of tryptophan to LTMB

Six laboratories studied the effect on the indole reaction of adding tryptophan (0.1 g/l) to LTMB. The results were recorded in parallel with the indole tests in LTMB without added tryptophan (Table 6). It should be noted that Tables 4 and 5 concern only LTMB and LTLB results without added tryptophan. The addition of tryptophan to LTMB yielded many more positive results ($P < 0.0001$), and in many instances the indole reactions were stronger.

DISCUSSION

The most important difference between BGB and LTLB was that LTLB gave significantly fewer false-negative gas results at 44 °C. The only other difference was that LTLB gave more false positive results at 37 °C than BGB. The latter is however not an important disadvantage in water examination since it is an error on the side of safety. Both media gave the correct answer in about 99% of tests at 37 °C and in 97% at 44 °C. Thus for gas production at 37 °C, either medium is satisfactory, but at 44 °C LTLB appears significantly less inhibitory and is therefore to be preferred.

LTLB was shown to be unsuitable for demonstrating indole formation at 44 °C since it gave such a high proportion (53%) of false-negative results, and it cannot therefore be recommended for this purpose. However, the substitution of mannitol for lactose and the addition of extra tryptophan gives a satisfactory single-tube

confirmatory medium for both gas and indole formation. Such a medium cannot however be expected to give a positive indole reaction in every instance, and if a false-negative indole reaction is suspected, the test should be repeated separately in tryptone water. It must also be remembered that LTMB detects mannitol fermentation, and discrepant gas results may therefore need to be repeated separately in tubes of both lactose and mannitol peptone water. In practice, however, the use of a medium containing mannitol may be advantageous in permitting the confirmation of unusual strains of *E. coli* which fail to ferment lactose, and which may be subcultured from a mixture on MacConkey plates or from membranes.

This work was partly funded by the Department of the Environment through Research Contract DGR/480/481.

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