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Confirmatory tests for coliform organisms

By the Public Health Laboratory Service Standing Committee on the Bacteriological Examination of Water Supplies*

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INTRODUCTION

During a comparison of media for the isolation of coliform organisms from water (Report, 1968) it was necessary to know whether the reactions produced in the media were due to coliform organisms or not. One of the methods used for this purpose was the subculture of each tube showing a presumptive positive reaction to a tube of brilliant green lactose bile broth (BGB) which was then incubated for up to 48 hr. at 37° C. and examined for the production of gas (W.H.O. 1961, 1963). This was in addition to the subculture of each presumptive positive tube to BGB for incubation at 44° C. for the detection of *Escherichia coli*. Each presumptive positive tube was also subcultured to a MacConkey agar plate, so that if a tube of BGB did not produce any gas after 48 hr. at 37° C., colonies from the corresponding MacConkey agar plate could be picked to lactose peptone water (LPW) for incubation at 37° C. for up to 48 hr. A presumptive positive tube was regarded as giving a false reaction only if colonies picked from MacConkey agar failed to produce acid and gas from LPW after 48 hr. incubation.

It soon became apparent that a considerable number of presumptive positive tubes which on subculture failed to produce gas in BGB at 37° C. nevertheless produced colonies on MacConkey agar which were capable of producing acid and gas in LPW at 37° C. In view of this, BGB incubated at 44° C. for the detection of *Esch. coli* was investigated in the same way and similar discrepancies were discovered.

Since this work had all been done with a single batch of dehydrated BGB, similar experiments were carried out with other batches of dehydrated BGB and with BGB made up in individual laboratories. Although there were differences between these media, none of the BGB media detected coliform organisms from as many presumptive positive tubes as were detected by subculturing the tubes

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to MacConkey agar and picking individual colonies to LPW. Other liquid media were therefore investigated for possible use as rapid confirmatory media at 37 and 44° C.

MATERIALS AND METHODS

Choice of media for investigation

Since the majority of the organisms causing false presumptive positive reactions both in MacConkey broth and in glutamic acid media, particularly with samples of chlorinated water, are spore-bearing organisms (Report, 1968) the first medium tried was formate ricinoleate broth (A.P.H.A., 1960) which inhibits the growth of these organisms. It soon became apparent, however, that this medium was not satisfactory as a confirmatory medium since it allowed non-lactose fermenting organisms to develop and produce gas.

Some preliminary experiments in which formate was omitted from this medium gave promising results and it was therefore decided to investigate a lactose ricinoleate medium more thoroughly. In addition to a lactose ricinoleate medium containing 1 % lactose, it was decided to investigate a lactose ricinoleate medium containing 5 % lactose, since Lowe & Evans (1957) have shown that late lactose-fermenting organisms produce acid and gas more rapidly in a medium containing 5 % lactose than in one containing 1 % lactose.

Media used

The media used were: (1) MacConkey agar, (2) 1 % lactose peptone water, both prepared in accordance with the instructions given in Report no. 71 (Report, 1956), (3) Oxoid dehydrated brilliant green bile (2 %) broth (CM 31) prepared according to the manufacturer's instructions, (4) 1 % lactose ricinoleate broth and (5) 5 % lactose ricinoleate broth which were prepared as follows: peptone (Evans), 5 g.; sodium ricinoleate, 1 g.; lactose, 10 g. or 50 g.; distilled water to 1000 ml.; pH 7·6 sterilized by autoclaving at 115° C. for 20 min. after tubing together with a Durham tube.

Methods

From each tube giving a presumptive positive reaction two tubes of BGB, two tubes of 1 % lactose ricinoleate broth (1 % LR), two tubes of 5 % lactose ricinoleate broth (5 % LR) and a MacConkey agar plate were inoculated.

The MacConkey agar plate was incubated at 37° C. for 24 hr.

Tests at 37° C.

One tube of BGB, one of 1 % LR, and one of 5 % LR were incubated at 37° C. for 48 hr., and the results recorded. With all the media any amount of visible gas in the concavity of the Durham tube was regarded as positive.

If all the media gave a positive result no further tests were carried out. If all the media gave negative results or if there was any disagreement between them, one or more colonies were picked from the corresponding MacConkey agar plate

and inoculated into separate tubes of LPW which were incubated for 48 hr at 37° C. and examined for the production of acid and gas.

Tests at 44° C.

One tube of BGB, one of 1 % LR and one of 5 % LR were incubated at 44° C. in a water-bath for 24 hr. and the results recorded. Any amount of visible gas in the concavity of the Durham tube was regarded as positive. If all the media gave positive results no further tests were carried out. If all the media gave negative results or if there was any disagreement between them, one or more colonies were picked from the corresponding MacConkey agar plate and inoculated into separate tubes of LPW which were incubated for 24 hr. at 44° C. and examined for the production of acid and gas.

Table 1. *Comparison of brilliant green bile broth* (*BGB*), 1 % *lactose ricinoleate broth* (1 % *LR*) *and* 5 % *lactose ricinoleate broth* (5 % *LR*) *as confirmatory media for the coliform organisms*

	Tests at 37° C.				
	1 % LR + 5 % LR +	1 % LR + 5 % LR −	1 % LR − 5 % LR +	1 % LR − 5 % LR −	Total
BGB + LPW +	306*	2	1	—	309
BGB + LPW −	—	—	—	—	—
BGB − LPW +	12	—	1	—	13
BGB − LPW −	3	—	10	64	77
Total	321	2	12	64	399

* Since the other three media were in agreement these strains were not tested in LPW.

RESULTS

Tables 1 and 2 present the detailed results of 399 tubes giving presumptive positive reactions examined at one laboratory. Table 1 presents the results of tests at 37° C., Table 2 the results of tests at 44° C.

It can be seen from Tables 1 and 2 that there were no instances, at either temperature, in which a positive result was obtained in LPW when all three of the media under investigation gave negative results.

In the test at 37° C. for coliform organisms it can be seen from Table 1 that there were 29 instances in which the three media did not agree with each other. The major differences between the three media were the 12 true coliform cultures which gave positive results with 1 % and 5 % LR and negative results with BGB; and the 10 non-coliform cultures which gave negative results with BGB and 1 % LR but false positive results with 5 % LR. Thus 1 and 5 % LR are significantly better ($P \leqslant 0{\cdot}01$) than BGB in detecting true coliform organisms and of these two media 1 % LR is better than 5 % LR in showing up false positive reactions.

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In the tests for *Esch. coli* at 44° C. it can be seen from Table 2 that there were 39 instances in which the three media under investigation did not agree with each other. The major differences were the 26 *Esch. coli* cultures which gave positive results with 1 and 5 % LR and negative results with BGB. Here again 1 and 5 % LR are significantly better ($P \leqslant 0{\cdot}01$) than BGB in detecting *Esch. coli* and of these two 1 % LR would appear to be better than 5 % LR in not giving false positive reactions.

Table 2. *Comparison of brilliant green bile broth (BGB), 1 % lactose ricinoleate broth (1 % LR) and 5 % lactose ricinoleate broth (5 % LR) as confirmatory media for* Escherichia coli

	Tests at 44° C.				
	1 % LR + 5 % LR +	1 % LR + 5 % LR −	1 % LR − 5 % LR +	1 % LR − 5 % LR −	Total
BGB + LPW +	168*	1	3	—	172
BGB + LPW −	—	—	—	2	2
BGB − LPW +	26	2	—	—	28
BGB − LPW −	1	—	4	192	197
Total	195	3	7	194	399

* Since the other three media were in agreement these strains were not examined in LPW.

Table 3. *Comparison of brilliant green bile broth (BGB), 1 % lactose ricinoleate broth (1 % LR) and 5 % lactose ricinoleate broth (5 % LR) as confirmatory media for coliform organisms and* Escherichia coli

	'Failures' in each of the three media			
Medium	False + ve	False − ve	Total false	No. of strains tested
	Tests at 37° C.			
BGB	0	13	13	399
1 % LR	3	2	5	399
5 % LR	13	2	15	399
	Tests at 44° C			
BGB	2	28	30	399
1 % LR	1	3	4	399
5 % LR	5	3	8	399

The number of failures in each medium at each temperature, assuming LPW to give the correct result, are shown in Table 3. Here again in the overall results 1 % LR is significantly better ($P \leqslant 0{\cdot}01$) than the other two media.

In view of the encouraging results with 1 % LR in this investigation, it was considered that much wider comparison of 1 % LR and BGB at both 37 and 44° C.

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should be carried out. An investigation using the procedure given under Methods above, save that 5% LR was omitted, was accordingly carried out in nine laboratories and the results in terms of 'failures' in each medium at each temperature are recorded in Table 4. There were considerable differences between the results obtained in different laboratories. The results, by laboratories, are set out in Table 5.

Table 4. *Comparison of brilliant green bile broth (BGB) and* 1% *lactose ricinoleate broth* (1% *LR) as confirmatory media for coliform organisms and* Escherichia coli

Tests carried out in nine laboratories. 'Failures' in each of the two media

Medium	False +ve	False −ve	Total false	No. of strains tested
		Tests at 37° C.		
BGB	12	139	151	2447
1% LR	42	91	133	2447
		Tests at 44° C.		
BGB	8	151	159	2822
1% LR	16	50	66	2822

DISCUSSION

In one laboratory comparisons at 37° C. were made at 24 hr. It was apparent, however, that 24 hr. at 37° C. was not a sufficiently long period of incubation even for 1% LR and this point was not pursued further.

In the results recorded in Table 4, 1% LR is significantly better ($P \leqslant 0{\cdot}01$) than BGB at both 37 and 44° C. and where 1% LR fails it is in calling a number of false presumptive positive reactions true coliform reactions. This at least is an error in the right direction since in water bacteriology the most important point is not to miss any true coliform reactions.

It will be seen from Table 5 that in the majority of the laboratories taking part in the investigation 1% LR was better than BGB. It should be borne in mind also that the number of presumptive positive tubes in which either medium gave a false result was small. BGB gave the correct answer in 2296 (93·8%) tubes out of 2447 at 37° C. and 2663 (94·4%) out of 2822 at 44 °C, whereas 1% LR gave the correct answer in 2314 (94·6%) out of 2447 tubes at 37° C. and 2756 (97·7%) out of 2822 at 44° C.

It is apparent, therefore, that in general either BGB or 1% LR would be satisfactory as a confirmatory medium at both 37 and 44° C. It may be that the different results obtained in the different laboratories taking part in the investigation were largely due to the nature of the organisms in the samples examined. In certain samples the difference between the results in the two media was quite striking, and it may be that the choice of which medium to use is one that will have to be made by each laboratory depending on the known behaviour of the waters that it is examining. It is, however, true that over-all 1% LR produces fewer false negative reactions than BGB at both 37 and 44° C and that its use as an alternative confirmatory medium to brilliant green bile broth can be recommended.

Table 5. *Confirmatory tests*

Comparison of brilliant green bile broth and 1% lactose-ricinoleate broth at 37 and 44° C.
Results in individual laboratories

Laboratory	False reactions at 37° C.			Medium	False reactions at 44° C.			No. of strains examined	
	Total	False +	False −		False −	False +	Total	37° C.	44° C.
Oxford	39	1	38	BGB	62	2	64	825	834
	9	5	4	1% lactose ricinoleate	7	1	8		
Newport	9	0	9	BGB	7	0	7	119	566
	7	0	7	LR	7	0	7		
Southend	8	5	3	BGB	—	—	—	175	0
	28	17	11	LR	—	—	—		
Brighton	24	0	24	BGB	2	0	2	334	334
	23	5	18	LR	0	1	1		
Hull	4	0	4	BGB	1	0	1	139	139
	3	1	2	LR	0	1	1		
Conway	29	5	24	BGB	26	5	31	346	348
	36	10	26	LR	24	10	34		
Metropolitan Water Board	2	1	1	BGB	0	0	0	66	158
	2	1	1	LR	0	1	1		
Wakefield	3	0	3	BGB	9	0	9	38	38
	1	0	1	LR	3	0	3		
Manchester	33	0	33	BGB	44	1	45	405	405
	24	3	21	LR	9	2	11		
Totals as already analyzed	151	12	139	BGB	151	8	159	2447	2822
	133	42	91	LR	50	16	66		

SUMMARY

In nine laboratories 1 % lactose ricinoleate broth has been investigated as a possible alternative to brilliant green bile broth as a confirmatory medium for coliform organisms and *Escherichia coli*.

Although the results varied considerably from one laboratory to another, in the sum of the results and in the majority of the participating laboratories 1 % lactose ricinoleate produced fewer false negative reactions than brilliant green bile broth as a confirmatory medium at both 37 and 44° C.

Although both media are satisfactory, 1 % lactose ricinoleate broth can be recommended as an alternative to brilliant green bile broth for the confirmation of coliform organisms and *Esch. coli* from presumptive positive tubes in the examination of water samples, as it is not subject to the known variability of brilliant green and ox-bile.

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