J. Hyg., Camb. (1980), **85**, 51
Printed in Great Britain

Single tube confirmatory tests for *Escherichia coli*

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

(*Received* 17 *July* 1979)

SUMMARY

In a multi-laboratory trial, lauryl tryptose mannitol broth (LTMB) and minerals-modified glutamate medium with added tryptophan (MMGM + T) were compared as single tube tests for the confirmation of *Escherichia coli* in water; the confirmed results were also compared with the production of gas from minerals-modified glutamate medium without added tryptophan (MMGM). LTMB and MMGM + T gave similar gas and indole results with about 90 % of the water samples in most of the laboratories. When compared with the 'correct' results as judged by acid and gas production from lactose peptone water and indole from tryptone water, the difference in the rate of false positive reactions between LTMB and MMGM + T was insignificant; but LTMB gave a significantly lower rate of false negative reactions than MMGM + T. Gas production from MMGM without added tryptophan gave significantly higher rates of both false positive and false negative reactions. Lauryl sulphate is therefore a suitable inhibitory surfactant for use in single tube media for the confirmation of *E. coli*, which can be recommended.

INTRODUCTION

In the bacteriological examination of water samples for coliform organisms, it is usual to subculture from tubes with positive acid and gas reactions at 37 °C to fresh tubes of a confirmatory medium for incubation at 44 °C to detect *Escherichia coli*. In Britain, the media currently recommended for this purpose include brilliant green bile broth and lactose ricinoleate broth (PHLS, 1968; Report 1969). Although the production of gas in these media can be taken in Britain as strong presumptive evidence for the presence of *E. coli*, this is not necessarily so in other countries or for every type of water. A tube of tryptone water inoculated at the same time to demonstrate indole formation also at 44 °C provides additional evidence for the presence of *E. coli*.

* The Joint Committee consisted of members of the Water Committee of the Public Health Laboratory Service and Panel 7.3A of the Standing Committee of Analysts. The members responsible for this work, and their affiliation, were: Dr G. I. Barrow, PHLS Truro (Chairman); Dr J. A. Rycroft, PHLS Chelmsford (Secretary); Dr J. Dadswell, PHLS Reading; Dr M. Hutchinson, South West Water Authority, Exeter; Mr F. Jones, North West Water Authority, Warrington; Dr M. J. Lewis, PHLS, Nottingham; Dr E. B. Pike, Water Research Centre, Stevenage; Dr. J. W. Ridgway, Water Research Centre, Medmenham; Miss J. K. Stevens, Thames Water Authority, London; Dr J. G. Wallace, PHLS, Lincoln; Miss J. M. Watkinson, PHLS, Manchester; and Dr H. E. Tillett, Statistician, PHLS, Colindale.

† The Standing Committee of Analysts is a joint technical committee of the Department of the Environment and the National Water Council.

Requests for reprints should be addressed to Dr J. A. Rycroft, Public Health Laboratory, New Writtle Street, Chelmsford CM2 0QH.

0022-1724/80/0069-1979 $01.00

Various media have been described in which both gas and indole formation can be demonstrated in a single tube. There is also a close relation between the fermentation of lactose and mannitol by *E. coli*; indeed occasional non-lactose fermenting strains of *E. coli* are known to ferment mannitol. In 1956, Schubert described a non-inhibitory medium containing mannitol for the rapid identification of *E. coli*; this was very similar to the basal lactose medium used in experiments to study the effect of carbohydrates on the production of indole (Boyd & Lichstein, 1955). By the addition of inhibitory agents such as sodium ricinoleate or Teepol 610, and the substitution of tryptone for casein peptone, Fennel (1972) made Schubert's medium more suitable for the detection of *E. coli* in mixed cultures. Later, a simpler medium containing tryptone, mannitol and sodium ricinoleate (Pugsley, Evison & James, 1973) was found to give similar results, besides being easier to prepare. Media containing ricinoleate, however, are usually turbid and so obscure gas formation in tubes. This inhibitor has therefore fallen out of favour and commercial manufacture of media containing it has been discontinued. In addition, production of Teepol 610 has also ceased. However, sodium lauryl sulphate, which is one of the active ingredients of Teepol, has been widely used in North America and elsewhere as the inhibitor in media for water examination. It has the advantage of being chemically defined and thus not subject to variation in quality as are bile-salts; it can also be incorporated directly in dehydrated media. Preliminary work showed that the substitution of mannitol for lactose in standard lauryl tryptose lactose broth produced a medium (LTMB) suitable for showing both gas and indole production at 44 °C. In fact this medium is very similar to that of Pugsley *et al.* (1973), but contains a different inhibitor, and is buffered to a slightly lower pH. In contrast, in preliminary work, standard lauryl tryptose lactose broth sometimes failed to give positive indole reactions at 44 °C in tests with pure cultures of *Escherichia coli*, as also occurred with the tryptone lactose ricinoleate broth of Mara (1973). As an alternative, the PHLS Water Committee in previous unpublished work with a selective medium of a quite different type – the chemically defined, minerals-modified version of Gray's improved formate lactose glutamate medium (Gray, 1964; PHLS, 1969) – found that this, with the addition of tryptophan, also showed promise as a single tube confirmatory medium.

A multi-laboratory trial of these two media – lauryl tryptose mannitol broth (LTMB) and minerals-modified glutamate medium with added tryptophan (MMGM + T) – was therefore conducted to determine their suitability for use as single tube tests for gas and indole production at 44 °C for the confirmation of *E. coli*. At the same time, minerals-modified glutamate medium (PHLS, 1969), which does not contain tryptophan, was also used to find out whether gas production alone in this medium at 44 °C was sufficient presumptive evidence in Britain to confirm the presence of *Escherichia coli*. The results obtained in this trial are presented in this paper.

MATERIALS AND METHODS

Media

The following media were used: (1) lauryl tryptose mannitol broth (LTMB) prepared in dehydrated form by Oxoid; this had the same composition as CM 451, but with mannitol 5 g/l instead of lactose; (2) minerals-modified glutamate medium (MMGM) prepared in dehydrated form by Oxoid as for CM 289 but without indicator; (3) the same medium as (2) but with 0·2 g/l of L-tryptophan added (MMGM + T); (4) tryptone water (TW); (5) 1 % lactose peptone water (LPW); and (6) MacConkey agar.

The first three were from single batches of dehydrated media specially prepared by Oxoid and reconstituted according to the manufacturer's instructions. The last three media were those used routinely by each participating laboratory.

Methods

The trial was conducted at the same time as a comparison between lauryl tryptose lactose broth and minerals-modified glutamate medium for the enumeration of coliform organisms and *E. coli* in water (PHLS/SCA, 1980), and the same samples of water were used. From each tube showing positive acid and gas reactions at 37 °C, subcultures were made into tubes of LTMB, MMGM + T, MMGM and TW as well as to a plate of MacConkey agar. These tubes were incubated at 44 °C for 24 h and then inspected for gas formation. Any visible gas in the inverted inner (Durham) tube was regarded as a positive result. A few drops of Kovac's reagent were then added to the tubes of LTMB and MMGM + T. The development of at least a pink colour in the reagent layer was taken as indicating indole production. If the gas and indole results at 44 °C were all positive in the appropriate media, no further tests were done. If there were any discrepancies or if the tests at 44 °C were negative, an attempt was made to determine which results were correct by subculturing typical colonies from the MacConkey plate to tubes of LPW and TW at 44 °C and 37 °C to confirm gas and indole formation. Although LPW could not be used to confirm gas formation in the medium containing mannitol, the number of lactose-negative but mannitol-positive strains encountered in practice is very small.

RESULTS

The results of confirmatory tests for *Escherichia coli* made by subculture from 2299 tubes giving presumptive reactions, from 156 samples of water, were obtained from a total of 11 laboratories. The gas and indole results were recorded for LTMB and MMGM + T media, and gas only for MMGM. Indole was tested from TW, but LPW was not used if all the other three gas tests were positive. In nine of the laboratories the overall results obtained were similar. In the other two laboratories there were much greater numbers of gas-positive but indole-negative results with both LTMB and MMGM + T, and their results have therefore been analysed separately.

The various combinations of total results from the two double-test methods are shown in Table 1 for nine laboratories and in Table 2 for the other two laboratories.

Table 1. *Overall gas and indole confirmatory test results from nine laboratories*

Medium	Gas and indole results	Minerals-modified glutamate medium with tryptophan (MMGM + T) G + I +	G + I −	G − I +	G − I −	Total
Lauryl tryptose mannitol broth (LTMB)	G + I +	1126	25	22	13	1186 (68%)
	+ −	26	54	3	21	104 (6%)
	− +	3	.	23	5	31 (2%)
	− −	2	11	21	385	419 (24%)
	Total	1157 (66%)	90 (5%)	69 (4%)	424 (24%)	1740 (100%)

Table 2. *Overall gas and indole results from two laboratories*

Medium	Gas and indole results	Minerals-modified glutamate medium with tryptophan (MMGM + T) G + I +	G + I −	G − I +	G − I −	Total
Lauryl tryptose mannitol broth (LTMB)	G + I +	74	80	.	3	157 (28%)
	+ −	78	180	.	20	278 (50%)
	− +	1	.	2	.	3 (1%)
	− −	.	8	1	112	121 (22%)
	Total	153 (27%)	268 (48%)	3 (1%)	135 (24%)	559 (100%)

There is considerable agreement between LTMB and MMGM + T in Table 1 where 1588 (91%) of 1740 tests gave identical results, but less so in Table 2 where only 368 (66%) of 559 tests gave the same results. MMGM without tryptophan yielded a total of 1214 gas-positive (70%) of 1740 test results from the nine laboratories (Table 3) and 415 gas-positive (74%) of 559 tests from the other two laboratories (Table 4).

In the light of results from all media, including subculture of single colonies to LPW when done, each tube was interpreted as containing (i) *E. coli*, (ii) not *E. coli*,

https://doi.org/10.1017/S002217240002708X Published online by Cambridge University Press

Table 3. *Overall interpretation of single tube confirmatory test results from nine laboratories*

Medium	Gas and indole results	Interpretation			Total
		E. coli	?*E. coli*	Not *E. coli*	
Lauryl tryptose mannitol broth (LTMB)	G I				
	+ +	1174	1	11	1186
	+ −	44	3	57	104
	− +	8	1	22	31
	− −	3	.	416	419
	Total	1229	5	506	1740
Minerals modified glutamate medium with tryptophan (MMGM+T)	G I				
	+ +	1148	3	6	1157
	+ −	45	.	45	90
	− +	22	.	47	69
	− −	14	2	408	424
	Total	1229	5	506	1740
Minerals-modified glutamate medium (MMGM)	G				
	+	1172	3	39	1214
	−	57	2	467	526
	Total	1229	5	506	1740

Table 4. *Overall interpretation of single tube confirmatory test results from two laboratories*

Medium	Gas and indole results	Interpretation			Total
		E. coli	?*E. coli*	Not *E. coli*	
Lauryl tryptose mannitol broth (LTMB)	G I				
	+ +	155	.	2	157
	+ −	213	12	53	278
	− +	.	1	2	3
	− −	2	1	118	121
	Total	370	14	175	559
Minerals modified glutamate medium with tryptophan (MMGM+T)	G I				
	+ +	138	13	2	153
	+ −	222	1	45	268
	− +	.	.	3	3
	− −	10	.	125	135
	Total	370	14	175	559
Minerals modified glutamate medium (MMGM)	G				
	+	361	13	41	415
	−	9	1	134	144
	Total	370	14	175	559

https://doi.org/10.1017/S002217240002708X Published online by Cambridge University Press

or (iii) ? *E. coli* – probably but not definitely *E. coli* (Table 3). The result was interpreted as *E. coli* if all the test results were positive; if there were any discrepancies, then reference was made to the LPW and TW reactions and the result interpreted as *E. coli* if both were positive; if not done, it was assumed that gas production from LPW would have been positive provided that all three other gas results were positive. The result was regarded as not '*E. coli*' if gas was not produced in LPW, or if all the indole tests were negative. The interpretation was uncertain (? *E. coli*) if the TW-indole result was negative but one or both of the other indole tests were positive, and LPW was either positive or not done. Table 3 shows the overall results after interpretation for tubes examined by nine laboratories according to the three confirmatory media under study; the results from the other two laboratories are shown in Table 4.

LTMB gave positive gas and indole results when the growth was not *E. coli* on 11 (0·9 %) of 1186 occasions and MMGM + T similarly on 6 (0·5 %) on 1157 occasions (Table 3). This difference in false positive results is not significant. MMGM without added tryptophan gave false positive results in 39 (3 %) of 1214 tests – a significantly higher false positive rate. LTMB gave negative gas and indole results when the growth was *E. coli* on 3 (0·7 %) of 419 occasions, and MMGM + T on 14 (3·3 %) of 424 occasions – a significantly greater proportion of false negative results than with LTMB. MMGM without tryptophan gave false negative results in 57 (10·8 %) of 526 tests – a significantly higher rate of false negative results than with either LTMB or MMGM + T. When LTMB and MMGM + T gave gas-positive but indole-negative results, the growth was found to be *E. coli* on about half the occasions. When LTMB and MMGM + T gave gas-negative but indole-positive results, the correct interpretation was likely not to be *E. coli* more than twice as often as it was likely to be *E. coli*. In total, these ambiguous results accounted for only 135 (8 %) of the tests with LTMB and 159 (9 %) tests with MMGM + T in the nine laboratories.

In the other two laboratories (Table 4), these ambiguous results accounted for about half of the total, almost all of them being gas-positive but indole-negative. For both LTMB and MMGM + T, gas-positive but indole-negative results proved to be *E. coli* between four and five times more often than not *E. coli*. The overall rate of false positive and false negative results were generally similar to those given by the other nine laboratories. LTMB and MMGM + T each gave two false positive results – a rate of about 1 %. In contrast, MMGM gave 41 (10 %) false positive gas results – a significantly higher rate than 39 out of 1214 results shown in Table 3. LTMB gave only two (2 %) false negative results out of 121 tests, whereas MMGM + T gave 10 (7 %) out of 135. The rate of false negative results with MMGM was 9 (6 %) out of 144 tests, and this was not significantly different from that shown in Table 3.

DISCUSSION

In the assessment of confirmatory media for *E. coli*, it is more important that false negative results should not occur than false positive results, since the latter err on the side of safety. Since MMGM, when used to show gas formation at 44 °C,

gave an unacceptable number of both false negative and false positive results, it was therefore considered unsuitable for the confirmation of *E. coli*.

When compared, the two single tube media LTMB and MMGM + T gave similar results in about 90% of tests in most of the participating laboratories, and each medium gave about the same number of false positive reactions. However, MMGM + T gave a significantly greater number of false negative results, and LTMB is therefore the more suitable medium for use in a single tube test to show both gas and indole production. The performance of LTMB is in fact comparable with that of the media described by Schubert (1956, 1958) and Pugsley *et al.* (1973).

If MMGM is used as the primary isolation medium in the multiple tube test, it is desirable that the subsequent confirmatory medium should contain a surface-active agent, so that this additional property of coliform organisms is demonstrated, besides the production of acid and gas from lactose and other suitable substrates. Growth in LTMB thus shows that lauryl sulphate is suitable for this purpose, as well as in giving copious gas production and usually strong indole reactions. During the trial it was observed that the addition of tryptophan to LTMB would often enhance the strength of the indole reaction. Further work is, therefore, in progress not only with LTMB to improve its performance, but also with media containing lactose – such as standard lauryl tryptose broth supplemented with tryptophan – and also simpler media in which the amount of carbohydrate is reduced. Meanwhile, the use of single tube tests is recommended as an economical way of demonstrating the production of both gas and indole for the confirmation of *E. coli* in positive presumptive reactions.

REFERENCES

Boyd, W. L. & Lichstein, H. C. (1955). The effect of carbohydrates on the tryptophanase activity of bacteria. *Journal of Bacteriology* **69**, 584.

Fennel, H. (1972). A single tube confirmatory test for *E. coli* at 44 °C. *Water Treatment and Examination* **21**, 13.

Gray, R. D. (1964). An improved formate lactose glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. *Journal of Hygiene* **62**, 495.

Mara, D. D. (1973). A single medium for the rapid detection of *Escherichia coli* at 44 °C. *Journal of Hygiene* **71**, 783.

PHLS Standing Committee on the Bacteriological Examination of Water Supplies (1968). Confirmatory tests for coliform organisms. *Journal of Hygiene* **66**, 641.

PHLS Standing Committee on the Bacteriological Examination of Water Supplies (1969). A minerals-modified glutamate medium for the enumeration of coliform organisms in water. *Journal of Hygiene* **67**, 367.

Public Health Laboratory Service and Standing Committee of Analysts (1980). 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 test. *Journal of Hygiene.* **85**, 35.

Pugsley, A. P., Evison, L. M. & James, A. (1973). A simple technique for the differentiation of *Escherichia coli* in water. *Water Research* **7**, 1431.

Report (1969). The Bacteriological Examination of Water Supplies. *Reports on Public Health and Medical Subjects*, no. 71. London: H.M.S.O.

Schubert, R. (1956). Die coliform Bakterien in der Wasserdiagnostik und ihre Differenzierungen. *Zeitschrift für Hygiene und Infektionskrankheiten* **142**, 476–86.

Schubert, R. (1958). Studien zur Differenzierung coliformen Bakterien in der Wasserdiagnostik. *Zeitschrift für Hygiene und Infektionskrankheiten* **144**, 485–95.