J. Hyg., Camb. (1982), **89**, 149
Printed in Great Britain

Rapid single-tube confirmatory test for *Escherichia coli*

By J. L. SMITH and S. ROCKLIFF

Public Health Laboratory, Capital Territory Health Commission, Canberra, Australia

(*Received* 11 *August* 1981; *accepted* 2 *March* 1982)

SUMMARY

A single-tube confirmatory test that allows a result to be obtained in 4 h has been developed from the single-tube confirmatory test recommended by the Joint Committee of the Public Health Laboratory Service and the Standing Committee of Analysts (PHLS/SCA, 1980). A variety of river, lake and reservoir samples were examined for the presence of *E. coli* using either most probable number (MPN) or membrane filtration (MF) technique, and the PHLS/SCA recommended confirmatory medium (LTMB) was evaluated against traditional methods. To improve the performance of LTMB, the medium was modified and this modified medium when used in 0·1 ml volumes and incubated for 4 h at 44 °C provided 99% agreement with traditional methods.

INTRODUCTION

Isolates of *Escherichia coli* from water sources are traditionally confirmed using either British (Report, 1969) or American (APHA, 1975) methods. Initially both methods involve subculturing from a lactose-based presumptive isolation medium with positive acid and gas reactions at 37 °C to two further media in order to confirm both gas and indole reactions at 44 °C. Isolates with positive gas and indole reactions at 44 °C are considered to be *E. coli*.

The PHLS/SCA have recommended a single-tube medium for this purpose – lauryl tryptose mannitol broth (LTMB) (PHLS/SCA, 1980). We evaluated the recommended medium against traditional procedures in use, that is subculture from presumptive minerals-modified glutamate medium (MMGM) or teepol agar (TA) of presumptive positive isolations to tubes of brilliant green bile broth (BGBB) in conjunction with lactose broth (LB) to confirm gas production and tryptone water (TW) to confirm indole production. For the purpose of water bacteriology, the definition of *E. coli* includes the ability to ferment lactose at 44 °C (Report, 1969); however, a significant proportion of samples tested produced gas from mannitol in LTMB but not from lactose in either BGBB or LB. These isolates invariably proved indole-negative and were identified as either Citrobacter or Enterobacter using API 20E identification kits (Carter-Wallace Aust. Pty Ltd). Thus the use of mannitol in place of lactose was considered unsuitable for the waters under examination.

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press

To overcome this problem and to improve the production of indole, LTMB was modified as follows: (1) mannitol was replaced by lactose and the quantity of lactose halved to enhance the production of indole (Boyd, 1955); (2) tryptone with 10% higher tryptophane content replaced tryptose (Oxoid, 1979); (3) 0·1% tryptophane was added to further increase indole production.

This modified medium, lauryl sulphate tryptone tryptophane broth (LSTTB), was then evaluated against traditional procedures in the same manner as LTMB, and the results are presented in this paper.

MATERIAL AND METHODS

Media

The following media were used: MMGM Oxoid CM 289; BGBB Oxoid CM 31; MacConkey agar (MAC) Difco 0075-01; EMB Oxoid CM 69; membrane-enriched teepol broth (METB) Oxoid MM369; LTMB, TW, Kovac's reagent prepared in the laboratory: TA as follows – 57·15 g METB, 10 g agar BBL 11849, 1000 ml distilled water autoclaved 15 lb/15 min, final pH approx. 7·4; LSTTB as follows – tryptone Difco 0123-01 20 g, lactose 2·5 g, K_2HPO_4 2·75 g, NaCl 5 g, sodium lauryl sulphate 0·1 g, tryptophane 1·0 g, distilled water 1000 ml autoclaved 15 lb/15 min, final pH 6·8. Circulating water-baths and a water-jacketed incubator were used throughout the trials.

Most probable number (*MPN*) (*Report*, 1969).

Water samples were initially distributed into MMGM and incubated at 37 °C. At 24 and 48 h each tube showing positive acid and gas reactions was subcultured to LSTTB, BGBB, LB, TW, MAC and EMB. All tubes were placed in a circulating water bath at 44 °C for 24 h, after which a few drops of Kovac's reagent was added to TW tubes and to all LSTTB tubes with gas. Agar plates were incubated at 37 °C for 24 h and inspected for the presence of typical colonies. Development of any shade of pink in the reaction layer of the indole test was considered positive and visible gas in the inverted inner (Durham) tube or the rise of a steady stream of bubbles on tapping the tube was considered a positive gas reaction. If both methods produced identical gas and indole reactions no further tests were performed; however, if disagreement occurred colonies from the agar plates were identified using API 20E kits.

Membrane filtration (*MF*) (*Report*, 1969).

Samples were filtered through sterile Gelman GN6 0·45 μm gridded filters, placed on TA and incubated in an automatic temperature-change water bath for 4 h at 30 °C followed by 18 h at 44 °C. Presumptive *E. coli* colonies were picked with a straight wire and inoculated into LSTTB, BGBB, TW and streaked to EMB and MAC agar plates. After incubation at 44 °C all TW tubes together with LSTTB tubes showing gas production were tested with a few drops of Kovac's reagent for presence of indole. Agar plates were incubated at 37 °C for 24 h and inspected for

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press

Table 1. *Gas and indole confirmatory test results using MPN method: PHLS/SCA-recommended medium and traditional methods*

	Gas and indole results		Traditional methods (MMGM to BGBB and TW) G. + I. +	G. + I. −	G. − I. +	G. − I. −	Total
	G.	I.					
MMGM to LTMB	+	+	61	1	−	−	62 (44 %)
	+	−	8	2	5	32	47 (33 %)
	−	+	−	−	−	−	−
	−	−	−	−	1	32	33 (23 %)
Total			69 (49 %)	3 (2 %)	6 (4 %)	64 (45 %)	142 (100 %)

the presence of typical colonies. At the same time 0·1 ml volumes of LSTTB in disposable Durham tubes were inoculated from presumptive *E. coli* colonies on membrane filters, placed in a circulating water bath for 4 h, removed and tested for the presence of indole by the addition of a few drops of Kovac's reagent. Where disagreement occurred between the gas and indole reactions obtained by traditional methods and by LSTTB, colonies were further identified using API 20E kits.

RESULTS

Colonial appearance on EMB and MAC agar has not been recorded in the accompanying tables as all isolates giving positive gas and indole results at 44 °C using either traditional methods or single-tube methods produced typical colonies. No discrepancies were observed between the gas reactions of BGBB and LB and LB was dropped from the trials. Results have been tabulated (Tables 1–4) by gas and indole reactions, and for the purpose of water bacteriology columns 1 and rows 1 list those isolates classified as *E. coli*; that is, organisms that produce gas from lactose and indole from tryptophane at 44 °C together with typical colonies on EMB and MAC agar.

Table 1 illustrates the results of the original evaluation of LTMB using the MPN method. Using traditional methods, 49 % of isolates were identified as *E. coli* and 44 % using LTMB. From column 1, representing *E. coli* identified by traditional methods, it can be seen that 8/69 (12 %) were not identified by LTMB. This proportion of false negative results was considered too high. Positive gas with negative indole reactions in LTMB occurred in 47/142 (33 %) of isolates, but using traditional methods in only 1/42 (0·7 %). This too was considered unsatisfactory and the medium was redesigned as described in the Introduction in an attempt to improve performance.

With the new medium, LSTTB, using the MPN method (Table 2), both LSTTB and traditional methods identified 43 % of isolates as *E. coli*, false negative results

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press

Table 2. *Gas and indole confirmatory test results using MPN method: lauryl sulphate tryptone tryptophane broth and traditional methods*

	Gas and indole results		Traditional methods (MMGM to BGBB and TW) G. I. G. I. G. I. G. I. + + + − − + − −				Total
	G.	I.					
MMGM to LSTTB	+	+	833	4	−	3	840 (43 %)
	+	−	7	25	1	33	66 (3 %)
	−	+	−	1	1	−	2 (< 1 %)
	−	−	1	2	−	1036	1059 (53 %)
Total			841 (43 %)	32 (2 %)	2 (< 1 %)	1072 (55 %)	1947 (100 %)

Table 3. *Gas and indole confirmatory test results using MF method: lauryl sulphate tryptone tryptophane broth and traditional methods*

	Gas and indole results		Traditional methods (TA to BGBB and TW G. I. G. I. G. I. G. I. + + + − − + − −				Total
	G.	I.					
TA to LSTTB	+	+	288	−	−	−	288 (89 %)
	+	−	−	23	−	1	24 (7 %)
	−	+	−	−	−	−	−
	−	−	−	−	−	12	12 (4 %)
Total			288 (89 %)	23 (7 %)	−	13 (4 %)	324 (100 %)

were reduced to 8/1947 (0·4 %) and positive gas negative indole isolates to 66/1947 (3 %) with complete agreement between traditional methods and LSTTB in 1895/1947 (97 %) of isolates. This 3 % of gas-positive indole negative isolates were further identified using API 20E kits and belonged to either the genus *Citrobacter* or *Enterobacter*. A number of those isolates which were not confirmed as *E. coli* by traditional methods (row 1) were retested using colonies from the agar plates. Primary test results were confirmed and as colonial appearance was typical these isolates were considered to be *E. coli*, giving the traditional method approximately the same rate of false negative identifications as LSTTB (0·4 %).

Using MF methods (Table 3) both the traditional methods and LSTTB identified 89 % of isolates as *E. coli* and discrepancy between gas and indole reactions occurred in only 1/324 isolates. This organism was identified as *Citrobacter freundii*.

When LSTTB was used in 0·1 ml volumes with 4 h incubation gas production could not be assessed and the method relied on the production of indole in the medium. Both the mini-broth and the traditional methods identified 89 % of

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press

Table 4. *Gas and indole confirmatory test results using MF method: lauryl sulphate tryptone tryptophane broth in 0·1 ml volumes and traditional methods*

		Traditional methods (TA to BGBB and TW)								
	Gas and indole results	G.	I.	G.	I.	G.	I.	G.	I.	
	I.	+	+	+	−	−	+	−	−	Total
TA to LSTTB, 0·1 ml vol.	+	287		−		−		−		287 (93%)
	−	1		16		−		5		22 (7%)
Total		288 (93%)		16 (5%)		−		5 (2%)		309 (100%)

isolates as *E. coli*. False negative reactions in LSTTB occurred in only 1/288 (0·3%) of isolates and the excellent agreement between LSTTB and traditional methods is evident (Table 4).

DISCUSSION

With the waters tested in this laboratory LSTTB appeared acceptable as a single-tube confirmatory medium. The trials have been conducted over a 10-month period and are continuing; however, seasonal variation does not appear to be significant. The percentage of false negative identifications that occur when LSTTB is used does not differ significantly from that obtained using traditional methods, and can be tolerated in view of the materials and time saved by the use of a single-tube method. It is possible that the intensity of the indole reaction can be increased by a further reduction in the lactose content of the medium (Boyd & Lichstein, 1955). It has also been observed that when 0·1 ml volumes of LSTTB are used, the longer the incubation time available the stronger is the indole reaction. In most laboratories it would be possible to provide a 6 h incubation period for this test. Preliminary investigations into the capabilities of the 0·1 ml test indicate that when the medium is inoculated with one Pasteur pipette drop from either a MMGM or MacConkey broth culture that is positive for acid and gas after 24 h incubation at 37 °C, a positive indole reaction is possible after 6 h incubation at 44 °C. Further investigations into these areas are planned together with an evaluation of a lactose reduced modification of LSTTB.

Thanks are due to the Chairman of the Capital Territory Health Commission for permission to publish this article.

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press

REFERENCES

APHA (1975). *Standard Methods for the Examination of Water and Wastewater*, 13th ed. APHA AWWA WPCF.

BOYD, W. L. & LICHSTEIN, H. C. (1955). The effect of carbohydrates on tryptophanase activity of bacteria. *Journal of Bacteriology* **69**, 584–589.

OXOID (1979). *The Oxoid Manual*, 4th ed., pp. 214–215.

PUBLIC HEALTH LABORATORY SERVICE/STANDING COMMITTEE OF ANALYSTS (1980). Single tube confirmatory test for *Escherichia coli*. By a Joint Committee of the Public Health Laboratory Service and the Standing Committee of Analysts. *Journal of Hygiene* **85**, 51.

REPORT (1969). The bacteriological examination of water supplies. *Report on Public Health and Medical Subjects*, no. 71. London. H.M.S.O.

https://doi.org/10.1017/S0022172400070649 Published online by Cambridge University Press