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The assessment of two media for the confirmation of *Escherichia coli* in water samples in single-tube tests

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SUMMARY

Two single-tube media, lactose-tryptone-lauryl sulphate broth (LTLSB) and lauryl-tryptose-mannitol broth were tested in parallel in a series of 1111 tests on water samples from a variety of sources.

LTLSB was found to be superior both in gas and indole production from *Escherichia coli* when incubated at 44 °C and is recommended as the most suitable single-tube confirmatory test for this organism.

INTRODUCTION

Enumeration of *Escherichia coli* is an important procedure in water and food bacteriology. The production of gas from lactose and indole from tryptophane are the criteria for the presence of *E. coli* (Report no. 71, 1983). However, that lactose fermentation inhibits indole production (Boyd & Lichstein, 1955) makes the formulation of a single tube medium difficult.

To overcome this problem the substitution of mannitol for lactose has been proposed (Schubert, 1956; Fennel, 1972; Pugsley, Evison & James, 1973) as mannitol fermentation is closely associated with that of lactose in *E. coli*.

A variety of other single tube media have been developed. These include 0·3% lactose broth (Mara, 1973), lauryl-tryptose-mannitol broth (LTMB), minerals modified glutamate medium with tryptophane (MMGM/TP) (PHLS/SCA, 1980) and lactose-tryptone-ricinoleate broth (LTRB) (Papadakis, 1972). None of these media have proved totally satisfactory either from gas or from indole production.

The LTRB medium was modified by the substitution of lauryl sulphate for ricinoleate to lactose-tryptone-lauryl sulphate broth (LTLSB). This medium gave identical results when compared with the two tubes classical technique of Mackenzie, Taylor & Gilbert (1948) and was better than LTMB especially in indole production (Papadakis & Mavridou, 1982, 1985).

The inability of LTMB to detect indole consistently was well known and the performance of the medium was improved with the addition of tryptophane (PHLS/SCA, 1981). In this paper we compare our single tube medium with the improved mannitol broth in the routine examination of water samples.

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MATERIALS AND METHODS

Media

(*a*) Minerals modified glutamate medium (MMGM) was prepared from OXOID's dehydrated media.

(*b*) Lactose-tryptone-lauryl sulphate broth (LTLSB) was prepared as follows: tryptone (Oxoid) 20 g; tryptose 10 g,; K_2HPO_4 3 g; KH_2PO_4 1 g; lactose 1 g; lauryl sulphate 0·1 g; sodium formate 5 g; distilled water 1000 ml. Final pH 7·5 ± 0·1 after sterilization at 110 °C for 20 min.

(*c*) Lauryl-tryptose-mannitol broth with tryptophane was prepared as the standard lauryl-tryptose-lactose broth but with mannitol instead of lactose, supplemented with 0·2 g/l tryptophane (LTMB/TP) (Report no. 71, 1983).

(*d*) A series of standard media were also used for confirmatory tests, i.e. lactose broth, tryptone water, MR and VP, Christensen's citrate agar slopes, lysine decarboxylase medium and sugar fermentation media.

(*e*) The indole reaction was tested by Kovac's modification method and oxidase reaction by the standard method.

Water samples

Samples were obtained of drinking water (58%), sea water (32%), swimming-pool water (4%) and the rest from river water and effluents.

Methods

Water samples were inoculated into tubes containing double and single strength of MMGM which were incubated at 37 °C for 24 and 48 h (Report no. 71, 1983). For sea water samples the volume ratio of inoculated water to medium was 1/10 or less (Papadakis 1975, 1982) and for effluents the appropriate dilutions were made before inoculation.

All positive presumptive tubes of MMGM after incubation were subcultured with a 3 mm loop into LTLSB and LTMB/TP tubes which were then incubated in a water bath for 24 or 48 h at 44 ± 0·2 °C. Tubes of either media with gas were tested for indole. The intensity of indole reaction was scored.

Confirmatory tests (IMViC, gas production from lactose and indole at 44 °C, lysine decarboxylase, adonitol and cellobiose fermentation, oxidase) were made in all cases where doubtful results were obtained.

RESULTS

The comparison between LTLSB and LTMB/TP is based on a series of 1111 tests of which 640 were on drinking water, 354 on sea water and 117 on samples from swimming pools and effluents.

Table 1 shows the number of tubes with gas and indole production in each medium and category of samples.

The total number of tubes positive for *E. coli*, i.e. gas + indole + whatever the strength of the reaction, is the same for both media. The differences lie in (*a*) the frequency of gas production and (*b*) the intensity of indole reaction.

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Table 1. *Gas and indole from lactose-tryptone-lauryl sulphate broth (LTLSB) and lauryl-tryptose-mannitol broth with tryptophane (LTMB/TP) at 44 °C*

Samples	Media	No. of tests	Gas Ind.* + +++	Gas Ind.* + ++	Gas Ind.* + +	Gas Ind. + −	Gas Ind. − −
Drinking water	LTLSB	640	309	48	14	27	242
	LTMB/TP	—	235	58	78	90	179
Sea water	LTLSB	354	277	12	9	17	39
	LTMB/TP	—	241	20	37	26	30
Swimming pool, effluents, etc.	LTLSB	117	59	1	1	12	44
	LTMB/TP	—	50	4	7	16	40
Total	LTLSB	1111	645	61	24	56	325
	LTMB/TP	—	526	82	122	132	249

* Indole reaction has been scored as follows: + + +, very strong reaction; + +, clear positive reaction; + weak to doubtful reaction; − negative

In only 56 tubes of LTLSB was gas produced in the absence of *E. coli*; with LTMB/TP 132 were positive ($P < 0{\cdot}0001$). With drinking water 3·3 times as many tubes without *E. coli* produced gas with LTMB/TP as with LTLSB.

Lactose-tryptone-lauryl sulphate broth gave strong and clear indole reactions, classified as 2–3 plus, in 97% of all positive tubes while only 83% LTMB/TP tubes give reactions of the same strength ($P < 0{\cdot}001$).

With drinking water samples almost 21% of positive LTMB/TP tubes show weak to doubtful indole reactions in the presence of *E. coli*. The possibility that some of the very weak indole reactions may be recorded as negative was tested by giving a series of paired tubes of the two media, one of which usually gave a weak indole reaction, to two experienced laboratory staff members who independently recorded the results. While both workers scored the correct number of indole negatives with the LTLSB medium (159) one worker recorded 196 as negative with the LTMB/TP medium.

DISCUSSION

The formulation of a lactose single tube medium for *E. coli* confirmation test at 44 °C has met the difficulty caused by lactose fermentation inhibiting indole formation. Even media with reduced lactose concentration (Mara, 1973) do not always produce indole and sometimes show very small amounts of gas. Further reduction of lactose results in very poor and usually undetectable gas production.

The substitution of mannitol for lactose in single tube media was a solution to the problem (Schubert, 1956; Fennel, 1972; Pugsley, Evison & James, 1973) and the use of lauryl sulphate as inhibitory substance allowed for good growth and copious gas production at 44 °C by *E. coli* (PHLS/SCA, 1980). However, indole production was not always satisfactory in mannitol media (PHLS/SCA, 1980; Papadakis & Mavridou, 1982; 1985) and the addition of tryptophane was an improvement (PHLS/SCA, 1981). Nevertheless as it is stated 'such a medium cannot however be expected to give a positive indole reaction in every instance'

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and also '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'.

The present results confirm the above statements. Indeed mannitol-tryptophane medium in comparison with our lactose single tube formula was found to have disadvantages both in lack of specificity for gas production at 44 °C only from *E. coli*, and in weak indole production such that it sometimes may be recorded as negative. Nearly 20% of tubes from drinking water samples with gas production in mannitol media were found both in the present and previous comparable studies (Papadakis & Mavridou, 1985) not to contain lactose or mannitol fermenting *E. coli* strains.

It is worth noting that although LTLSB contains only 0·1% lactose it constantly gives abundant gas formation because of the enhancing property of sodium formate. Moreover the very low lactose concentration permits good indole production which is always readily identifiable and comparable in intensity to that obtained with pure tryptone water.

It is concluded that LTLSB has overcome the difficulties of lactose single tube tests and incorporates the most suitable lactose formulation for *E. coli* differentiation tests in water and food bacteriology.

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