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A single medium for the rapid detection of *Escherichia coli* at 44° C

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SUMMARY

Lactose tryptone ricinoleate broth (0·3 % lactose, 2 % Oxoid tryptone, 0·1 % Na ricinoleate; pH 7·1) was found to be as good as the currently recommended combination of lactose ricinoleate broth and tryptone water for the detection of *Escherichia coli* in the positive tubes of presumptive coliform counts.

INTRODUCTION

British practice (Report, 1969) for the rapid detection of *Escherichia coli* in positive tubes of presumptive coliform counts requires that a tube of lactose ricinoleate broth (LRB) containing 1 % lactose and a tube of tryptone water (TW) should be inoculated with a loopful of culture from the positive presumptive tube; *E. coli* is judged to be present if gas is produced in LRB and indole in TW after 24 hr. at 44° C. The work involved in media preparation and subculturing and the requirement for water bath space would be halved if there were a single medium for the detection of lactose fermentation and indole production. The concentration of lactose in such a medium is important since Boyd & Lichstein (1965) found that the tryptophanase activity of *E. coli* was inhibited in media containing more than 0·02 M carbohydrate. This note describes a low lactose, tryptone rich ricinoleate medium suitable for the detection of both gas and indole by *E. coli* after incubation at 44° C for 20–24 hr.

MATERIALS AND METHODS

Samples of sewage effluent from Kariobangi sewage treatment works, Nairobi, of polluted water from various streams in Nairobi and of upland catchment water from Ruiru Dam, Nairobi, were used to inoculate suitable volumes of Oxoid MacConkey broth (purple). Some samples of polluted stream water were subjected to the chlorination treatment described by Burman (1967); others were stored at 4–6° C. for 14 days. These treatments were done to obtain physiologically debilitated organisms in an attempt to create conditions under which false reactions (either positive or negative) might occur. After these had been incubated for 14–48 hr. at 37° C., loopfuls of each resulting culture were transferred to tubes of LRB, TW and lactose tryptone ricinoleate broth (LTRB). LTRB contained 0·3 %

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Table 1. *Experimental details and results*

Sample source	No. of presumptive coliform cultures obtained	No. of cultures containing *E. coli* based on	
		LRB + TW	LTRB
Sewage effluent	50	50	50
Ruiru dam	25	0*	1*
Polluted waters:			
(i) untreated	60	60	60
(ii) chlorinated	20	20	20
(iii) stored at 4–6° C	25	7*†	8*†

* Disparity explained in text.
† Only one disparity.

lactose, 2 % Oxoid tryptone and 0·1 % sodium ricinoleate (pH 7·1); it was sterilized at 115° C. for 20 min. After incubation for 20–24 hr. at 44° C., the tubes were examined for gas and indole production, Kovac's reagent (Report, 1969) being used for the latter. Immediately before the addition of Kovac's reagent, plates of Oxoid eosin methylene blue (EMB) agar (Levine) were inoculated with the LTRB cultures; these were incubated at 37° C.

RESULTS AND DISCUSSION

One hundred and eighty coliform cultures were obtained: their sources and the number of cultures containing *E. coli* (as judged by both LRB + TW and LTRB) are given in Table 1. In only two cases was there a difference between the two methods. In each of these cases it was found to be caused by a false negative reaction in LRB + TW (no indole production in TW; however, when the positive LRB cultures were transferred into fresh TW, indole production at 44° C occurred). All the positive LTRB cultures formed green colonies with a metallic sheen, typical of *E. coli*, on EMB agar; none of the negative cultures did.

Gas production in LTRB was at least as copious as that in LRB. This suggests that the formulation of LRB with 1 % lactose is unnecessarily extravagant. It may be noted that American media for the presumptive and completed tests are formulated with only 0·5 % lactose (APHA, 1971). The medium for the confirmed test, however, is formulated with 1 % lactose.

The present results indicate that LTRB is at least as good as LRB + TW in confirming the presence of *E. coli* in presumptive positive tubes. Its greater convenience suggests that it is a practical alternative to the presently recommended procedure.

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