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COMPARISON OF SELECTIVE MEDIA FOR THE DETECTION OF *ESCHERICHIA COLI* IN WATER

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Abstract

The performance of selective media LTB, (leauryl tryptose broth), MMGM (minerals modified glutamate medium), BGBB (brilliant green bile broth) , for the detection of *E.coli* in water, were compared and the following results were obtained:

The MMGM found to be unsuitable as confirmatory medium for the detection of *E.coli* at 44°C, due to the occurrance of an unacceptable number of the false-negative and false-positive results.

The comparison of the two confirmatory medium LTB, BGBB for the confirmation of *E.coli* at 44°C , it was found that LTB was less inhibitory than BGBB for the recovery of *E.coli* in mixed populations. The occurrence of a higher number of false-negatives in BGBB is a more serious disadvantage since it would lead to an underestimate of the actual number of *E.coli*. Therefore, the results of this study showed that LTB is a more suitable confirmatory medium than BGBB.

Introduction

The infectious diseases transmitted by drinking water are primarily those which have been associated with contamination of water by human or animal faeces. A variety of diseases of microbial origin, such as typhoid fever, cholera, bacterial dysentery, as well as viral diseases, hepatitis A and polio transmitted by water.

To enumerate all wasteborne pathogens, the microbiologist would have to perform a variety of complex, time consuming, costly and often tentative procedures for each sample analyzed. A more realistic approach is to use

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a bacterial indicator (*Escherichia coli*) to detect and quantify faecal pollution from all warm-blooded animals.

The selective media for the enumeration and detection of *Escherichia coli* are MMGM, LTB and BGBB (1,3,4,5,6). The purpose of this research was to compare the performance of these media in detection of *Escherichia coli* in sewage.

Materials and methods

The media used were Minerals Modified Glutamate Medium (MMGM, Oxoid), Lauryl Tryptose Broth (LTB, Oxoid) and Brilliant Green Bile Broth (BGBB, Oxoid). The constituents and preparation of these media can be found in the manufacturers manuals.

10 ml of Minerals Modified Glutamate Medium was dispensed into each of 300 screw-capped (1-oz) bottles, containing an inverted Durham tube. Autoclaving was carried out as recommended by the manufacturers.

1 ml of sewage samples was inoculated into ecah bottle. All the bottles were incubated at 37°C for 24 hours.

The bottles were examined at 24 hours for gas and acid production. Each of the positive gas and acid bottles were labelled, then subcultured into MMGM, LTB, BGBB and tryptone water (TW) at 44°C for 24 hours. After the incubation period, positive gas and acid was recorded with MMGM, LTB, BGBB, and indole test was performed on tryptone water. A loopful from each positive acid and gas bottle was streaked on Eosine Methylene Blue Agar (EMB) plates. Such plates were incubated at 37°C for 24 hours. Gram reaction and morphology of isolets were determined.

The IMViC tests were carried out for the identification of *E.coli*. The positive gas production in each of MMGM, LTB, BGBB, indole in tryptone water at 44°C, and IMViC(++--) tests was interpreted as confirming the presence of *E.coli*.

A known strain of *E.coli* was used as a control, through the experiments.

Results

The results of gas and acid production, together with colonial apperance on EMB agar plates are given in Table 1.

Out of 300 isolates, 221 (73.5%) produced gas and acid, and showed green metallic sheen colonies on EMB agar. All of these isolates were gram negative rod.

The biochemical characteristics of cultures isolated on MMGM at 37°C and

subcultured on LTB, MMGM and BGBB at 44°C are given in Table 2. As it shown in Table 2, MMGM gave a higher number of false-positive results than LTB and BGBB, while BGBB gave more false-negative results.

A total of 9 isolates on MMGM were produced gas at 44°C, but were not identified as *E.coli* and 7 isolates failed to produce gas on BGBB at 44°C, although they were identified as *E.coli*. A total of 4 isolates on LTB produced gas at 44°C, but were not identified as *E.coli*.

Discussion

The higher number of false-positive results were obtained with MMGM at 44°C. This was expected since MMGM does not contain any chemical inhibitors. In the U.K., MMGM is recommended as a first choice medium over LTB in the presumptive test for the detection of coliforms (7,8,9). The results of this study also indicated that MMGM was less inhibitory than LTB, and more suitable than LTB to be for the presumptive test.

The confirmatory media (BGBB, LTB), used for the detection of *E.coli* were compared. The results showed that LTB gave fewer false-negatives, but more false-positives than BGBB.

The higher number of false - negatives associated with BGBB might be due to the more inhibitory effect of the brilliant green and bile salts combination present in the BGBB than the sodium lauryl sulphate which is present in LTB.

The occurrence of a higher number of false-positives in LTB is not an important disadvantage, since it led to the higher counts on the safety side.

The occurrence of a higher number of false-negatives in BGBB is a more serious disadvantage, since it would lead to an underestimate of the actual number of *E.coli* . Therefore , it seems from these results than LTB is a more suitable confirmatory medium than BGBB. Similar results were obtained in a comparative trial by APHA (1,2).

Table 2 - The Biochemical characteristics of cultures isolated on MMGM at 37°C, subcultured on LTB, MMGM, BGBB and Tryptone water at 44°C

Isolation media	No. of isolates	A + G at 44°C		No. of non - *E.coli* producing gas (false-positive)	No. of non - *E.coli* not producing gas (false-negative)	I at 44°C	MR (+)	VP (-)	C (-)
		A	G						
MMGM	221	217	217	14	3	208	209	209	209
LTB	221	206	206	6	2	199	200	201	200
BGBB	221	201	198	1	13	210	210	209	210

I= Indole test
MR= Methyl - red test
VP = voges - proskauer test
A= Acid
G= Gas
C= Citrate test

Table 1- The Morphological and Biochemical characteristics of cultures isolated on MMGM at 37°C from sewage samples

No. of isolates	Isolation medium	Incubation temperature	Colony appearance on EMB agar	Morphology of GMS colonies	Gram-reaction of GMS colonies	No. of acid and gas production
300	MMGM	37°C	221 GMS 79 NGMS	All rods	All negative	221

GMS = Green metallic sheen colonies
NGMS = non-green metallic sheen colonies

References

1- American Public Health Association (1981) : *Standard methods for the examination of water and waste-water*, 15th , ed. American Public Health Association, Washington, D.C.

2- American Public Health Association (1995) : *Standard methods for the examination of water and waste-water*, 19th , ed. American Public Health Association, Washington, D.C.

3- Anderson JG, Meadows PS , Mullins BW, Patel K. (1980): Gas production by *Escherichia coli* in selective lactose fermentation media. *FEMS Microbiology letters.* **8**: 12-7.

4- DOE/SCA (1996): Methods for the Isolation and Identification of *Escherichia coli* 0157/H7 from waters.

5- Hajna AA, Perry CA (1943): Comparative study of presumptive and confirmative media for bacteria of the coliform group and faecal streptococci. *American Journal of Public Health.* **33** : 554 - 60.

6- Mallman WL, Darby CW (1941): Uses of a Lauryl Sulphate Tryptose Broth for the detection of coliforms. *American Journal of Public Health.* **31**: 127 - 34.

7- PHLS (1981): A comparison of confirmatory media for coliform organisims and *Escherichia coli* in water , by a joint committee of the Public Health Laboratory Service and the Standing Committee of Analysts. *Journal of Hygiene.* **87**: 369 - 75.

8- Report 71 (1985): The Bacteriological Examination of Water Supplies, HMSO, London.

9- Report (1994): The Microbiology of Water, Part 1-Drinking water, Reports on Public Health and Medical Subjects, No. 71. HMSO, London.