

# Rapid Recovery of *Escherichia coli* from Estuarine Water

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The efficiencies of two 24-hr elevated-temperature tests to recover *Escherichia coli* from estuarine water were compared simultaneously with the 72-hr standard methods procedure of the American Public Health Association (APHA). From 1,710 tubes, *E. coli* was recovered 222 times in lauryl tryptose medium incubated at  $44 \pm 0.2$  C for 24 hr, 261 times in an experimental medium incubated at  $44.5 \pm 0.2$  C for 24 hr, and 257 times by the 72-hr APHA method. The number of false positives enumerated was similar in all three tests. The data indicated that *E. coli* in raw seawater could be determined in 24 hr without a significant loss of accuracy.

The need for a rapid, reliable method for determining the bacterial quality of shellfish-growing waters is an important aspect of the continuing search for methods improvement. The standard methods procedure for the examination of water recommended by the American Public Health Association (APHA) requires a maximum 48-hr enrichment period in lactose broth or lauryl sulfate tryptose broth (LST) to obtain optimum recovery of fecal coliforms. Positive tubes are then transferred to EC medium (Difco) to determine the most probable number (MPN) of fecal coliforms. Gas production within 24 hr in the fermentation vial of a tube of EC medium incubated in a water bath at  $44.5 \pm 0.2$  C is considered a positive reaction indicating an organism of fecal origin. Tubes that do not produce gas indicate that the organisms are derived from sources other than the tract of a warm-blooded animal (1). Working with frozen foods, Fishbein et al. (3) reported that LST incubated directly at 44 C gave *E. coli* recovery similar to that of the standard methods procedure of the APHA. A preliminary investigation in our laboratory demonstrated that a newly formulated medium also gave *E. coli* recovery comparable to that of the APHA method. The purpose of this study was to compare simultaneously the reliability of *E. coli* recovery from field seawater samples by use of (i) Fishbein's elevated-temperature test, (ii) an experimental 24-hr elevated-temperature test with the newly for-

mulated medium, and (iii) the 72-hr APHA standard methods procedure.

## MATERIALS AND METHODS

Sixty-eight samples were collected over a 2-year period from a relatively small estuary of Mobile Bay approximately 1 mile long and one-half mile wide. Water depth ranged from 1 to 50 feet. During the period of sampling, salinities varied from  $<1.8$  to 14 parts per thousand, and temperatures ranged from 12.3 to 31 C. The estuary receives the effluent from a gypsum manufacturing plant and from various dock facilities located on the western shore. Sampling stations were located approximately one-quarter mile from any source of pollution. All samples were iced and tested within 24 hr after collection. Five-tube decimal dilutions were performed in phosphate-buffered water (pH 7.2) as far as necessary to obtain a determinant MPN of coliforms. LST was incubated at  $35 \pm 0.5$  C in a walk-in incubator for 24 to 48 hr, and positive tubes were transferred to EC medium and incubated in a water bath at  $44.5 \pm 0.2$  C for 24 hr in accordance with the APHA standard methods procedure for the examination of water (1). In the two rapid tests, LST and Medium A-1 (the newly formulated medium) were inoculated and incubated directly in water baths at  $44 \pm 0.2$  C and  $44.5 \pm 0.2$  C, respectively. Medium A-1 included 5 g of lactose, 20 g of tryptone, and 5 g of NaCl found in most of the commercially prepared dehydrated media; it also contained 1 ml of Triton X-100 (Rohm and Haas Co.), a surfactant, and 0.5 g of salicin (Difco), a carbohydrate readily utilized by *Escherichia* species (2), added to 1,000 ml of distilled water.

All positive tubes from the elevated temperatures were streaked on Levine's eosin methylene blue agar and incubated at  $35 \pm 0.5$  C for 24 hr. Representative colonies of each type were picked to lactose

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broth and incubated at 35 C for 24 and 48 hr. All positive tubes (gas formation) were inoculated into 1% tryptone, MR-VP medium, and Koser citrate medium and were subjected to indole, methyl red, Voges-Proskauer, and citrate (IMViC) determinations at  $35 \pm 0.5$  C. All positive lactose broth tubes were also reinoculated into EC medium and incubated at  $44.5 \pm 0.2$  C for 24 to 48 hr to confirm the ability of the pure culture to grow and produce gas at the elevated temperature.

To facilitate a comparison with the data of Fishbein et al. (3), we decided to use their terminology, which is as follows: positive tube, a tube that ferments lactose with the production of gas within 24 to 48 hr; negative tube, a tube that does not produce gas within 24 to 48 hr; *E. coli*<sup>+</sup>, a tube from which *E. coli* type I (++) or type II (-+-) is isolated; false-positive tube (FP), a tube that produces gas within 24 or 48 hr, but from which *E. coli*<sup>+</sup> is not isolated; false positive (group), a tube that produces gas within 24 or 48 hr, but from which a coliform organism other than *E. coli*<sup>+</sup> is isolated; false positive (synergistic), a tube that produces gas within 24 or 48 hr, but from which neither *E. coli*<sup>+</sup> nor any coliform is isolated.

## RESULTS AND DISCUSSION

In Table 1, the positive tube reactions of the four tests are compared. LST incubated at 35 C produced the largest number of positive tubes; all the elevated-temperature tests produced considerably fewer positive tubes. LST incubated directly at 44 C produced the fewest positive tubes (631), whereas EC medium and Medium A-1 incubated at 44.5 C produced a similar number of positive tubes (665 and 674).

*E. coli* recovery data are given in Table 2.

TABLE 1. Positive tube reactions

Test	Number of positive tubes	Percentage of tubes positive <sup>a</sup>
LST, <sup>b</sup> 35 C, presumptive . . .	1,011	59.12
EC, 44.5 C, confirmatory . . .	665	38.89
LST, 44 C . . . . .	631	36.91
Medium A-1, 44.5 C . . . . .	674	39.42

<sup>a</sup> Based on the number of positive tubes divided by the total number of tubes (1,710).

<sup>b</sup> Lauryl sulfate tryptose broth.

TABLE 2. Recovery of *Escherichia coli*

Test	<i>E. coli</i> <sup>+</sup> tubes	<i>E. coli</i> <sup>+</sup> /total no. of tubes producing gas (%)
EC, 44.5 C, confirmatory . .	257	38.65
LST, <sup>a</sup> 44 C . . . . .	222	35.18
Medium A-1, 44.5 C . . . . .	261	38.72

<sup>a</sup> Lauryl sulfate tryptose broth.

Not as many *E. coli* were recovered from the LST incubated at 44 C as from the EC medium and Medium A-1 incubated at 44.5 C. The productivity ratios (column 3) were obtained by dividing the number of tubes from which *E. coli*<sup>+</sup> was isolated by the total number of gassing tubes. The observed differences in productivity ratios of the three tests were not considered significant.

Data for the recovery of coliforms, exclusive of *E. coli* types I and II, are given in Table 3. These data indicate no significant difference in the number of false-positive tubes enumerated by the three elevated-temperature tests.

The results of this study are consistent with those of Fishbein et al. (3), who found that LST incubated at 44 C gave *E. coli* recovery comparable to that obtained by the APHA standard methods procedure. Although with LST incubated at 44 C we did not recover as many coliforms (631 contrasted to 665) and *E. coli* (222 contrasted to 257) as were recovered with the standard methods procedure, the differences were not significant.

We wish to emphasize that 44 C, rather than 44.5 C, was used as the temperature of direct incubation of the LST in the water bath, inasmuch as this was the temperature used by Fishbein et al. (3). Furthermore, we believe the use of 44 C is justified since the recovery of *E. coli* and the enumeration of false positives by the LST at this temperature were not significantly different from those by the APHA method. With Medium A-1, however, a temperature of 44.5 C was used because this is the most acceptable temperature for the incubation of the fecal coliform group (1). The effects of the direct incubation of LST and Medium A-1 over a range of temperatures will be the subject of a subsequent report. When the overall performance of Medium A-1 was compared with that of the APHA procedure, re-

TABLE 3. Coliform group recoveries<sup>a</sup>

Test	Total no. of FP <sup>b</sup>	No. of FP (group)	No. of FP (synergistic)	FP tubes/total no. of tubes producing gas (%)
EC, 44.5 C, confirmatory	406	385	21	61.06
LST, <sup>c</sup> 44 C	407	389	18	64.50
Medium A-1, 44.5 C	405	380	25	60.09

<sup>a</sup> All *Enterobacter* and *Escherichia* species exclusive of *E. coli* types I and II.

<sup>b</sup> FP = false positives; gas-producing tubes from which *E. coli* was not recovered.

<sup>c</sup> Lauryl sulfate tryptose broth.

sults by both methods were similar in enumerating coliforms (674 and 665) and *E. coli* (261 and 257). When the two rapid methods were compared to each other, Medium A-1 recovered more coliforms (674 compared to 631) and *E. coli* (261 compared to 222) than did LST incubated at 44 C. Both rapid tests merit further investigation because of the savings in time and cost of media without an accompanying loss in accuracy. It would be particularly significant if a rapid, reliable method could be adopted as a standard methods procedure for determining the bacterial quality of shellfish-growing waters as well as the bacterial content of oysters, clams, and other potentially marketable seafoods.

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