Comparison of the Elevated Temperature Plate Count (ETPC) and MPN Procedure to Estimate the Densities of Fecal Coliforms and *Escherichia coli* in Soft-Shell Clams

S. VARGA, R. E. DOBSON, and R. EARLE

Environment Canada Fisheries and Marine Service
Technology Branch, Box 429
Halifax, Nova Scotia B3J 2R3, Canada

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**ABSTRACT**

The Elevated Temperature Plate Count procedure of Hefferman and Cabell was assessed to measure the sanitary quality of soft shell clams. The method, compared to the standard MPN procedure, underestimated the densities of fecal coliforms by about 10%. The estimated cell densities of *Escherichia coli* and *Enterobacter aerogenes* in saline suspension were about 20% lower than the estimate obtained on nutrient agar plates. Thus, the fecal coliform standard used in the assessment of sanitary quality of clams would need to be modified when the analysis is conducted by the Elevated Temperature Plate Count procedure.

Depuration of soft-shell clams (*Mya arenaria*) from moderately contaminated water (231-700 coliforms/100 ml) has been of interest in Nova Scotia in recent years. A pilot plant jointly-supported by industry, Federal, and Provincial Government agencies was operated in Digby, Nova Scotia in 1976 (5). The bacteriological quality of clams before and following depuration was determined by the standard MPN fecal coliform procedure (1) which requires 72 h to complete. During the analyses of samples, depurated shellfish must be stored. This delay is costly; hence a faster method of assessing the sanitary quality of clams was of interest.

Since some rapid procedures to estimate fecal coliform densities in samples were described by other workers (2, 3, 4); our laboratory was requested to assess the usefulness of the elevated temperature plate count technique (ETPC) of Hefferman and Cabell, (4) with the hope that this procedure might be used to estimate the sanitary quality of depurated clams.

**MATERIALS AND METHODS**

Samples of contaminated clams were taken from the estuary of the Cornwallis River in Nova Scotia. Clams were placed in polyethylene bags, chilled with ice in a cooling box, and transported to the laboratory for microbiological examination within 24 h of harvesting.

For analysis, clams were thoroughly washed and scrubbed in running potable water. A 100-g quantity of meat and liquor was homogenized for 2 min with sterile saline (ratio of sample and saline 1:9). The fecal coliform densities in the samples were estimated by the standard 5-tube MPN procedure of APHA (1) in triplicate. Pre-enrichment was in Lautry Sulphate Tryptose (LST) broth for 48 h at 35°C. The positive tubes were cultured in EC broth for 24 h at 44.5°C.

The ETPC of fecal coliforms were obtained in modified (4) MacConkey’s agar after 24-h incubation in air at 44.5 ± 0.5°C. Only brick-red colonies >0.5 mm in diameter were counted and the fecal coliform density/100 g sample was calculated. Each sample was plated in duplicate. The modified single strength MacConkey agar consisted of: proteose peptone 20 g, sodium deoxycholate 0.75 g, lactose 10 g, neutral red 0.03 g, crystal violet 0.001 g, agar 13.5 g, distilled water 1000 ml. The pH of the agar adjusted to 7.1. For plating, a 6-ml aliquot of homogenate was mixed with 54 ml of prewarmed (45°C) saline which in turn was added to 60 ml of double-strength tempered (45°C) agar. After mixing, the contents were poured into eight petri plates and incubated in air at 44.5 ± 0.5°C for 24 h.

Each counted colony from the agar plates was subcultured into lactose broth. After 24 h of incubation at 35°C, the cultures were streaked on Eosin Methylene Blue (EMB) agar. Typical *Escherichia coli* or coliform colonies from the EMB agar were purified on nutrient agar (NA) and subsequently they were typed by the IMVIC procedure.

The fecal coliform and *E. coli* counts were calculated for each sample. The log mean of replicate analyses for each sample was obtained and the results from the MPN and ETPC procedures were correlated with the least sum of squares method.

To estimate the effect of temperature shock of ETPC procedure, suspensions of typical *E. coli* (++- -) and *Enterobacter aerogenes* (- +++) (able to ferment lactose at 44.5°C) were enumerated on nutrient agar plates at 35°C and by the ETPC technique described. The mean and the confidence limits of the mean estimates at P 0.05 were calculated for the purposes of comparison. The results are described in Table 1.

**RESULTS AND DISCUSSION**

The regression correlation of the paired log means of fecal coliform density estimates for 35 samples of soft shell clams by the MPN and ETPC procedures is shown in Fig. 1. The percent correlation is significant at p < 0.05. The standard error, however, is rather high. The mean fecal coliform estimate of samples by the ETPC is 6% lower than the mean of the MPN procedure.

Figure 2 illustrates the regression correlation between the paired log means of MPN and ETPC estimates of *E. coli* densities from the 35 soft shell clam samples. The
percent correlation between the means is significant at p < 0.05, the standard error about the regression line is high. The mean of ETPC E. coli counts is about 10% lower than the mean counts of E. coli obtained by the MPN procedure. The mean of MPN E. coli estimates is 17% lower than the mean of MPN fecal coliform estimate. The mean of ETPC E. coli estimate is 19% lower than the mean of ETPC fecal coliform results.

Table 1 describes the estimate of cell densities of E. coli (+ + -) and E. aerogenes (- - +) in saline suspensions by the ETPC and standard plate count (surface inoculation) technique. Table 2 illustrates the IMVIC types of isolated cultures.

The results from Table 1 and Fig. 1 and 2 indicate that the heat shock and the ensuing selective growth environment employed by the ETPC procedure leads to an underestimation of the actual density of fecal coliforms in samples. This underestimation on the basis of these results appears to be about 10-20%. Thus use of the ETPC procedure for assessment of sanitary quality of soft shell clams would result in applying 10-20% lower acceptance criteria. This could be rectified however, by multiplying the ETPC value by a factor of 1.2. Considering the speed of the ETPC technique, this option would be quite attractive. An MPN procedure employing LST or even EC broth at 44.5°C incubation without pre-enrichment would probably give a better estimate of fecal coliforms than ETPC method. This alternative procedure, however, should first be thoroughly evaluated on soft shell clam samples and its comparability to the standard procedures established.

REFERENCES