Rapid Method for Detection and Enumeration of Fecal Coliforms in Fresh Chicken

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A rapid method for enumerating fecal coliforms in foods was developed employing an agar pour-plate medium. After 7 h of incubation at 41.5 ± 0.05°C, this medium effectively allows the growth of fecal coliforms only. This rapid method was compared with the Association of Official Analytical Chemists multiple-tube dilution method for Escherichia coli, by using 21 samples of fresh, cut-up chicken and a surface rinsing procedure for sample preparation. Verification of picked colonies was carried out in EC broth using parallel incubation temperatures of 45.5 and 44.5 ± 0.05°C. Verifications for these temperatures averaged 79 and 98%, respectively. All positively verified isolates were E. coli types I and II, as were the negatives. Geometric means for the verified 7-h plate count were within 12% of the standard means for both EC broth incubation temperatures.

Various methods that have accelerated bacteriological assessment of the sanitary quality of foods have been described (7, 17). However, the Association of Official Analytical Chemists (A.O.A.C.) multiple-tube dilution method for Escherichia coli (6) has remained the accepted method for judging quality of many foods. Several attempts have been made to improve this laborious and time-consuming procedure (1, 5, 11), but thus far no really rapid method has been devised.

The fecal coliform group of bacteria, comprised primarily of E. coli type I (13), defined according to criteria listed in Standard Methods for the Examination of Water and Wastewater (3) as those bacteria that produce gas in EC medium when incubated at 44.5°C for 24 h.

The use of the fecal coliform group as an index of the sanitary quality of water stems from their identification as indicators of fecal contamination by man and other warm-blooded animals (13) and from the correlation of their densities with the presence of pathogens such as salmonellae (14). Geldreich et al. (12) developed a 24-h membrane filter procedure to quantify fecal coliforms in water supplies. Several other investigators have modified this technique, bringing about a significant decrease in the incubation time required for a confirmed count (15, 16; D. J. VanDonsel, R. M. Twedt, and E. E. Geldreich, Bacteriol. Proc., p. 25, 1969).

The purpose of this study was to modify the rapid water fecal coliform procedure for use with foods and to evaluate it by comparing it with the A.O.A.C. standard method.

MATERIALS AND METHODS

Medium. The modified medium was formulated after intensive preliminary studies by using pure cultures of fecal and nonfecal coliforms suspended in buffer and in various food products. Ingredients and techniques were adjusted to give maximum recovery of fecal strains and still screen out nonfecals. The final formulation of the medium is as follows: proteose peptone no. 3, 5.0 g; yeast extract, 3.0 g; lactose, 10.0 g; NaCl, 7.5 g; sodium lauryl sulfate, 0.06 g; bromothymol blue, 0.3 g; agar, 15.0 g; glass-redistilled water, 1,000.0 ml.

In preliminary studies, the use of glass-redistilled water was shown to give more consistent results. The sodium lauryl sulfate concentration is moderately inhibitory to noncoliforms, but selectivity is primarily effected by the incubation time-temperature combination. To insure complete dissolution, bromothymol blue is dissolved separately in a portion of the measured quantity of water which is then added to the rest of the ingredients in the solution. The pH is adjusted to 7.3 before the addition of agar. The medium is heated to boiling to dissolve the agar, dispensed into sterile bottles, and stored at 4°C, with a shelf life of at least 1 month. Enumeration is carried out in standard pour plates that are overlaid with the medium, heat-sealed in plastic pouches, and incubated submerged in a water bath for 7 h at 41.5 ± 0.05°C. After incubation, the plates are counted on a

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Quebec colony counter, with fecal coliform colonies appearing yellow to orange, with yellow haloes against the bluish-green background color of the medium.

**Methods of sample preparation.** After surveying various foods, we chose fresh, cut-up chicken as a representative food for evaluating our method, since fresh chicken containing suitable levels of fecal coliforms could be obtained consistently. For sample preparation, we modified the rinsing procedure recommended by the American Public Health Association (A.P.H.A.) (2). Our procedure consisted of rinsing the chicken part in a 1-gallon plastic food storage bag with Butterfield's A.P.H.A. sterile phosphate buffered diluent (4), and subsequently enumerating the fecal coli rinsed off the surface of the sample.

A recovery efficiency study was performed to evaluate this bag-rinsing method. Packages of six drumsticks served as single samples; five were surface-inoculated with a known level of an EC broth positive strain of type I *E. coli* recently isolated from fresh ground beef. The sixth drumstick was left uninoculated to serve as a base-level control. The inoculated pieces were rinsed with five ratios of rinse volume to sample weight: 2:1, 1:1, 1:1.5, 1:2, and 1:4. Since the results of the analysis showed recoveries for all ratios to be > 90%, the ratio of 1:4 was chosen because it produced the greatest concentration of organisms and thus the maximum sensitivity for our method.

**Comparative evaluation study.** Twenty-one samples of fresh, cut-up chicken were enumerated for fecal coliforms by the rapid fecal coliform procedure and the A.O.A.C. standard method. The evaluation procedure, which enumerated fecal coliforms and *E. coli* by both methods, was carried out as outlined in Fig. 1.

Rinse from each sample prepared as described above was used for both plating and most probable number (MPN) tube inoculation. Plating was done in duplicate, by using $10^3$, $10^4$, and $10^5$ dilutions. All 10-fold dilutions were made by pipetting 11 ml into 99-ml dilution blanks. A 5-tube MPN series was set up by pipetting five 1-ml portions of each dilution into each of five tubes containing 9 ml each of lauryl sulfate tryptose (LST) broth. Dilutions made were $10^3$ through $10^{-4}$. Counts are expressed per milliliter of rinse.

Representative numbers of colonies were picked from 7-h plates into LST broth. All colonies were picked when the plate count was 10 or below; 10 colonies were picked from plates at a count greater than 10. Positive LST tubes, in addition to presumptive positives from the standard method MPN series, were confirmed in brilliant green lactose bile broth and EC broth. Since preliminary work had shown that the EC broth incubation temperature of 45.5 C failed to verify about 20% of the *E. coli* found in chicken, it was decided to include a parallel EC broth incubation temperature of 44.5 C for both the rapid and standard procedures.

All positive EC tubes from both methods and also EC negatives from colonies picked from 7-h plates were subjected to the standard completed test.

**RESULTS**

The results of this study are shown in Tables 1 to 3. Table 1 shows counts for 21 individual samples of fresh chicken for both the rapid and standard procedures with verification in EC broth at both 44.5 and 45.5 C. A paired *t* test was computed to compare methods, with the first value of the rapid method duplicate counts being used for this purpose. Significant difference between methods could not be demonstrated at $\alpha = 0.01$ for either temperature (Table 2). The geometric mean difference between our method and the A.O.A.C. standard method, by using first value means, was 11.5% at 44.5 C verification and 5.2% at 45.5 C. By using the means for duplicate counts, they were 12.1 and 9.8%, respectively, for the two temperatures.

Log variance for the rapid method duplicates was 0.00224 for 44.5 C and 0.00896 for 45.5 C. Overall percentage of verification of colonies picked from 7-h plates was 98% at the 44.5 C EC broth incubation temperature and 79% at 45.5 C (Table 2). At 44.5 C, 97% of the EC positives were *E. coli* type I, and 3% were type II; all of the EC negatives were type I. At 45.5 C, 98% of the EC positives were *E. coli* type I, and 2% were type II; 91% of the EC negatives were type I, and 9% were type II (Table 3).
Table 1. Comparison of the rapid fecal coliform method and the A.O.A.C. standard procedure with verification in EC broth at 44.5 and 45.5 C for 21 samples of fresh chicken

| Sample | Rapid method avg. count verified at: | A.O.A.C. MPN count verified at: |
|--------|-------------------------------------|----------------------------------|
|        | 44.5 C | 45.5 C | 44.5 C | 45.5 C |
| 1      | 18     | 18     | 11     | 11    |
| 2      | 50     | 46     | 33     | 33    |
| 3      | 105    | 63     | 109    | 109   |
| 4      | 65     | 36     | 22     | 11    |
| 5      | 34     | 26     | 24     | 24    |
| 6      | 94     | 84     | 109    | 109   |
| 7      | 6      | 6      | 5      | 5     |
| 8      | 20     | 20     | 49     | 49    |
| 9      | 18     | 10     | 24     | 24    |
| 10     | 36     | 26     | 80     | 50    |
| 11     | 14     | 12     | 22     | 22    |
| 12     | 9      | 6      | 7      | 5     |
| 13     | 28     | 19     | 50     | 50    |
| 14     | 18     | 16     | 23     | 13    |
| 15     | 30     | 30     | 79     | 14    |
| 16     | 20     | 19     | 13     | 13    |
| 17     | 6      | 6      | 13     | 3     |
| 18     | 14     | 10     | 13     | 2     |
| 19     | 32     | 20     | 33     | 23    |
| 20     | 146    | 146    | 172    | 79    |
| 21     | 36     | 29     | 79     | 22    |

* Counts are per milliliter of rinse.

Table 2. Comparison of the rapid fecal coliform method and the A.O.A.C. standard procedure

| Source          | EC broth verification temp |
|-----------------|---------------------------|
|                 | 44.5 C | 45.5 C |
| Geometric mean: |        |        |
| First value of rapid method | 27.0   | 20.3   |
| A.O.A.C. MPN     | 30.5   | 19.3   |
| Paired t result  | -1.07* | 0.34*  |
| Critical difference | 38%   | 49%    |
| Geometric mean of rapid method duplicate counts | 26.8  | 21.2   |
| Log variance of rapid method duplicates | 0.00224 | 0.00896 |

* Not significant at α = 0.01.

DISCUSSION

When n = 21, the paired t test could only demonstrate significance of a difference between the rapid and standard methods greater than 40 to 50%. This result stems primarily from the great variability inherent in the MPN procedure, despite the fact that we made use of the five-tube technique rather than the usual three-tube. More than 200 samples would be required to detect a difference of 10%, and this effort is patently not feasible.

However, given the nature of the MPN procedures, a 50% difference between methods was not considered unacceptable. Furthermore, since the geometric means of the two methods were no more than 12% apart, we felt that our method performed very well in comparison with the standard procedure.

The log variance of 0.00224 for the 7-h count verified at 44.5 C was good. At 45.5 C, however, with a 19% drop in verification, the log variance increased to an unacceptably high value of 0.00896 (0.005 is considered an acceptable upper limit) (8).

Preliminary studies employing the EC broth incubation temperature of 45.5 C specified by A.O.A.C. resulted in a verification of picked colonies averaging only about 80%. Nevertheless, all the EC-negative picked colonies proved to be E. coli types I and II. Consequently, in an effort to improve our percentage of verification and possibly demonstrate that 45.5 C is too high a temperature for fecal coliform verification, we included parallel EC broth verification at 44.5 C.

Our data show the improvement effected by the 1 C reduction in EC broth incubation temperature—both a 19% increase in verification for the rapid method, with an improved log variance, and a substantial increase in the verified standard MPN count. Fishbein (9, 10), working with pure cultures of E. coli and Aerobacter strains and also with frozen foods and nutmeats, concluded that 44.5 C was too low a temperature for EC broth incubation. Many Aerobacter strains and a significant number of non-E. coli strains isolated from the food products studied were verified as positive. To screen out these interfering organisms, an incu-

Table 3. Verification in EC broth at both 44.5 and 45.5 C of 420 representative colonies picked from 7-h fecal coliform agar plates

| Item                              | EC broth incubation temp |
|-----------------------------------|--------------------------|
|                                  | 44.5 C | 45.5 C |
| No. of EC broth positives         | 410    | 331    |
| Type I                            | 398    | 325    |
| Type II                           | 12     | 6      |
| No. of EC broth negatives         | 10     | 89     |
| Type I                            | 10     | 82     |
| Type II                           | 7      |        |
| Percent verification              | 97.6   | 78.8   |
bation temperature of 45.5 C was recommended. From our experience with fresh chicken, however, no organisms other than E. coli types I and II were encountered; thus, we feel that, with this product at least, 44.5 C is the preferred temperature for EC broth incubation.

Some difficulties were encountered in counting 7-h method plates. These arose primarily when dealing with the 10^1 dilution. The pH of the rinse was found to vary from 6.0 to 6.9; samples having a rinse pH in the low end of the range caused an overall yellowing of the medium color. Such loss of medium contrast against yellowish fecal coliform colonies sometimes made these colonies hard to see. Also, debris in the rinse could either obscure fecal coliform colonies or be confused with them. Experience in counting helped to overcome these difficulties.

We feel that the value and potential of this rapid method has been well demonstrated. It has value as a rapid screening procedure for the detection and enumeration of organisms indicative of fecal contamination of fresh chicken, and it has potential application to other foods. This method has been evaluated with other food products including fresh frozen and dried foods as well as dairy products. Data obtained have provided us with confidence that the procedure can be useful for a variety of foods. Continuing studies in this laboratory are directed toward evaluating the wider applicability of the rapid method for enumeration of total, as well as fecal, coliforms.

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