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# Comparative Evaluation of Modified m-FC and m-TEC Media for Membrane Filter Enumeration of *Escherichia coli* in Water

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**Two media used to detect fecal coliforms in water by membrane filtration, m-FC and m-TEC, were modified and supplemented with the chromogenic substrate 5-bromo-6-chloro-3-indoyl-β-D-glucuronide (BCIG) and were compared for quantitative recovery of *Escherichia coli*. Student's *t* test of data from 181 water samples of sewage, rivers, lakes, and wells did not demonstrate any statistically significant differences ($P = 0.05$) in the enumeration of *E. coli* with these media. Target colonies were confirmed to be *E. coli* at rates of 98.6 and 97.3% by using FC-BCIG and TEC-BCIG media, respectively. Glucuronidase-negative isolates of *E. coli* were encountered at the same frequency (6.0%) on both media. This collaborative study demonstrated that either modified basal medium could be used successfully for detection of *E. coli* in various nontreated waters within 24 h.**

Testing for evidence of fecal contamination in water has been traditionally accomplished by detection or enumeration of fecal coliforms (1). Unfortunately, the specificity of fecal coliforms to indicate fecal pollution can vary considerably, depending on environmental conditions and the presence of industrial effluent (1). The presence of *Escherichia coli* in water is nearly always associated with recent fecal pollution and is the preferred indicator organism for this purpose (1). Conventional procedures for verification of fecal coliforms as *E. coli* when fecal contamination is questionable are very laborious and time-consuming. Recent developments in substrate technology have simplified the direct detection of *E. coli* within a 24-h period. These methods are based on detecting β-glucuronidase activity by using various substrates incorporated into microbiological media. The fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) has been used successfully for membrane filter enumeration of *E. coli* (3, 4, 10). This method requires a UV lamp for detecting fluorescence as a result of hydrolysis of the MUG substrate. Interpretation of fluorescent colonies on solid media containing MUG can be difficult because of diffusion of fluorescence around the colony and background fluorescence of membrane filters (3, 12). Alternately, the addition of the chromogenic substrates indoxyl-β-D-glucuronide and 5-bromo-6-choro-3-indolyl-β-D-glucuronide (BCIG) into microbiological media results in β-glucuronidase-positive organisms producing visible blue colonies (5, 7, 8, 11). A desirable feature of these substrates is the formation of an insoluble indigo blue complex within the colony as a result of β-glucuronidase activity which does not diffuse into the surrounding media. At the time of this study, incorporation of BCIG into existing fecal coliform media for enumeration of *E. coli* in untreated water had not yet been extensively evaluated. Two media which are widely used for enumeration of fecal coliforms in water by membrane filtration are m-FC and m-TEC media (2, 6). The present paper describes a collaborative study involving provincial government and municipal laboratories for evaluating modified m-FC and m-TEC media supplemented with BCIG for detection of *E. coli* in various nontreated waters throughout Ontario, Canada.

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## MATERIALS AND METHODS

**Medium preparation.** FC basal medium contained the following ingredients (grams per liter): tryptose (10.0), Proteose Peptone no. 3 (5.0), yeast extract (3.0), sodium chloride (5.0), bile salts no. 3 (1.5 g), and agar (15.0). For the purposes of this study, Difco Laboratories prepared one custom batch of this medium as dehydrated powder which was distributed among the laboratories involved in the collaborative study. FC-BCIG medium was prepared by suspending 39.5 g of dehydrated FC basal medium (Difco Laboratories) and 0.1 g of BCIG (Diagnostic Chemicals Ltd.) in 1.0 liter of purified water, and then the suspension was heated to boiling to dissolve completely. The final pH of the medium was 7.4 ± 0.2 at 25°C. The medium was dispensed into petri dishes (50-mm diameter; 10 ml per plate), allowed to harden, sealed in plastic containers, and stored at 4.0°C. Prepared FC-BCIG medium was used within 2 weeks.

TEC basal medium contained the following ingredients (in grams per liter): Proteose Peptone no. 3 (5.0), yeast extract (3.0), lactose, (1.0), sodium chloride (7.5), potassium phosphate, mono-basic (1.0), potassium phosphate, dibasic (3.3), sodium lauryl sulfate (0.2), sodium deoxycholate (0.1), and agar (15.0). A single batch of this dehydrated medium was specially prepared by Quelab Laboratories, Montreal, Canada, and distributed among the laboratories involved in the collaborative study. TEC BCIG medium was prepared by suspending 36.0 g of TEC basal medium and 0.1 g of BCIG (Diagnostic Chemicals Ltd.) in 1.0 liter of purified water. By being heated gently with frequent agitation, the mixture was boiled for 1.0 min and then sterilized for 15 min at 121°C. The final pH of the medium was 7.2 ± 0.2 at 25°C. This medium was dispensed and stored in the same manner as the FC-BCIG medium.

**Samples.** The laboratories participating in this study were strategically selected to provide reasonable geographical representation in the province of Ontario. Water samples were collected from four different environmental sources, which included sewage, river, lake, and well water (Table 1). Selection of samples to be tested was based on historical information indicating sites which had been previously contaminated with *E. coli*. Each laboratory was instructed to test as many different locations as possible. Water samples were held at 4°C and tested within 24 h of sample collection. This approach was taken to maximize obtaining quantitative comparisons of *E. coli* colony counts on FC-BCIG and TEC-BCIG media from a wide variety of samples.

TABLE 1. Confirmation of colony types on FC-BCIG and TEC-BCIG media

| Water type | No. of samples | No. of colonies tested | Colonies biochemically confirmed as *E. coli* (%) | | | |
|---|---|---|---|---|---|---|
| | | | FC-BCIG | | TEC-BCIG | |
| | | | Target[a] | Nontarget[b] | Target[a] | Nontarget[b] |
| Sewage | 40 | 200 | 97.9 | 5.0 | 97.4 | 4.0 |
| River | 43 | 215 | 99.5 | 9.8 | 95.3 | 9.3 |
| Lake | 70 | 300 | 97.7 | 7.8 | 98.3 | 8.8 |
| Well | 28 | 100 | 98.6 | 2.3 | 98.1 | 3.6 |
| Total | 181 | 815 | Mean 98.4 | 6.2 | 97.3 | 6.4 |

[a] Blue colonies.
[b] Nonblue colonies.

**Membrane filtration procedure.** Selection of the volume of water to be tested varied with the sample source in order to obtain as many countable *E. coli* CFU on both media with the same volume. Each sample was filtered in duplicate by using 4.5-cm-diameter, 0.45-μm-pore-size, gridded, cellulose-nitrate membrane filters. One filter from each pair was transferred to the surfaces of FC-BCIG and TEC-BCIG plates, respectively, and the plates were incubated at 44.5 ± 0.2°C for a period of 20 ± 2 h. The preincubation period of 2 h at 35°C which was used in the original procedure with m-TEC agar was not used with either medium. A binocular dissecting microscope (magnification, ×10 to ×15) was used to count blue colonies as presumptive *E. coli* on both media. Background counts of nonblue colonies were noted on selected samples from different water sources as a means of subjectively determining differences in selectivity between FC-BCIG and TEC-BCIG media.

**Colony verification and identification.** As many as five presumptive *E. coli* (blue colonies) and five non-*E. coli* (nonblue colonies) colonies were randomly selected from membrane filters from each medium per sample. Each selected colony was subcultured onto nutrient agar containing 100 μg of MUG per ml, and the cultures were incubated at 35°C for 18 to 20 h. Growth on the nutrient-MUG plates was examined for blue-white fluorescence under long-wavelength (366-nm) UV light to detect β-glucuronidase activity (9). All isolates were tested for oxidase and indole production according to the *Standard Methods for the Examination of Water and Wastewater* (1). Oxidase-negative isolates which were positive for β-glucuronidase and indole were considered *E. coli* and required no further biochemical confirmation. Oxidase-negative isolates which were positive for only β-glucuronidase or indole production were identified with the API20E system (Analtab Products, Plainsview, N.Y.) or by conventional biochemical tests. Isolates which were negative for both β-glucuronidase and indole production or which were oxidase positive were not considered *E. coli* and were discarded.

**Statistical analysis.** *E. coli* colony counts from both media were converted to log 10 values for statistical analysis. A correlation coefficient ($r$) and a paired $t$ test of the transformed data were performed to determine whether *E. coli* counts from FC-BCIG and TEC-BCIG differed significantly.

## RESULTS AND DISCUSSION

A scatterplot of the data comparing FC-BCIG and TEC-BCIG media for enumeration of *E. coli* in water samples is shown in Fig. 1. The graph depicts a strong positive linear correlation between the two enumeration methods with a correlation coefficient ($r$) of 0.89. *E. coli* counts of fewer than 100/100 ml (log 2.0) demonstrate a much greater degree of scattering of values about the theoretical line of equivalence. This phenomenon can be attributed to the lack of precision often associated with low bacterial counts on membrane filters (1). Statistically, there was no significant difference between *E. coli* results obtained between the two media by the paired $t$ test ($P = 0.05$). Comparative results from 181 water samples of sewage, rivers, lakes, and wells are outlined in Table 1. The mean confirmation rates of target blue colonies for *E. coli* were 98.4 and 97.3% for FC-BCIG and TEC-BCIG media, respectively. The similar confirmation rates indicate the high specificities of both media for identification of blue colonies as *E. coli*. Other investigators have shown a good correlation between β-glucuronidase detection and *E. coli*. Feng and Hartman (4) found that 97% of *E. coli* strains exhibited β-glucuronidase when lauryl tryptose broth (LTB)-MUG broth was used. Sarhan and Foster (10) reported that 96.3% of fluorescent, β-glucuronidase-positive colonies on MUG-7 medium were confirmed to be *E. coli* by using a membrane filter technique. Our investigation suggests that when BCIG is added to either FC or TEC basal medium, the results are comparable to those from studies involving MUG-based media for detection of the β-glucuronidase activity of *E. coli*. The advantage of using media containing BCIG is that detection of β-glucuronidase can be determined by direct examination for visible blue colonies. By comparison, MUG-based media require the use of UV light (365 mm) to determine fluorescence indicating β-glucuronidase activity.

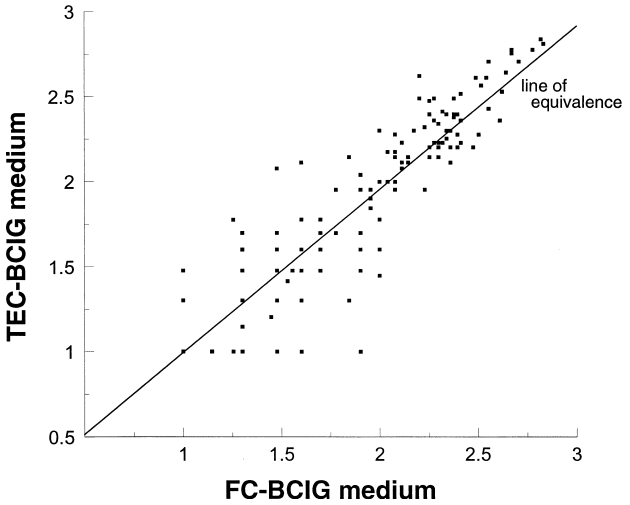


FIG. 1. Scatterplot of *E. coli* counts (log 10) per 100 ml.

The blue β-glucuronidase-positive colonies which were not biochemically confirmed to be *E. coli* are listed in Table 2. The predominant organisms which contributed to false-positive results were *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Aeromonas hydrophila*. The incidence of β-glucuronidase activity in *K. pneumoniae*, and *C. freundii* strains has been previously reported (7); however, the presence of β-glucuronidase activity in thermotolerant strains of *A. hydrophila* growing at 44.5°C has not been documented in the literature to our knowledge. Overall, the low false-positive rates of 1.6% (FC-BCIG) and 2.7% (TEC-BCIG) demonstrate that the presence of the organisms listed in Table 2 in these water sources has a negligible impact on the specificities of these media.

The β-glucuronidase-negative colonies which were biochemically confirmed to be *E. coli* occurred at almost identical rates of 6.2 and 6.4% for FC-BCIG and TEC-BCIG media, respec-

TABLE 2. Identification of target (non-*E. coli*) colonies

| Organism | No. of isolates |
|---|---|
| *Klebsiella pneumoniae* | 17 |
| *Citrobacter freundii* | 17 |
| *Aeromonas hydrophila* | 15 |
| *Enterobacter cloacae* | 3 |
| *Klebsiella pneumoniae* | 2 |
| *Enterobacter sakazakii* | 1 |
| *Enterobacter agglomerans* | 1 |
| Total | 56 |

tively. In all instances, the lack of development of blue colonies among the *E. coli* colonies growing on the BCIG-containing media occurred as a result of their inability to express β-glucuronidase and was not due to inhibitory properties of the selective media. This observation was confirmed by a lack of β-glucuronidase activity after subculturing to the nonselective medium, nutrient-MUG agar (9). Slightly higher incidences of β-glucuronidase-negative *E. coli* isolates occurred with river (9.8 and 9.3%) and lake (7.8 and 8.8%) samples with both FC-BCIG and TEC-BCIG, respectively. Overall, the incidences of β-glucuronidase-negative *E. coli* colonies recovered by both FC-BCIG (6.2%) and TEC-BCIG (6.4%) media are very similar to that found by other investigators with the MUG substrate. Feng and Hartman (4) determined that 4.0% of *E. coli* strains did not exhibit β-glucuronidase with MUG in LTB broth, while Sarhan and Foster (10) identified a false-negative rate of 6.1% for *E. coli* growing on MUG-7 medium.

The close similarities between the performances of FC-BCIG and TEC-BCIG media can be partially attributed to the use of the same substrate (BCIG) to detect β-glucuronidase activity. Although each medium uses different selective agents, there were no significant differences observed in levels of background growth between the two media among the different types of water samples tested. Characteristically, *E. coli* produces slightly larger dark-blue colonies (1.5- to 2.0-mm diameter) on FC-BCIG medium; however, this difference did not prove to be an advantage or disadvantage in counting colonies. Counting *E. coli* colonies can be accomplished easily on either medium because of the distinct blue of *E. coli* colonies in contrast to the white-tan growth of nontarget colonies.

Both FC and TEC basal media were modifications of m-FC and m-TEC media, respectfully. Aniline blue was excluded from FC basal medium, while bromocresol purple and bromophenol red were omitted from TEC basal medium. These previously mentioned dye indicators were removed to prevent color interferences occurring in the development of blue colonies as a result of β-glucuronidase hydrolyzing the BCIG substrate. In addition, lactose was omitted from FC basal medium and reduced to 0.1% in TEC basal medium to avoid potential hindrance of β-glucuronidase expression due to production of acid from lactose fermentation (3, 10). The small concentration of lactose in TEC basal medium serves as an energy source and inducer of β-galactosidase for further biochemical identification when required. The slight difference in lactose concentration did not have any significant impact on recovery levels and confirmation rates for *E. coli* between FC-BCIG and TEC-BCIG media.

Overall, FC-BCIG and TEC-BCIG media were equivalent in performance characteristics. However, FC-BCIG medium is slightly more convenient to prepare since it requires only boiling compared with the requirement of autoclaving by TEC-BCIG medium. Currently, arrangements are being made with Difco Laboratories (Detroit, Mich.) to produce FC basal medium commercially. Storage life studies conducted in our laboratory have shown that prepared FC-BCIG will remain stable for a period of as long as 6 weeks when it is stored at 4°C in closed plastic containers. We recommend using the FC-BCIG medium within a 4-week period to allow for slight differences in storage conditions among laboratories.

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## ADDENDUM

Since this study, 12 Ministry of Health Laboratories have adopted use of FC-BCIG medium for determining *E. coli* levels in recreational and nontreated drinking waters. Our experience with FC-BCIG medium has been very positive, and we have not encountered any problems with the performance of this medium. We have no hesitation in recommending the use of FC-BCIG medium whenever enumeration of *E. coli* in untreated water is required as an alternative to MUG-based membrane filtration methods.

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