Comparing Defined-Substrate Coliform Tests for the Detection of Escherichia coli in Water

Terry C. Covert, Eugene W. Rice, Scott A. Johnson, Donald Berman, Clifford H. Johnson, and Paralee J. Mason

Two commercially available defined-substrate coliform tests were compared with EC medium plus 4-methylumbelliferyl-β-D-glucuronide (MUG) for detecting chlorine-exposed Escherichia coli in spiked water samples. Statistical analyses of the test results indicated no significant differences in detection of E. coli between the Autoanalysis Colilert test and EC medium with MUG. There were, however, significant differences in detection of E. coli between the ColiQuik test and EC medium with MUG in the free-chlorine-exposed pure culture studies and when all the data were combined. All methods were capable of detecting 1 cfu/100 mL of E. coli.

The total coliform group of organisms is the principal indicator used to assess the microbiological quality of drinking water. The sanitary significance of coliform organisms and the characteristics of their culture have been studied extensively.1-5 The presence of any member of the coliform group in treated water suggests either contamination after disinfection or inadequate treatment. Members of the coliform group are considered a reliable indicator of the adequacy of treatment, but their presence does not necessarily indicate fecal contamination or pathogen occurrence. This shortcoming may be due partly to poor detection of stressed coliforms and to interference by heterotrophs.6-11 Coliforms, fecal coliforms, and Escherichia coli are all used as indicators of fecal pollution. Among these, E. coli is often preferred as an indicator because it indicates recent fecal contamination and the possibility of enteric pathogens because enteric pathogens often coexist with fecal coliforms or E. coli. The presence of E. coli is indicative of fecal contamination. The other members of the coliform group (Klebsiella, Citrobacter, Enterobacter) may be isolated in feces, but their presence does not always suggest fecal contamination.

The presence of any member of the coliform group in treated water suggests either contamination after disinfection or inadequate treatment.

The US Environmental Protection Agency (USEPA) recently amended the National Primary Drinking Water Regulations (NPDWRs) published June 1, 1990,12 incorporating the maximum contaminant level (MCL), monitoring requirements, and analytical requirements for total coliform bacteria, including fecal coliforms and E. coli. The USEPA also promulgated an MCL goal of zero for total coliforms, including fecal coliforms and E. coli. The total coliform group remains the primary bacterial indicator. However, for each total coliform-positive sample, a fecal coliform or E. coli analysis must be performed. The NPDWRs published June 1, 1990,12 proposed three analytical methods based on β-glucuronidase (GUR) activity for detecting E. coli in drinking water. One of these methods was the minimal medium o-nitrophenyl-β-D-galactopyranoside-4-ethylumbelliferyl β-D glucuronide (MMO-MUG) or AC* test previously approved for detecting total coliforms in the revised total coliform rule published June 29, 1989.

The USEPA approved two of the methods previously proposed for E. coli detection in the NPDWRs of Jan. 8, 1991,14 but deferred approval of the MMO-MUG test because of concerns about its ability to detect low densities of injured E. coli.

Several studies have shown that the AC test is comparable to the Standard Methods total coliform membrane filter (MF) test, multiple tube fermentation (MTF) test and presence-absence (P-A) coliform test in detecting total coliforms.14-18 However, there have been only limited studies evaluating the AC test and other similar MUG-based test procedures, e.g., CK,4 for detecting E. coli in disinfected distribution water. Thus far two commercially available o-nitrophenyl-β-D-galactopyranoside (ONPG)-MUG formulations—AC and CK—appear to be the most prevalent in the marketplace; however, others are rapidly being developed. Both test sys-
The use of GUR activity to identify E. coli
lauryl tryptose broth with MUG (LTB-
held for 24 h and for five days after disin-
Aliquots from six of the samples were
Bulow.' The association between GUR
was first described by Kilian and
species in a variety of environmental,
and E. coli has been used to identify this
MUG*)23 for detection of E. coli.

Materials and methods

Samples. A total of 33 samples from 27
sources was analyzed (Tables 1 and 2). All
samples from six of the samples were
held for 24 h and for five days after disinfec-
tion. The samples were analyzed for E. coli
injury prior to disinfection. Because of dif-
ficulties in locating a sufficient number of
disinfection there were sufficient E. coli
cells to detect. The samples of public
drinking water supply systems analyzed in
this study were E. coli–positive, i.e., no
spiking was required.

The thermotolerant (EC-positive),
MUG-positive E. coli used for spiking
were isolated from the environment and
identified. The E. coli were inoculated
into heart infusion broth* and incubated
for 24 h at 5°C. The culture was washed
twice with OFTDW to remove nu-
trients from the cells. The cells were re-
suspended with OFTDW and held for 48
h at 20°C to simulate low-nutrient stress.
The suspension was further diluted in
OFTDW prior to disinfection.

Feces samples were used as a high
density E. coli source by blending ap-
proximately 1 g of feces with 200 mL of
OFTDW in a sterile blender for 1 min at
high speed. The feces suspension was
further diluted with OFTDW and was
then filtered through sterile filters† to
remove large particles, lower the turbid-
ity, and lessen the chlorine demand. The
suspension was stored at 5°C for 24 h
prior to disinfection.

Primary effluents were collected from
waste treatment plants that receive pri-
priarily domestic influent. Samples were
* Difco Laboratories, Detroit, Mich.
† Whatman No. 40, Maidstone, U.K.
‡ Analytab Products, Plainview, N.Y.
§ Standard Methods, 19th ed. (1992), APHA.
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TABLE 2
Comparison of EC-MUG, AC, and CK for detecting E. coli—natural sample studies

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Source</th>
<th>Disinfectant</th>
<th>Log Reduction</th>
<th>Holding Time</th>
<th>Dilution</th>
<th>EC-MUG</th>
<th>AC</th>
<th>CK</th>
<th>MPN* E. coli/100 mL</th>
<th>MPN* E. coli/tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Source water</td>
<td>Free chlorine 0.30 mg/L</td>
<td>1.2</td>
<td>0</td>
<td>10 mL</td>
<td>10/10</td>
<td>10/10</td>
<td>9/10</td>
<td>&gt;23</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>10</td>
<td>Feces</td>
<td>Free chlorine 0.32 mg/L</td>
<td>3.7</td>
<td>0</td>
<td>1 mL</td>
<td>1/10</td>
<td>3/10</td>
<td>0/10</td>
<td>10</td>
<td>0.11</td>
</tr>
<tr>
<td>11</td>
<td>Feces</td>
<td>Free chlorine 0.40 mg/L</td>
<td>4.1</td>
<td>24 h</td>
<td>10 mL</td>
<td>2/10</td>
<td>1/10</td>
<td>0/10</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>12</td>
<td>Effluent</td>
<td>Monochloramine 2.5 mg/L</td>
<td>5.1</td>
<td>5 days</td>
<td>10 mL</td>
<td>4/10</td>
<td>3/10</td>
<td>4/10</td>
<td>5.1</td>
<td>0.51</td>
</tr>
<tr>
<td>13</td>
<td>Effluent</td>
<td>Monochloramine 3.1 mg/L</td>
<td>5.4</td>
<td>5 days</td>
<td>10 mL</td>
<td>9/10</td>
<td>10/10</td>
<td>4/10</td>
<td>23</td>
<td>2.3</td>
</tr>
<tr>
<td>14</td>
<td>Drinking water</td>
<td>No disinfectant</td>
<td></td>
<td>5 days</td>
<td>10 mL</td>
<td>1/10</td>
<td>1/10</td>
<td>0/10</td>
<td>1.1</td>
<td>0.11</td>
</tr>
<tr>
<td>15</td>
<td>Drinking water</td>
<td>No disinfectant</td>
<td></td>
<td>48 h</td>
<td>10 mL</td>
<td>10/10</td>
<td>10/10</td>
<td>5/10</td>
<td>&gt;23</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>16</td>
<td>Drinking water</td>
<td>No disinfectant</td>
<td></td>
<td>48 h</td>
<td>10 mL</td>
<td>10/10</td>
<td>10/10</td>
<td>8/10</td>
<td>&gt;23</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>17</td>
<td>Drinking water</td>
<td>No disinfectant</td>
<td></td>
<td>48 h</td>
<td>10 mL</td>
<td>10/10</td>
<td>8/10</td>
<td>7/10</td>
<td>&gt;23</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>18</td>
<td>Effluent</td>
<td>Monochloramine 2.5 mg/L</td>
<td>6.3</td>
<td>24 h</td>
<td>1 mL</td>
<td>4/10</td>
<td>7/10</td>
<td>0/10</td>
<td>0.61</td>
<td>0.61</td>
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<tr>
<td>19</td>
<td>Effluent</td>
<td>Monochloramine 2.5 mg/L</td>
<td>5.12</td>
<td>24 h</td>
<td>1 mL</td>
<td>5/10</td>
<td>1/10</td>
<td>0/10</td>
<td>69</td>
<td>0.7</td>
</tr>
<tr>
<td>20</td>
<td>Effluent</td>
<td>Monochloramine 3.0 mg/L</td>
<td>5.42</td>
<td>24 h</td>
<td>1 mL</td>
<td>2/10</td>
<td>3/10</td>
<td>1/10</td>
<td>22</td>
<td>0.22</td>
</tr>
<tr>
<td>21</td>
<td>Effluent</td>
<td>Monochloramine 2.7 mg/L</td>
<td>6.04</td>
<td>24 h</td>
<td>10 mL</td>
<td>0/10</td>
<td>4/10</td>
<td>2/10</td>
<td>&lt;1.1</td>
<td>&lt;0.11</td>
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<tr>
<td>22</td>
<td>Effluent</td>
<td>Monochloramine 2.50 mg/L</td>
<td>5.70</td>
<td>24 h</td>
<td>10 mL</td>
<td>4/10</td>
<td>3/10</td>
<td>0/10</td>
<td>5.1</td>
<td>0.5</td>
</tr>
<tr>
<td>23</td>
<td>Effluent</td>
<td>Monochloramine 2.67 mg/L</td>
<td>4.84</td>
<td>24 h</td>
<td>1 mL</td>
<td>3/10</td>
<td>4/10</td>
<td>1/10</td>
<td>30.0</td>
<td>0.3</td>
</tr>
<tr>
<td>24</td>
<td>Effluent</td>
<td>Monochloramine 2.00 mg/L</td>
<td>4.89</td>
<td>24 h</td>
<td>10 mL</td>
<td>9/10</td>
<td>9/10</td>
<td>4/10</td>
<td>23.0</td>
<td>2.3</td>
</tr>
<tr>
<td>25</td>
<td>Effluent</td>
<td>Monochloramine 2.81 mg/L</td>
<td>5.10</td>
<td>24 h</td>
<td>1 mL</td>
<td>7/10</td>
<td>7/10</td>
<td>7/10</td>
<td>129</td>
<td>1.2</td>
</tr>
<tr>
<td>26</td>
<td>Effluent</td>
<td>Monochloramine 2.76 mg/L</td>
<td>5.40</td>
<td>24 h</td>
<td>1 mL</td>
<td>1/10</td>
<td>8/10</td>
<td>7/10</td>
<td>110</td>
<td>1.1</td>
</tr>
<tr>
<td>27</td>
<td>Effluent</td>
<td>Monochloramine 2.36 mg/L</td>
<td>5.19</td>
<td>24 h</td>
<td>1 mL</td>
<td>10/10</td>
<td>10/10</td>
<td>8/10</td>
<td>&gt;230</td>
<td>&gt;2.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>114/220</td>
<td>126/220</td>
<td>80/220</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Based on EC-MUG
*Chlorine residual
†Contact time

In the first set of experiments, the level of inactivation was determined immediately at the end of the exposure time (samples 1–9, 9, and 10). Subsequently, the neutralized samples were held at a temperature of 5°C for 24 h and for five days prior to assay (samples 6–8, 11–13). These holding periods were done to ascertain the true titer present in the inactivated sample for purposes of later dilutions to low levels of E. coli and to determine the effect of holding in the absence of a disinfectant residual on the surviving E. coli population.

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**AC and CK coliform tests.** AC and CK tubes containing sufficient defined substrate for 10 mL of sample were prepared as a 10 tube MPN test. A sample (10, 1.0, or 0.1 mL) was added to each tube, and the powder was dissolved with agitation. To AC and CK tubes that received 1 or 0.1 mL of sample, 0.9 mL of 9.9 mL, respectively, of sterile water or buffer was added, consistent with the manufacturers' instructions.

The MPN AC and CK tubes were incubated at 35 ± 0.5°C for 24 ± 0.5 h. Each tube was exposed to a hand-held long-wavelength (366-nm) 6-W UV light.* Fluorescence indicated the presence of *E. coli* (MUG test). Doubtful MUG-positive tubes were incubated for an additional 4 h and were also compared with a color comparator to assess any degree of fluorescence so as to not underestimate the *E. coli* density. A positive control (*E. coli*) was included with each sample.

**LTB-MUG and EC-MUG tests.** The 10-tube MTI test was performed by adding 10, 1, or 0.1 mL of sample to LTB-MUG tubes. The tubes were incubated at 35 ± 0.5°C, and positive tubes showing gas or heavy growth within 24 or 48 ± 0.5 h were read for fluorescence and transferred to EC-MUG with sterile hardwood applicator sticks. The EC-MUG tubes were incubated at 44.5 ± 0.2°C in a gable-covered water bath for 24 ± 0.5 h. All LTB-MUG and EC-MUG tubes were exposed to a hand-held long-wavelength (366-nm) UV light. Fluorescence indicated the presence of *E. coli* (MUG test).

**MUG-negative tubes.** All LTB-MUG-, EC-MUG-, AC-, and CK-MUG-negative tubes (no fluorescence) were membrane filtered according to Standard Methods using a modification of the M-TEC method for *E. coli*. LTB-MUG-negative tubes were filtered in the pure culture studies, and EC-MUG-negative tubes were filtered with the natural sample comparison studies. LTB-MUG-negative tubes were filtered in the pure culture studies because none of the EC-MUG tubes failed to show a positive MUG response upon transfer from the LTB-MUG tubes. One- and 9-mL portions of each MUG-negative tube were filtered, and the MF was placed in petri dishes (50 × 9 mm) containing 5 mL of plate count agar and incubated for 2 h at 35 ± 0.5°C to allow chlorine-exposed organisms a chance to repair. The MFs were then placed on petri dishes (50 × 9 mm) containing 5 mL of M-TEC agar and incubated for 22 h at 44.5 ± 0.2°C in sealed plastic bags in a gable-covered circulating water bath. Presumptive *E. coli* colonies (yellow colonies) on the MFs were streaked for isolation on MacConkey agar and incubated at 35°C for 24 ± 0.5 h. The isolates were rubbed with a sterile swab, a small portion of the surface of MacConkey agar plates was rubbed and subsequently streaked for isolation with a sterile loop, and LTB-MUG tubes were inoculated. The tubes were incubated at 35 ± 0.5°C, and positive tubes showing gas or heavy growth within 24 or 48 ± 0.5 h were transferred to EC-MUG with sterile hardwood applicator sticks. The EC-MUG tubes were incubated at 44.5 ± 0.2°C for 24 ± 0.5 h. All LTB-MUG and EC-MUG tubes were exposed to a hand-held long-wavelength (366-nm) UV light. Fluorescence indicated the presence of *E. coli* (MUG test). Isolates from samples that were spiked with natural sources were further identified as *E. coli.*

**Statistical analyses.** The *E. coli* recoveries by the EC-MUG, CK, and AC tests were evaluated by the Wilcoxon signed rank test.27 The Wilcoxon signed rank test was performed by arranging the 10 samples (N = 10) in order of size, ignoring their signs. Rank numbers were then assigned to these absolute differences, rank 1 being given to the smallest difference, rank 2 to the next smallest, etc., and rank N to the largest. The signs of the original differences were then restored to the rank numbers, and T, the sum of the positive rank numbers, is the test statistic. In the event that ties occur among the differences, the same procedure as in the rank sum test is used. The tied differences are each given the average rank numbers that would have been assigned had the differences not been tied. The hypothesis tested was that there is no difference in detection rates when reinoculated into LTB-MUG and EC-MUG tubes.

**Results**

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>EC-MUG</th>
<th>AC</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure culture</td>
<td>2.6</td>
<td>6.4</td>
<td>32.9</td>
</tr>
<tr>
<td>Natural sample</td>
<td>16.4</td>
<td>23.4</td>
<td>18.6</td>
</tr>
<tr>
<td>All samples</td>
<td>10.7</td>
<td>14.9</td>
<td>26.0</td>
</tr>
</tbody>
</table>

*Percentage of MUG-negative tubes by each method for which isolates of these tubes were MUG-positive when reinoculated into LTB-MUG and EC-MUG.

*UVP Inc., San Gabriel, Calif.
**Millipore Corp., Bedford, Mass.
**CPI, Oak Park, Ill.
*Wilcoxon et al.* All statistical tests were performed at an alpha level of 0.05.
with the pure culture studies. However, there were no statistically significant differences in detection of *E. coli* between EC-MUG and CK with the natural samples. The EC-MUG method yielded more positive tubes than the AC test in 12 samples, whereas the AC test yielded more positive tubes in 10 of the samples. Both methods resulted in equal numbers of positive tubes in 10 samples. The EC-MUG method yielded more positive tubes than the CK coliform test in 28 samples, whereas the CK method resulted in more positive tubes in only four of the samples. Both EC-MUG and CK resulted in the same number of positive tubes in two of the samples.

**False-negative tubes.** Table 3 shows the percentage of MUG-negative tubes by each method in which isolates of these tubes were MUG positive when reinoculated into LTBMUG and EC-MUG (false negative). With the exception of CK (32.9), the lowest false-negative rates were observed with EC-MUG (2.6) and AC (6.4) using a free-chlorine-exposed pure culture of *E. coli*. The false-negative rates with natural populations of *E. coli* ranged from 16.4 percent with EC-MUG, followed by 18.6 percent with the CK test to 23.4 percent using the AC test. The lowest percentage of false-negative tubes overall was with EC-MUG (10.7), followed by the AC test (14.9) and CK (26.0). There were instances in which isolates from MUG-negative tubes were MUG-positive with EC-MUG, but *E. coli* was not isolated from MacConkey agar plates. This was because, in many cases, there was confluent growth on the MFs from filtration of the MUG-negative tubes, and *E. coli* was detected by swabbing the surface of the MF and transferring that to LTBMUG. However, because of the limited number of colonies picked for identification, *E. coli* colonies were undoubtedly present but were sometimes missed. This was not a frequent occurrence, i.e., less than 20 percent of these isolates were not identified as *E. coli*. The predominant background organism was Klebsiella pneumoniae. The number of MUG-positive tubes given in Tables 1 and 2 was not corrected for the false-negative results.

**Undetected target error.** Table 4 shows the percentage error introduced in specificity resulting from undetected *E. coli* calculated by ASTM standard D 3870-79. The closer each calculated value is to zero percent, the more specific the method. The lowest calculated value, i.e., best specificity, was with EC-MUG (7.8 percent) for all samples, followed by AC (9.7 percent) and CK (37.2 percent). The lowest value (1.3 percent) was with EC-MUG using pure cultures of free-chlorine-exposed *E. coli*. Generally, the percentages were higher in the natural sample studies compared with

<table>
<thead>
<tr>
<th>Source</th>
<th>EC-MUG</th>
<th>AC</th>
<th>CK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure culture</td>
<td>1.3</td>
<td>4.3</td>
<td>42.3</td>
</tr>
<tr>
<td>Natural sample</td>
<td>16.6</td>
<td>18.4</td>
<td>30.2</td>
</tr>
<tr>
<td>All samples</td>
<td>7.8</td>
<td>9.7</td>
<td>37.2</td>
</tr>
</tbody>
</table>

*Calculated by ASTM Standard D 3870-79

**Effect of holding disinfected samples.** Table 5 presents the results of holding studies. Six of the samples were held 24 h and 5 days after disinfection to determine the effects of holding in the absence of disinfectant residual on the surviving *E. coli* population. With the possible exception of sample 6, which was indeterminate (i.e., MPN >23), the remaining samples showed no significant changes in *E. coli* density within the five-day holding period.

**Characteristics of OFTDW.** Table 6 shows the characteristics of the OFTDW. The values for the analytes were below the MCLs of the primary and secondary drinking water regulations, with the exception of turbidity, which exceeded the MCL of 0.5 ntu.

**Discussion**

Both the AC and CK tests are novel departures from classical total coliform cultural methods that depend on lactose fermentation to detect the presence of coliforms. The AC and CK coliform tests use the substrate ONPG (for total coliforms) and MUG (for *E. coli*) both for essential nutrients and as the indicator system (yellow color and fluorescence). The tests are designed so that no additional confirmation tests are needed. Positive ONPG tubes are relatively easy to read. A positive MUG test using AC is easy to detect because of the brilliantly fluorescing tubes; however, the MUG reaction is sometimes difficult to interpret with CK, LTBMUG, and EC MUG tubes showing heavy growth.

Statistical analyses using the Wilcoxon signed rank test show that there was no significant statistical difference (P>0.05) between the EC-MUG method and AC for detecting *E. coli*. There was a significant difference (P<0.05) between EC-MUG and the CK coliform test, with the EC-MUG method showing better detection of free-chlorine-exposed *E. coli*. The results of this study do not agree completely with the findings of other similar CK and AC evaluation studies. Ziel and Mick found AC and CK comparable to LTBMUG for detection of *E. coli* in spiked distribution samples. McCarty et al found CK and AC equivalent to LTBMUG for the recovery of *E. coli* from spiked disinfected distribution samples. Clark et al showed that there was a significant difference between the MFC method, the AC test, and the CK test for detecting *E. coli* in treated water samples, with the MFC method being more sensitive. Gable and Broberg found in their evaluation of AC that the minerals-modified glutamate MIT test was significantly better in detecting and enumerating *E. coli* in both untreated and chlorinated water samples. They used the same statistical test used in this study. Differences in the outcomes of

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Holding Time</th>
<th>MPN* E. coli/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>24 h</td>
<td>&gt;23</td>
</tr>
<tr>
<td>7</td>
<td>24 h</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>24 h</td>
<td>5.1</td>
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<tr>
<td>11</td>
<td>24 h</td>
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<tr>
<td>12</td>
<td>24 h</td>
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<td>13</td>
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<td>5.1</td>
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<tr>
<td>6</td>
<td>5 days</td>
<td>3.6</td>
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<td>7</td>
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<tr>
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<td>1.1</td>
</tr>
<tr>
<td>11</td>
<td>5 days</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Based on EC-MUG
these studies may be attributed to different sample types, different media comparison combinations, and different forms of stress to the organisms.

The results of this study corroborate those of Edberg and Edberg, who were able to detect 1 cfu/100 mL of chlorine-exposed \( E. \text{coli} \) with a MUG-based substrate. All samples in this study, with the exception of the drinking water samples, received chlorine disinfection. Using the AC test, \( E. \text{coli} \) was detected in all samples. It was detected in 32 of the 33 samples using EC-MUG and in 24 of the samples using the CK coliform test. After disinfection, the mean \( E. \text{coli} \) count was 23 cfu/100 mL. The mean was somewhat skewed by the relatively high levels of \( E. \text{coli} \) in the first several samples of the study. The median \( E. \text{coli} \) count was 22 cfu/100 mL. The median number of \( E. \text{coli} \) organisms per tube was 1.2, and the median was 0.9.

Six of the disinfected samples were held five days after disinfection (Table 5) to assess any repair that may have occurred, as evidenced by significant changes in \( E. \text{coli} \) density. Any differences observed between the \( E. \text{coli} \) levels at 24 h and at five days after disinfection were not significant. The 24-h and five-day MPN values for each sample were within the 95 percent confidence limits. However, the laboratory-simulated nutritional and disinfection stress may not closely approximate the stress applied to \( E. \text{coli} \) in treated drinking water. These data do not completely agree with other studies that examined the effects of holding time and temperature on the survival of coliforms. McDanel and Bördner examined the survival of total coliforms in municipal drinking water distribution system samples held at both ambient temperature (22°C) and 5°C. Coliform populations declined significantly at both temperatures after 24 h. Average losses in 24 h were 34 percent at 5°C and 87 percent at 22°C. However, it is not known whether these samples contained \( E. \text{coli} \). McFeters et al. reported survival times for \( E. \text{coli} \) of one to five days in well water, and Plan reported survival times of up to 260 days at temperatures from 4 to 25°C for \( E. \text{coli} \) introduced into filtered sterilized river water. Many other holding time studies with total coliforms or \( E. \text{coli} \) have been reported, but basic differences in the conditions of the studies make comparisons difficult.

A major factor in the disparity of the results of the studies comparing EC-MUG to the other tests was the occurrence of false-negative tubes. Increasing the incubation time from 24 to 28 h did not result in significant changes in the number of MUG-positive tubes by any of the methods. This is similar to the results of Clark et al. Sixteen percent of the isolates from MUG negative EC MUG, 23 percent of the isolates from AC MUG-

### TABLE 6

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Analytical Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity</td>
<td>ntu</td>
<td>1.3</td>
</tr>
<tr>
<td>Chloride</td>
<td>mg/L</td>
<td>19.5</td>
</tr>
<tr>
<td>Nitrite</td>
<td>mg/L</td>
<td>90</td>
</tr>
<tr>
<td>Nitrate-N</td>
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<tr>
<td>Sodium</td>
<td>mg/L</td>
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<tr>
<td>Calcium</td>
<td>mg/L</td>
<td>29.9</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mg/L</td>
<td>10.6</td>
</tr>
<tr>
<td>Hardness as CaCO₃</td>
<td>mg/L</td>
<td>150</td>
</tr>
<tr>
<td>Alkalinity as CaCO₃</td>
<td>mg/L</td>
<td>67.6</td>
</tr>
<tr>
<td>pH</td>
<td>pH units</td>
<td>8.05</td>
</tr>
<tr>
<td>Copper</td>
<td>mg/L</td>
<td>-0.02</td>
</tr>
<tr>
<td>Manganese</td>
<td>mg/L</td>
<td>-0.06</td>
</tr>
<tr>
<td>Lead</td>
<td>mg/L</td>
<td>-0.02</td>
</tr>
<tr>
<td>Iron</td>
<td>mg/L</td>
<td>-0.04</td>
</tr>
<tr>
<td>Zinc</td>
<td>mg/L</td>
<td>-0.01</td>
</tr>
<tr>
<td>Specific Conductance</td>
<td>micromhos at 25°C</td>
<td>410</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>cfu/100 mL</td>
<td>1</td>
</tr>
<tr>
<td>Mean heterotrophic plate count</td>
<td>cfu/mL</td>
<td>1,700</td>
</tr>
</tbody>
</table>

MUG-based substrates are limited. Clark et al. reported false-negative occurrences of 12 and 19 percent with CK and AC, respectively, with untreated waters and 61 and 81 percent (CK and AC, respectively) with treated water samples positive for \( E. \text{coli} \). Covert et al. reported the percentage of false negatives in their evaluation of the AC test for total coliforms to be 20.5 percent; however, there were too few \( E. \text{coli} \)-positive samples to evaluate the efficacy of the AC test for detecting \( E. \text{coli} \). In this study, the false-negative rates for EC-MUG, CK, and AC using chlorine-exposed natural populations of \( E. \text{coli} \) were 16.4, 18.6, and 23.4, percent respectively. Using ASTM standard practice D 3870-79 for establishing the performance characteristics of microbiological methods, the percentages of undetected target errors using chlorine-exposed natural populations of \( E. \text{coli} \), the AC test and EC-MUG were similar.

### Summary

Statistical analyses of the data indicated no significant difference in detection of \( E. \text{coli} \) between the AC test and EC-MUG; however, there were statistically significant differences between the CK coliform test and EC-MUG using a free-chlorine-exposed pure culture of \( E. \text{coli} \) and when the data for all samples were combined. The AC test was equivalent to EC-MUG in detecting free-chlorine-exposed \( E. \text{coli} \) using a pure culture and monochloramine-exposed natural populations of \( E. \text{coli} \). There were no statistically significant differences in detection of \( E. \text{coli} \) with CK using monochloramine-exposed natural populations of \( E. \text{coli} \). All the methods evaluated were capable of detecting 1 cfu/100 mL of \( E. \text{coli} \). The lowest false-negative rate or undetected target error was with EC-MUG. In view of the lack of published studies addressing false-negative occurrences or
rates, more definitive studies are needed to establish the false-negative rates with MUG-based methods using chlorine-exposed environmental populations of E. coli. Also, the observation that E. coli exposed to halogen disinfection may sometimes be unable to utilize MUG substrate warrants additional study.

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References


About the authors: All the authors are employed by the US Environmental Protection Agency, 26 W. Martin Luther King Dr., Cincinnati, OH 45268. Terry C. Covert is a research microbiologist with the Environmental Monitoring Systems Laboratory (EMSL). Covert is a graduate of Ohio State University in Columbus (BS degree) and of the University of Cincinnati (MS degree). He is a member of ASTM and Sigma Xi, and his work has been published previously by Applied and Environmental Microbiology. Eugene W. Rice is a microbiologist with the Risk Reduction Engineering Laboratory (RREL). Scott A. Johnson is a biological laboratory technician with the EMSL, Donald Berman is a microbiologist with the RREL, Clifford H. Johnson is a biologist with the RREL, and Parallei J. Mason is a biological laboratory technician with the RREL.