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# NOTES

## Assay for β-Glucuronidase in Species of the Genus *Escherichia* and Its Applications for Drinking-Water Analysis

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**Recently, *Escherichia* species other than *Escherichia coli* have been isolated from potable water. Environmental isolates as well as clinical isolates of *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermannii*, and *E. vulneris* were assayed for the enzyme β-glucuronidase by using EC MUG medium and the Colilert system. None of the isolates were positive for the enzyme by either method.**

Since the beginning of this century *Escherichia coli* has been considered a specific bacterial indicator of fecal pollution of water and food. The U.S. Environmental Protection Agency recently issued major changes in the Safe Drinking Water Act Total Coliform Rule that places *E. coli* as a critical indicator of fecal pollution of drinking water (8). The total coliform group will remain the primary bacterial indicator. However, for each positive total coliform test, a fecal coliform or *E. coli* analysis must be performed for that sample. The U.S. Environmental Protection Agency is basing *E. coli* methods on β-glucuronidase (GUR) activity (9).

Kilian and Bulow (12) first reported the association of GUR with the identification of *E. coli*. This enzyme is limited to the genera *Escherichia* and *Shigella* and approximately 10% of *Salmonella* species. The association between GUR and *E. coli* has been used to develop a means of identifying this species from a variety of environmental and clinical sources (4, 5, 10, 13, 14).

During the past several years, a variety of coliform bacterial isolates from potable water have been submitted to our laboratories for definitive identification. These isolates were most often forwarded because they were positive for β-galactosidase activity, as measured by the *o*-nitrophenyl-β-D-galactopyranoside (ONPG) test, but were not readily identified as members of the coliform group. When identified by conventional biochemical means (6), approximately 50% were *Citrobacter diversus* and other indole-positive total coliforms, whereas the remaining isolates consisted of *Escherichia* species other than *E. coli*.

*Escherichia* species including *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermannii*, and *E. vulneris* have been isolated from a variety of clinical and environmental sources (2, 3, 6, 11, 14). The sources of the clinical isolates were predominantly infections of peripheral limbs secondary to trauma. These species are not routinely isolated from stool specimens. Their presence is not considered to necessarily be indicative of fecal contamination. In drinking-water analysis, it is therefore essential to differentiate these species from *E. coli*. Previous reports, based upon a relatively small number of isolates, suggested that *Escherichia* species other than *E. coli* are negative in assays for GUR (10, 14). To determine whether negative reactions for GUR assays were specific for these species, we analyzed clinical and environmental isolates for GUR activity.

Forty-two *Escherichia* isolates from the Enteric Diseases Branch culture collection at the Centers for Disease Control were analyzed. When first received, the majority of these isolates did not yield a definitive bacterial identification and were designated as enteric groups 1, 10, and 11. Subsequently, on the basis of DNA relatedness studies, they were designated as separate species in the genus *Escherichia* (2, 3, 7). Once the species were established by genetic means, phenotypic biochemical tests were chosen to provide optimum separation among these *Escherichia* species.

Isolates from water supply systems were also included in the study (Table 1). Standard bacteriologic analyses of the water samples from which the isolates were recovered revealed no apparent recent fecal contamination, since *E. coli* was absent. All isolates were identified by conventional biochemical tests (6).

GUR assays were performed by using the 4-methylumbelliferyl-β-D-glucuronide (MUG) substrate. MUG at a concentration of 75 mg · liter$^{-1}$ in a mixture of salts, cofactors, and dispersant was used in the Colilert system (Access Analytical Systems, Branford, Conn.) (4, 5). EC medium containing MUG at 50 mg · liter$^{-1}$ was used in the fecal coliform test (10). All strains were streaked for isolation on plate count agar and incubated at 35°C for 24 h. An isolated colony was lightly touched with a sterile loop or wooden applicator stick and used as the inoculum for both procedures. Colilert tubes were hydrated with 10 ml of sterile distilled water and, after inoculation, were incubated at 35°C for 24 h. Lauryl tryptose broth (LTB) tubes (1) were inoculated and then incubated at 35°C for 24 to 48 h. After growth was observed, each culture was transferred to EC-MUG medium and incubated at 44.5°C for 24 h. Fluorescence was observed in both procedures by exposing the tubes to long-wave UV light (366 nm). Any fluorescence was considered positive. A recent environmental isolate of *E. coli* served as a positive control and routinely yielded a strong fluorescence. A *Klebsiella pneu-*

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TABLE 1. Distribution of *Escherichia* spp. isolated from drinking water

| Species | No. of isolates | Location | Sample type |
|---|---|---|---|
| *E. hermannii* | 1 | Florida | New pipe |
| | 1 | Alberta | Hydrant, flushing |
| | 3 | Massachusetts | Distribution system |
| | 2 | Kentucky | Distribution system, dead-end location |
| | 1 | California | Holding cistern |
| | 1 | Washington | Holding tank |
| | 1 | Arizona | Distribution system, dead-end location |
| | 1 | Georgia | Holding reservoir |
| | 3 | Illinois | Holding reservoir |
| *E. fergusonii* | 1 | Florida | New pipe |
| | 1 | Alberta | Distribution system |
| | 1 | California | Distribution system, dead-end location |
| | 2 | Connecticut | Distribution system |
| | 1 | South Carolina | Distribution system |
| *E. vulneris* | 2 | Alberta | Hydrant, flushing |
| | 1 | Florida | New pipe |
| | 1 | Louisiana | Distribution system, dead-end location |
| *E. adecarboxylata* | 1 | Massachusetts | Distribution system |

*moniae* isolate served as a negative control and was always GUR negative.

Fecal coliform tests were also performed in accordance with standard procedures (1). An isolated colony of each strain was transferred to LTB and incubated at 35°C for up to 48 h. After growth was observed, each culture was transferred to EC medium, incubated at 44.5°C for 24 h, and observed for growth and gas production. The results of fecal coliform testing and GUR assays are presented in Table 2.

The results from this study indicate that assays for GUR by using EC-MUG medium or the Colilert system are able to distinguish *E. coli* from other *Escherichia* species. These non-*E. coli* species also do not produce a positive fecal coliform test. The Colilert system provides more rapid results and requires only one incubation temperature, as opposed to the EC-MUG and fecal coliform procedures.

TABLE 2. Results of fecal coliform and GUR tests for *Escherichia* spp.

| Species | No. of isolates | No. showing fecal coliform growth | No. showing fecal coliform gas | No. positive in GUR assay with: | |
|---|---|---|---|---|---|
| | | | | EC-MUG | Colilert-MUG |
| CDC[a] clones | | | | | |
| *E. hermannii* | 17 | 0 | 0 | 0 | 0 |
| *E. fergusonii* | 11 | 0 | 0 | 0 | 0 |
| *E. vulneris* | 13 | 1 | 0 | 0 | 0 |
| *E. blattae* | 1 | 0 | 0 | 0 | 0 |
| Environmental isolates | | | | | |
| *E. hermannii* | 14 | 0 | 0 | 0 | 0 |
| *E. fergusonii* | 6 | 0 | 0 | 0 | 0 |
| *E. vulneris* | 4 | 0 | 0 | 0 | 0 |
| *E. adecarboxylata* | 1 | 0 | 0 | 0 | 0 |

[a] CDC, Centers for Disease Control.

These methods are in keeping with the requirements of the new Safe Drinking Water Act Total Coliform Rule (8). The use of these procedures will permit public health authorities to make decisions regarding acute violations and possible boil-water orders based on methods which have been shown to be specific for *E. coli* detection and are not subject to false-positive results.

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