IDEXX
Literature Cover Sheet

IDEXX #: 10B

Title: Identification of *Escherichia coli* by Colilert *B*-Glucuronidase Assay: Sensitivity, Specificity, and Implications for Water Testing

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Source: American Water Works Association Research Foundation

Topic: Colilert is a superior *E. coli* test

Highlights:

- Human and animal *E.coli* were tested and the sensitivity and specificity of Colilert was excellent. There were no false positives and only one false negative out of 620 *E. coli* isolates.
- Some of the other advantages of the Colilert system are:
  1. Rapid test procedure producing definitive results within 24-28 hours.
  2. Incubation of Colilert at 35°C instead of the 44°C temperature in the Fecal Coliform Test favors the recovery of stressed organisms.
  3. Colilert detects anaerogenic strains of *E. coli*. They are missed by conventional procedures that rely on lactose fermentation.
  4. *E.coli* is generally considered to be a more reliable indicator since its presence directly relates to fecal contamination and the implied threat of the presence of enteric pathogens.

* See pages 2,5& 7

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Identification of *Escherichia coli* by Colilert B–Glucuronidase Assay: Sensitivity, Specificity, and Implications for Water Testing

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ABSTRACT

In 1976 Killian and Bulow described the association of B-glucuronidase with the genus *Escherichia* (97% positive) and suggested its assay would be a useful identification test. Since this report papers have appeared examining the sensitivity and specificity of this enzyme for the identification of *E. coli* from clinical, food, sea water, potable water supplies, and various environmental sources. The reservoir of *E. coli* from both environmental, and clinical samples, is the mammalian gut. A study was undertaken to determine the sensitivity and specificity of the defined substrate technology B-glucuronidase (Colilert) assay for the identification of this species from fecal samples. A total of 460 human, 105 cow, and 55 horse *E. coli* isolates were tested. Results showed 96.5% B-glucuronidase positive in 24 hours incubation; only one *E. coli* isolate was negative. There were no significant differences in the percentage of B-glucuronidase positive isolates among the human or animal isolates. There were no non-*E. coli* isolates that were positive. All subjects carried Colilert positive *E. coli*. These results demonstrate that the detection of *Escherichia coli* by the Colilert test had excellent sensitivity and specificity. The Colilert *E. coli* assay is also compatible with the new Safe Drinking Water Act regulations, which require the assay of this species, or fecal coliforms, from each water sample that shows the presence of total coliforms.

INTRODUCTION

Escherichia coli has been utilized as an indicator of the fecal pollution of water for nearly 100 years (13). This member of the family *Enterobacteriaceae* has been shown to be present in high numbers in the colons of all mammalian species (4). In the early part of this century, when it was virtually impossible to detect *E. coli* from mixed bacterial populations, a surrogate group of indicators, the total coliforms (TC), became widely used as a sentinel of fecal contamination of water and potential health risk. While not necessarily a part of the fecal flora TC served as a useful indicator of water treatment processing. Within the last decade renewed interest in the direct enumeration of the more specific indicator, *E. coli*, has occurred. The United States Environmental Protection Agency has proposed that water utilities assay for the presence of either fecal coliforms or *Escherichia coli* from any drinking water sample containing total coliforms (14). The presence of even a single *E. coli* occurrence would require the utility to contact public health authorities for consultation (15).

There have been a number of surrogate systems employed for the determination of *E. coli*, including the original fecal coliform temperature elevation test (12). Methods that detect an end product likely to be associated with this species, such as indole have also been used (23). However, all of these have been shown to suffer from a lack of both sensitivity (1) and specificity (3). In this group, only the m-TEC *E. coli* MF method has been recommended by the U.S. E.P.A. as specific for *E. coli* (4).

Kilian and Bulow first described the association of the enzyme B-glucuronidase with the tribe *Escherichia* (20). B-glucuronidase has been shown to be limited to *E. coli*, *Shigella* species, and *Salmonella* species in the family *Enterobacteriaceae* (21). Since this report B-glucuronidase substrates have been incorporated in diverse media to detect *E. coli* from a variety of sources including environmental (16), food (17, 26), sea water (24), and clinical (6, 7).

B-glucuronidase assays have included both constitutive and inducible enzyme tests. Constitutive enzyme methods, which require a large bacterial inoculum (106 cells/mL) and are useful only for pure colonies, utilize a hydrolyzable substrate that releases a colored aglycone. The endpoint is detectable within one or two hours after inoculation. Inducible enzyme tests use a B-glucuronidase substrate present in complete media and detect the endpoint of enzyme hydrolysis as the organisms grow. The base medium containing the substrate can significantly affect the sensitivity of the assay (17). The sensitivity of B-glucuronidase assays also varies depending on whether constitutive or inducible enzymes are being measured. Constitutive enzyme tests demonstrated 87 to 97% of *E. coli* were positive and inducible procedures showed 91 to 100% positivity (8, 18, 22, 25, 26, 27). A recent

Division of Sherwood Medical, Plainview, NY). Supplemental tests were employed when required (19).

Colilert system. The Colilert system was obtained in powdered form in the MPN tube configuration (Access Analytical Systems, Branford, CT). Each MPN tube contained enough Colilert powder to receive 10 mL of a water sample. For the identification of bacteria from colonies, each Colilert tube was reconstituted with 10 mL of sterile distilled water. The powder was mixed to dissolve it. Each Colilert tube was inoculated within two hours of hydration. The formula becomes yellow if total coliforms are present, and fluorescent if *E. coli* is present.

Protocol. From each stool specimen five lactose fermenting colonies were identified by the API 20E system and subcultured to both MacConkey's and Mueller-Hinton agars for purity. MacConkey's agar was utilized as a sugar containing medium and Mueller-Hinton agar was utilized because it does not contain a fermentable carbohydrate. From each subculture the top of the colony was lightly touched with a sterile wooden applicator stick. This inoculum was transferred to the Colilert tube and thoroughly agitated to disperse the bacteria. Colilert tubes were incubated at 35°C in ambient air. Tubes were read for fluorescence after 24, 28, and 48 hours of incubation. Fluorescence, which indicates the hydrolysis of the B-D-glucuronidase substrate, was determined by exposing the test tubes to a four-watt long range ultraviolet generator (366 nm) (Edmund Scientific Co., Barrington, NJ) with the tubes held approximately 2 inches from the light. Tubes that were negative for fluorescence were incubated for five days. Fluorescence was graded on a scale from negative to 4+. Negative tubes showed no discernible fluorescence by eye and were quality controlled by inoculating a *Klebsiella pneumoniae* (always B-glucuronidase negative) with every test run. Weak fluorescence, or 1+, equaled fluorescence just discernible to the observer and equal to a standard comparator tube (Access Analytical Systems). *E. coli* ATCC 25922 served as a positive control which routinely yielded the strongest fluorescence, or 4+. Gradings of 2+ and 3+ were taken to be intermediate between the 1+ and 4+ readings.

RESULTS

This study was designed to evaluate the efficacy of the B-glucuronidase assay for the detection of *E. coli* using the Colilert autoanalysis procedure. A total of 720 enteric bacterial isolates were obtained from the feces of 106 human subjects, 24 cows and 14 horses. Of the 720 enteric isolates, 620 (86%) were identified as *E. coli*. The test was highly specific with 592 (95.5%) of the *E. coli* yielding a positive response within 24 hours. This number increased to 617 (99.5%) with an additional four hours of incubation. None of the other fecal isolates, which included members of the genera *Enterobacter, Klebsiella, Citrobacter* and *Serratia*, gave a positive reaction within 28 hours. Only one of the 620 *E. coli* strains failed to produce a positive response. Subculturing from the media with (MacConkey's) or without (Mueller-

teriaceae. The misidentification of *E. coli* using commercially available kits has also been cited in reference to other enteric organisms (5). Research is currently underway to more thoroughly characterize the B-glucuronidase activity of species of the genus *Escherichia* other than *coli*. Lastly, the hypothesis put forth by Chang et al. that clinical specimens may contain a larger percentage of MUG positive *E. coli* strains than other sources (2) is not supported by the current findings, which also examined strains from domestic animals as well as healthy humans.

The ability to detect the presence of fecal contamination in public water supplies is a matter of urgent concern for water utilities and public health authorities. Under the new coliform rule of the Safe Drinking Water Act, potable water samples containing total coliforms must also be analyzed for either *E. coli* or fecal coliforms (14, 15). *E. coli* is generally considered to be a more reliable indicator since its presence directly relates to fecal contamination and the implied threat of the presence of enteric disease agents. Any test procedures used for the analysis of environmental samples must possess the required sensitivity and specificity to detect wild type *E. coli* from a variety of sources.

This study confirms previous findings indicating that the vast majority of wild type *E. coli* possess B-glucuronidase and can be detected using assays for that enzyme. The MUG assay used in the Colilert system offers many advantages over conventional cultural procedures for *E. coli*. The Colilert system is a rapid test procedure producing definitive results within 24-28 hours of initial inoculation. The sensitivity and specificity of this system are excellent. Incubation of the Colilert tubes at 35°C instead of the 44.5°C temperature in the Fecal Coliform Test favors the recovery of stressed organisms (4). Anaerogenic strains of *E. coli*, which are not detected by conventional procedures that rely on lactose fermentation, can be detected using the Colilert MUG assay. In summary, the Colilert B-glucuronidase assay offers a rapid and reliable methodology for the detection of *E. coli*.

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