APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1990, p. 1203–1205 Vol. 56, No. 5
0099-2240/90/051203-03$02.00/0
Copyright © 1990, American Society for Microbiology

Efficacy of β-Glucuronidase Assay for Identification of *Escherichia coli* by the Defined-Substrate Technology

EUGENE W. RICE,[1]* MARTIN J. ALLEN,[2] AND STEPHEN C. EDBERG[3]

Risk Reduction Engineering Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268[1]; American Water Works Association Research Foundation, Denver, Colorado 80235[2]; and Department of Laboratory Medicine, Yale University School of Medicine, New Haven, Connecticut 06510[3]

Received 16 November 1989/Accepted 29 January 1990

In 1976, Kilian and Bulow described the association of β-glucuronidase with the genus *Escherichia* (97% positive) and suggested that a β-glucuronidase assay would be a useful identification test. Since that report, papers about the sensitivity and specificity of this enzyme for the identification of *Escherichia coli* from clinical sources, food, seawater, potable-water supplies, and various environmental sources have appeared. A study was undertaken to determine the efficacy and specificity of the defined-substrate technology β-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 95.5% β-glucuronidase-positive isolates in 24 h and 99.5% positive after 28 h of incubation. Only one *E. coli* isolate was negative. There were no significant differences in the percentage of β-glucuronidase-positive isolates among the human or animal isolates. There were no non-*E. coli* isolates that were positive. All subjects carried β-glucuronidase-positive *E. coli*.

Escherichia coli has been used as an indicator of fecal pollution for nearly 100 years (11). This member of the family *Enterobacteriaceae* has been shown to be present in high numbers in the colons of all mammalian species (4). Within the last decade, there has been renewed interest in the direct enumeration of *E. coli*. The U.S. Environmental Protection Agency has proposed that water samples be assayed for the presence of either fecal coliforms or *E. coli* from any drinking water sample containing total coliforms (12). There have been a number of surrogate systems employed for the determination of *E. coli*, such as the original fecal coliform temperature elevation test (10). Methods that detect an end product likely to be associated with this species, such as indole, have also been used (20). However, all of these methods have been shown to suffer from a lack of both sensitivity (1) and specificity (3).

Kilian and Bulow first described the association of the enzyme β-glucuronidase with the *Escherichia-Shigella* group (17). β-Glucuronidase has been shown to be limited to *E. coli*, *Shigella* species, and *Salmonella* species in the family *Enterobacteriaceae* (18). Since that report, β-glucuronidase substrates have been incorporated in diverse media to detect *E. coli* in samples from a variety of sources, such as environmental (13), food (14, 23), seawater (21), and clinical (7, 9) sources. The sensitivities of β-glucuronidase assays vary, depending on whether constitutive or inducible enzymes are being measured. Constitutive enzyme tests demonstrate that 87 to 97% of *E. coli* isolates are positive, and inducible procedures show 91 to 100% positivity (8, 15, 19, 22, 23, 24). A recent report, however, indicates that *E. coli* from humans shows a β-glucuronidase positivity rate of only 66% (2).

The defined-substrate technology Colilert method uses 4-methylumbelliferyl-β-glucuronide (MUG) as the fluorogenic indicator nutrient for detecting *E. coli* (5). The metabolic activity of the target microbe is directed towards a specific substrate which the microbe utilizes for growth. Accordingly, there is no interference by nontarget microorganisms. The pH is controlled, thereby optimizing the production of fluorescence from the substrate. To determine the efficacy and specificity of source *E. coli* by this new method, a variety of stool specimens were analyzed. Wild-type *E. coli* isolates from humans, cows, and horses were examined.

MATERIALS AND METHODS

Isolate sources and identification. Isolates were obtained from both healthy and ill human beings (University Hospital, University of Cincinnati, Cincinnati, Ohio, and Yale University School of Medicine, New Haven, Conn.) and cows and horses maintained on nonantibiotic dietary control (New Haven, Conn.). Ages ranged from 6 months to 83 years for humans, from 1 month to 5 years for cows, and from 6 months to 11 years for horses. All stool samples were collected fresh in appropriate transport media (Culturette; Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) and plated on MacConkey agar within 24 h of collection. After 24 h of incubation at 35°C in ambient air, five randomly chosen lactose-fermenting colonies per subject were analyzed. Each of the five isolates was identified to the species level by the API 20E system (Analytab Products, Plainview, N.Y.). Supplemental tests were employed when required (16).

Colilert system. The Colilert system was obtained in powdered form in the multiple fermentation tube configuration (Access Analytical Systems, Branford, Conn.). For the identification of bacteria from colonies, each Colilert tube was reconstituted with 10 ml of sterile distilled water. The powder was mixed until dissolved. Each Colilert tube was inoculated within 2 h of hydration. The formula became yellow if total coliforms were present and fluorescent if *E. coli* was present.

Protocol. Five lactose-fermenting colonies per subject were randomly picked from the MacConkey agar plate used for initial isolation, identified by the API 20E system, and subcultured to both MacConkey and Mueller-Hinton agars for purity. MacConkey agar was used as a sugar-containing

* Corresponding author.

TABLE 1. Identification of fecal isolates by the Colilert β-glucuronidase test

Species	No. of isolates tested	No. (%) fluorescent at 366 nm at:		
		24 h	28 h	120 h
Escherichia coli	620	592 (95.5)	617 (99.5)	619 (99.8)
Enterobacter cloacae	55	0	0	0
Klebsiella pneumoniae	28	0	0	0
Citrobacter freundii	6	0	0	0
Citrobacter diversus	9	0	0	0
Serratia sp.	1	0	0	0
Unidentified	1	0	0	0

TABLE 2. Detection of β-glucoronidase from *E. coli* originating in different host species

Incubation time (h)	No. of positive isolates/no. tested (%) from:		
	Human	Cow	Horse
24	441[a]/460 (95.9)	99[b]/105 (94.3)	52[c]/55 (94.5)
28	457[d]/460 (99.3)	105[e]/105 (100)	55[f]/55 (100)
120	459/460 (99.8)		

[a] 80% 4+, 11% 3+, 6% 2+, 3% 1+.
[b] 85% 4+, 5% 3+, 5% 2+, 5% 1+.
[c] 88% 4+, 4% 3+, 4% 2+, 4% 1+.
[d] 91% 4+, 7% 3+, 1% 2+, 1% 1+.
[e] 93% 4+, 3% 3+, 3% 2+, 1% 1+.
[f] 92% 4+, 4% 3+, 2% 2+, 2% 1+.

medium and Mueller-Hinton agar was used as a medium that does not contain a fermentable carbohydrate. The Mueller-Hinton agar was used to determine if a decrease in the amount of fermentable substrate would affect the production of the β-glucuronidase enzyme. The top of the colony in each subculture was lightly touched with a sterile wooden applicator stick. This inoculum was transferred to a 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 120 h of incubation. Fluorescence, which indicates the hydrolysis of the β-D-glucuronidase substrate, was determined by exposing the test tubes to a 4-W long-range UV generator (366 nm; Edmund Scientific Co., Barrington, N.J.) from approximately 2 in. (ca. 5 cm) away. Tubes that were negative for fluorescence were incubated for 5 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* isolate (always β-glucuronidase negative) with every test run. Weak fluorescence, or 1+, was defined as fluorescence that was just discernible to the observer and equal to that of a standard comparator tube (Access Analytical Systems). *E. coli* ATCC 25922 served as a positive control and routinely yielded the strongest fluorescence, or 4+. Gradings of 2+ and 3+ were intermediate readings.

RESULTS AND DISCUSSION

This study was designed to evaluate the efficacy of the β-glucuronidase assay for the identification of *E. coli* by 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 results of the β-glucuronidase assays for all isolates are shown in Table 1. The test was highly specific, with 592 (95.5%) of the *E. coli* isolates yielding a positive response within 24 h. This number increased to 617 (99.5%) with an additional 4 h 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 h. Only one of the 620 *E. coli* strains failed to produce a positive response. Subculturing from the media with (MacConkey agar) or without (Mueller-Hinton agar) a fermentable carbohydrate did not affect the β-glucuronidase assay in the Colilert system.

The results of the β-glucuronidase test for the isolates from the different mammalian species are listed in Table 2. There were no meaningful differences in carriage for the *E. coli* strains from the three mammalian species. *E. coli* was the most common gram-negative bacterium isolated from each human and animal species (>90% of the enteric strains in the fecal samples). Of all the *E. coli* isolates from each host species, 90% or more gave a positive response within 24 h, with the percentages increasing to >99% positivity after 28 h of incubation. The gradation of the fluorescence response, which indicated the degree of hydrolysis of the MUG substrate by the β-glucuronidase enzyme, showed that a 4+ reaction occurred in 80% or more of the isolates from all sources after 24 h and in more than 90% of the isolates after 28 h of incubation.

These results indicate that the Colilert β-glucuronidase test is an efficacious and highly specific system for the identification of *E. coli*. This is a rapid assay; readily discernible results are produced within 24 to 28 h of initial inoculation. The findings reported here corroborate the reports of other investigators, which indicate that 90% or more of *E. coli* strains isolated from a variety of sources contain the enzyme β-glucuronidase (8, 15, 19, 22–24).

The results of the present study, coupled with the historical data base, are in contrast to the recent report by Chang et al. (2) which cites 66% positive β-glucuronidase activity for the strains of *E. coli* investigated. The sensitivity of the lactose-based MUG medium used in this study would not appear to entirely account for this discrepancy, since it was used as a confirmatory medium with large inocula of pure cultures. Another explanation might be that in attempting to pick different colony types from the initial isolation medium, organisms other than *E. coli* were overrepresented. One such group of organisms could be *Escherichia* species other than *E. coli*. While *E. coli* is the major gram-negative enteric bacterium in the mammalian gut, other species of this genus, such as *Escherichia fergusonii*, *Escherichia hermannii*, and *Escherichia vulneris*, have been isolated from feces and have been reported to be MUG negative (24). These *Escherichia* species are not readily differentiated by many commercial kits available for taxonomic identification of members of the family *Enterobacteriaceae*. The misidentification of *E. coli* with commercially available kits has also been cited in reference to other enteric organisms (6). 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 are from examinations of strains from domestic animals as well as healthy humans.

The ability to detect the presence of fecal contamination is a matter of urgent concern for public health authorities. *E. coli* is generally considered a more reliable sanitary indicator than fecal coliforms, since the presence of *E. coli* directly relates to fecal contamination and the implied threat of the presence of enteric disease agents. Any test procedure used for the analysis of environmental samples must possess the

required specificity to identify wild-type *E. coli* from a variety of sources.

This study confirms previous findings indicating that the vast majority of wild-type *E. coli* strains possess β-glucuronidase and can be detected by using assays for that enzyme. The Colilert system is a rapid test procedure producing definitive results within 24 to 28 h of initial inoculation. Incubation of the Colilert tubes at 35°C, instead of 44.5°C as in the fecal coliform test, favors the recovery of stressed organisms (4). Anaerogenic strains of *E. coli*, which are not detected by conventional procedures which rely on lactose fermentation, can also be detected by using the Colilert MUG assay.

ACKNOWLEDGMENTS

We acknowledge the assistance provided by Donald Berman, Janet C. Blannon, Elizabeth Creamer, Paralee J. Mason, Lois Shadix, and Cheryl Tow.

LITERATURE CITED

1. **Boyd, W. L., and H. C. Lichstein.** 1955. The effect of carbohydrates on the tryptophanase activity of bacteria. J. Bacteriol. **69**:584–589.
2. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of β-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335–339.
3. **Dufour, A. P., and V. J. Cabelli.** 1975. Membrane filter procedure for enumerating the component genera of the coliform group in seawater. Appl. Environ. Microbiol. **29**:826–833.
4. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152–1158.
5. **Edberg, S. C., M. J. Allen, D. B. Smith, and the National Collaborative Study.** 1988. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with the standard multiple tube fermentation method. Appl. Environ. Microbiol. **54**:1595–1601. (Erratum, **54**:3197.)
6. **Edberg, S. C., and L. Konowe.** 1982. Statistical and applied meaning of a clinical microbiology product: bacterial identification systems, p. 268–299. *In* V. Lorian (ed.), Clinical microbiology in patient care. The Williams & Wilkins Co., Baltimore.
7. **Edberg, S. C., and C. M. Kontnick.** 1986. Comparison of β-glucuronidase-based substrate systems for identification of *Escherichia coli*. J. Clin. Microbiol. **24**:368–371.
8. **Edberg, S. C., V. Piscitelli, and M. Cartter.** 1986. Phenotypic characteristics of coliform and noncoliform bacteria from a public water supply compared with regional and national clinical species. Appl. Environ. Microbiol. **52**:474–478. (Erratum, **53**: 214, 1987.)
9. **Edberg, S. C., and R. W. Trepeta.** 1983. Rapid and economical identification and antimicrobial susceptibility test methodology for urinary tract pathogens. J. Clin. Microbiol. **18**:1287–1291.
10. **Eijkman, C.** 1904. Die Garungs probe bei 46°C als Hilfsmittel bei der Trinkwasseruntersuchung. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **37**:742–752.
11. **Escherich, T.** 1885. Die Darmbakterien des Naugenborenen und Sauglings. Fortschr. Med. **3**:515–547.
12. **Federal Register.** 1989. National primary drinking water regulations: total coliforms (including fecal coliforms and *E. coli*). Fed. Regist. **54**:27544–27568.
13. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320–1329.
14. **Hartman, P. A., J. P. Petzel, and C. W. Kaspar.** 1986. New methods for indicator organisms, p. 175–218. *In* M. D. Pierson and N. J. Stern (ed.), Foodborne microorganisms and their toxins. Marcel Dekker, New York.
15. **Iritani, B., and T. J. Inzana.** 1988. Evaluation of a rapid tube assay for presumptive identification of *Escherichia coli* from veterinary specimens. J. Clin. Microbiol. **26**:564–566.
16. **Kelly, M. T., D. J. Brenner, and J. J. Farmer III.** 1985. *Enterobacteriaceae*, p. 263–277. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
17. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. I. Detection of bacterial glycosidases. Acta Pathol. Microbiol. Scand. Sect. B **84**:245–251.
18. **Kilian, M., and P. Bulow.** 1979. Rapid identification of *Enterobacteriaceae*. II. Use of β-glucuronidase detecting agar medium (PGUA agar) for the identification of *E. coli* in primary cultures of urine samples. Acta Pathol. Microbiol. Scand. Sect. B **87**:271–276.
19. **Koburger, J. A., and M. L. Miller.** 1985. Evaluation of a fluorogenic MPN procedure for determining *Escherichia coli* in oysters. J. Food Prot. **48**:244–245.
20. **Mara, D. D.** 1973. A single medium for the rapid detection of *Escherichia coli* at 44°C. J. Hyg. **71**:783–785.
21. **Mates, A., and N. Schaffer.** 1988. Quantitative determination of *Escherichia coli* from fecal coliforms in seawater. Microbios **53**:162–165.
22. **Moberg, L. J., M. K. Wagner, and L. A. Kellen.** 1987. Fluorogenic assay for rapid detection of *Escherichia coli* in chilled and frozen foods: collaborative study. J. Assoc. Off. Anal. Chem. **71**:589–602.
23. **Robison, B. J.** 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. Appl. Environ. Microbiol. **48**:285–288.
24. **Trepeta, R. W., and S. C. Edberg.** 1984. Methylumbelliferyl-β-D-glucuronide-based medium for rapid isolation and identification of *Escherichia coli*. J. Clin. Microbiol. **19**:172–174.