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# Rapid Glutamic Acid Decarboxylase Test for Identification of *Bacteroides* and *Clostridium* spp.

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A rapid 4-h test for glutamic acid decarboxylase is described for the identification of certain anaerobic bacteria. The test substrate consisted of 1.0 g of L-glutamic acid, 0.3 ml of Triton X-155, and 0.05 g of bromcresol green sodium salt in 1 liter of water. The substrate was dispensed in 0.5-ml amounts into test tubes, and a turbid suspension was made with the test organism. The test was then incubated aerobically at 35°C for 4 h. The development of a blue color was considered positive. A total of 345 strains of clinically isolated anaerobic bacteria were tested. All isolates of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Clostridium perfringens*, and *Clostridium sordellii* gave a positive reaction. Some isolates of *Bacteroides distasonis* and *Bacteroides vulgatus* were also positive. The use of this rapid test in conjunction with other rapid methods, such as the spot indol test, will enable laboratory workers to report these pathogens on the same day on which an inoculum of pure culture growth on agar is available.

The role of anaerobic bacteria in the disease state and normal flora of humans has been well documented (1, 3, 10). Therefore, it is important that these bacteria be accurately and rapidly identified. Conventional methods in which thioglycolate-based fermentation media (2) and prereduced, anaerobically sterilized media (5) are used have been shown to be highly reliable for the identification of anaerobic bacteria (7). However, these methods are time consuming to perform and require specialized equipment and, generally, 24 to 48 h of incubation under anaerobic conditions (7). Schreckenberger and Blazevic (8, 9) and Lindquist and Kjellander (6) have described a series of biochemical tests for the characterization of anaerobic bacteria which could be incubated aerobically and read after 4 h of incubation. In these tests, a large inoculum-to-substrate ratio is used to promote the enzymatic reactions and enable the results to be read within 2 to 4 h. This paper describes a rapid, one-step glutamic acid decarboxylase test for the identification of certain species of anaerobic bacteria.

## MATERIALS AND METHODS

The organisms used in this study consisted of 345 fresh or freshly frozen clinical isolates of anaerobic bacteria representing 11 genera and 49 species: *Actinomyces* (2 strains); *Bacteroides* (128 strains, including *B. asaccharolyticus*, 2; *B. bivius*, 13; *B. capillosus*, 9; *B. disiens*, 3; *B. distasonis*, 11; *B. fragilis*, 28; *B. melaninogenicus* group, 2; *B. oralis*, 5; *B. ovatus*, 3; *B. thetaiotaomicron*, 14; *B. uniformis*, 1; *B. vulgatus*, 9; *Bacteroides* species, 28); *Bifidobacterium* (1 strain); *Clostridium* (73 strains, including *C. bifermentans*, 2; *C. butyricum*, 1; *C. clostridiiforme*, 9; *C. innocuum*, 2; *C. perfringens*, 42; *C. putrefaciens*, 2; *C. ramosum*, 5; *C. sordellii*, 1; *C. sporogenes*, 2; *Clostridium* species, 7); *Eubacterium* (18 strains, including *E. aerofaciens*, 2; *E. contortum*, 1; *E. cylindroides*, 2; *E. lentum*, 9; *E. ventriosum*, 1; *Eubacterium* species, 3); *Fusobacterium* (23 strains, including *F. mortiferum*, 1; *F. naviforme*, 2; *F. necrophorum*, 2; *F. nucleatum*, 3; *Fusobacterium* species, 15); *Lactobacillus* (7 strains); *Peptostreptococcus* (61 strains, including *P. asaccharolyticus*, 11; *P. anaerobius*, 15; *P. magnus*, 17; *P. micros*, 2; *P. productus*, 2; *P. prevotii*, 8; *P. tetradius*, 6); *Propionibacterium* (4 strains); *Streptococcus* (16 strains, including *S. constellatus*, 2; *S. intermedius*, 13; *S. morbillorum*, 1); and *Veillonella* (12 strains). These organisms were identified by the procedures outlined in the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (5). Fresh isolates were stored in prereduced, anaerobically sterilized chopped-meat liquid (Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) at room temperature until used. Frozen cultures were kept in skimmed milk at −70°C. Three days before use, the frozen cultures were subcultured to fresh thioglycolate broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.5% yeast extract, 1% hemin, and 0.02% vitamin $K_1$ and incubated at 35 to 37°C. All organisms were then subcultured to fresh brain heart infusion agar plates (Difco Laboratories, Detroit, Mich.) supplemented with 5% sheep blood, 0.5% yeast extract, 1% hemin, and 0.02% vitamin $K_1$. The brain heart infusion agar plates were then incubated for 18 to 24 h at 35 to 37°C under anaerobic conditions in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) with an atmosphere of 5% carbon dioxide, 10% hydrogen, and 85% nitrogen.

The glutamic acid test substrate was prepared with 0.05 g of bromcresol green sodium salt (Eastman Kodak Co., Rochester, N.Y.)–1.0 g of L-glumatic acid (Sigma Chemical Co., St. Louis, Mo.)–0.3 ml of Triton X-155 in 1 liter of distilled water. The substrate was placed in a brown-glass bottle and refrigerated at 4 to 5°C until used.

The test was performed by scraping 18- to 24-h anaerobic cultures grown on brain heart infusion agar plates with a sterile, cotton-tipped applicator stick and suspending it in 0.5 ml of glutamic acid test substrate until a turbidity approximately equal to that of a no. 1 McFarland turbidity standard was obtained. The inoculated substrate was then incubated aerobically at 35 to 37°C. Readings were taken at 1, 2, 3, 4, and 24 h. A definite blue color was recorded as positive, whereas any other color was recorded as negative.

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## RESULTS

All isolates of *B. fragilis*, *B. thetaiotaomicron*, *B. uniformis*, *C. perfringens*, and *C. sordellii* decarboxylated glutamic acid. Three strains of *B. ovatus* tested were positive, whereas 63.6% (7 of 11) *B. distasonis* and 44.4% (4 of 9) *B. vulgatus* isolates were also positive for glutamic acid decarboxylase. No other species tested were found to be positive. Greater than 90% of the positive reactions occurred within 1 h, and all of the positive reactions occurred within 4 h. Incubation for 24 h did not alter the reactions or interfere with the readability of the reactions.

## DISCUSSION

Freier et al. (4), using the rapid glutamic acid decarboxylase method of Wauters and Cornelis (12) for testing aerobic and anaerobic bacteria, have suggested that glutamic acid decarboxylase may be of some use in the identification of anaerobes. Glutamic acid decarboxylase facilitates the decarboxylation of glutamic acid to yield gamma-aminobutyric acid and carbon dioxide. It is apparent that some type of lytic agent is essential for the liberation of the enzyme, since use of the glutamic acid substrate without any lytic agent gave inconsistent results. Wauters and Cornelis (12) and Freier et al. (4) treated the organisms with toluene to liberate this enzyme. Toluene, a lipid solvent, presumably destroys parts of the cell wall, thereby allowing contact between the enzyme and the substrate. Schreckenberger et al. (P. C. Schreckenberger, B. J. Jilly, and L. J. LeBeau, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C66, p. 288) have described a one-step, rapid method for detecting glutamic acid decarboxylase in anaerobic bacteria. In this procedure, the toluene treatment step is eliminated by placing the substrate in a hypertonic sodium chloride solution. This one-step procedure is quicker to perform and gives results comparable to those obtained with toluene treatment. Miranda et al. (C. J. Miranda, M. A. Edelstein, and D. M. Citron, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C36, p. 280) have evaluated a commercial prototype of this test and reported good results after 2 h of incubation. The drawbacks of this procedure are that the hypertonic NaCl tends to buffer the solution at a lower pH than is desirable and the NaCl tends to precipitate out of solution upon storage of the substrate. Therefore, other lytic agents were tested. The lipid solvent Triton X-155, unlike toluene, is soluble in water, thereby enabling it to be incorporated directly into the test substrate, resulting in a one-step procedure. This reagent is also very stable. Test substrate stored at 4 to 6°C showed good activity even after 12 months.

The results of this study confirm the works of previous authors. Wauters and Cornelis (12) reported that all of the *B. fragilis* (22 of 22) strains and 4 of 8 fusobacteria were positive for glutamic acid decarboxylase. This study, as well as that of Freier et al. (4), Schreckenberger et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, C66, p. 280), and Miranda et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, C36, p. 280), have also shown that *B. fragilis* is positive for this enzyme, but none of the *Fusobacterium* species were positive for glutamic acid decarboxylase. It is not clear in the report of Wauters and Cornelis (12) which species of fusobacteria were positive for glutamic decarboxylase. It is possible that the species they report as being positive would not be classified as a *Fusobacterium* species under present nomenclature.

The results of this study show that glutamic acid decarboxylase is useful in the identification of the *B. fragilis* group and *C. perfringens*. Further differentiation of the *B. fragilis* group may be accomplished with the spot indol test and other rapid methods (6, 8, 9, 11). The use of this rapid test for glutamic acid decarboxylase will enable laboratory workers to report these pathogens to physicians within 4 h after they have been isolated in pure culture.

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