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Characterization of Anaerobic Bacteria by Using a Commercially Available Rapid Tube Test for Glutamic Acid Decarboxylase

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A rapid glutamic acid decarboxylase microdilution test for presumptive identification of certain anaerobic bacteria was marketed recently by Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga. The test was evaluated with 474 clinical isolates, representing 11 genera and 54 species, and was found to be a useful aid in the presumptive identification of *Bacteroides fragilis*, *B. distasonis*, *B. vulgatus*, *B. thetaiotaomicron*, *B. ovatus*, *B. uniformis*, *B. eggerthii*, *Clostridium perfringens*, *C. barati*, *C. sordellii*, and *Eubacterium limosum*.

In 1974, Wauters and Cornelis described a simple, rapid, two-step procedure to test for glutamic acid decarboxylase (GDC) in certain gram-negative bacteria (8). A toluene treatment of the bacteria preceded the incubation of the organisms in the presence of the glutamic acid substrate. Interestingly, 22 of 22 strains of *Bacteroides fragilis* and 4 of 8 strains of organisms that were called "*Fusiformis*" species gave positive reactions, whereas 2 of 2 strains labeled *B. melaninogenicus* and 5 of 5 so-called "*Sphaerophorus*" strains were negative in this test (8). In 1976, Freier et al. tested several additional species of anaerobic bacteria with a toluene extraction procedure modified from that of Wauters and Cornelis and confirmed that the test was useful for presumptive identification of *B. fragilis* (4). Freier et al. also found that *Clostridium perfringens* gave a positive reaction and stated that the test was useful for identification of this species (4). In 1984, Jilly et al. described their results with 345 strains of anaerobic bacteria which they tested using a one-step glutamic acid test that eliminated the need for toluene treatment (6). Jilly et al. reported that all isolates of *B. fragilis*, *B. thetaiotaomicron*, *B. uniformis*, and *B. ovatus* were positive and that 7 of 11 *B. distasonis* strains and 4 of 9 *B. vulgatus* strains were positive. They also found that 42 of 42 isolates of *C. perfringens* and the only strain of *Clostridium sordellii* tested were positive for GDC (6). All other anaerobic bacteria, including several other *Bacteroides* and *Clostridium* species, were found to be negative in the test (6).

Recently, Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga., modified this procedure and developed a modified substrate to test for GDC in a semisolid medium contained in a microdilution tube. The medium included the following ingredients: 2.0 g of L-glutamic acid, 0.07 g of bromcresol green-sodium salt, 0.3 ml of Triton X-155, and 3.0 g of agar in 1 liter of water. The substrate was inoculated heavily with a pure culture of an organism, incubated aerobically for up to 4 h, and then observed for a color change from green to dark blue.

The object of this study was to examine the performance of a prototype of this new commercial, packaged microdilution procedure which is intended to aid in the rapid identification of certain anaerobic bacteria. We characterized a wider variety of anaerobic bacteria with the test than had been examined with the other GDC test procedures cited previously and found that in combination with some suggested additional tests, the GDC test provided useful information that could aid in rapid identification of commonly encountered anaerobes.

The bacteria tested included 474 strains of anaerobic bacteria, representing 11 genera and 54 species (Table 1). These clinical isolates were from a variety of sources, including blood, wounds and abscesses at various anatomic sites, pleural fluid, peritoneal fluid, and other specimens. The strains had been stored in chopped-meat medium, transferred to Centers for Disease Control anaerobe blood agar (Carr-Scarborough), repeatedly subcultured on that medium, tested repeatedly to ensure purity, and identified. Identifications were confirmed in the same time frame as the study, with traditional conventional reference laboratory media and procedures for identification, including Gram reaction, morphology, relationships to oxygen, and growth characteristics on agar (e.g., Centers for Disease Control anaerobe blood agar and egg yolk agar) and in liquid media. Reactions in peptone-yeast extract-based media were noted for determining biochemical characteristics, and gas-liquid chromatography was also used (1, 5).

The commercial GDC packaged microdilution tube was developed and kindly supplied to us by Dennis L. Carr of Carr-Scarborough. It consisted of a small microdilution tube (6 by 50 mm) containing the GDC substrate. The microdilution tube in which the test is done was inserted into a larger screw-cap carrier tube (13 by 82 mm) which contained a plug of agar in the bottom of the carrier tube only to provide support for the microdilution tube. These tubes were stored at 2 to 4°C in the dark and were not used after the expiration date of 3 months.

A heavy inoculum was prepared from a fresh, pure culture of the isolate to be tested that had been grown on Centers for Disease Control anaerobe blood agar incubated anaerobically for 48 h. A heavy paste was scraped from the culture plate by using a small inoculating loop, and the inoculum was stabbed and mixed into the upper one-fourth of the GDC microdilution tube medium. Following inoculation, the carrier tube containing the microdilution tube was incubated aerobically at 35°C for up to 4 h before a final reading was made (some isolates gave positive tests in less than 4 h). A positive GDC test was indicated by a color change from

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TABLE 1. Positive GDC test results[a]

Organism	No. of strains	
	Tested	Positive
Bacteroides distasonis	22	19
B. fragilis	28	28
B. ovatus	21	17
B. thetaiotaomicron	21	21
B. uniformis	22	21
B. vulgatus	21	20
B. eggerthii	2	2
B. splanchnicus	4	1
Fusobacterium gonidiaformans	3	1
F. mortiferum	7	1
F. necrophorum	10	7
F. varium	20	12
Eubacterium limosum	17	17
Propionibacterium acnes	23	11
Peptostreptococcus micros	15	8
Clostridium barati	15	15
C. difficile	12	3
C. perfringens	31	30
C. sordellii	10	10

[a] Negative reactions were obtained with *Bacteroides asaccharolyticus* (4 strains), *B. bivius* (7 strains), *B. disiens* (2 strains), *B. intermedius* (1 strain), *B. melaninogenicus* (5 strains), *B. melaninogenicus* group (6 strains), *B. oralis* (1 strain), *B. oris-B. buccae* (7 strains), *B. veroralis* (1 strain), *B. ureolyticus* (4 strains), *Capnocytophaga* species (4 strains), *Fusobacterium nucleatum* (11 strains), *F. russii* (1 strain), *E. lentum* (8 strains), *Actinomyces israelii* (1 strain), *Propionibacterium avidum* (2 strains), *P. granulosum* (2 strains), *Peptostreptococcus anaerobius* (8 strains), *P. asaccharolyticus* (10 strains), *P. magnus* (10 strains), *P. prevotii* (10 strains), *Veillonella parvula* (10 strains), *Staphylococcus saccharolyticus* (4 strains), *Streptococcus intermedius* (6 strains), *Clostridium bifermentans* (2 strains), *C. butyricum* (5 strains), *C. cadaveris* (2 strains), *C. clostridiiforme* (8 strains), *C. glycolicum* (1 strain), *C. innocuum* (9 strains), *C. paraputrificum* (3 strains), *C. ramosum* (9 strains), *C. septicum* (3 strains), *C. sporogenes* (1 strain), and *C. tertium* (2 strains).

green to deep blue in the inoculated portion of the medium. Light blue or no change from green was considered a negative test.

The GDC test was positive in a limited number of species, with the most consistent positive results in members of the *B. fragilis* group, *Eubacterium limosum*, *C. perfringens*, *Clostridium barati*, and *C. sordellii* (Table 1).

Our findings suggest that the commercial GDC microdilution tube test, like the one-step procedure described by Jilly et al. (6) from which it was modified, has potential usefulness for rapid identification of certain anaerobic bacteria. However, as pointed out by the manufacturer, this product is not a stand-alone system and provides a test result that should be interpreted only as part of a battery of additional differential test results (such as catalase and indole production) and characteristics such as Gram reaction, microscopic morphology, colony features, relation to oxygen, and other growth characteristics. The GDC test was simple to use and seems to provide a rapid presumptive clue to the presence of members of the most commonly isolated species of the *B. fragilis* group versus all the tested non-*B. fragilis* group species of the genus *Bacteroides* which were GDC negative. The test should also aid in rapid differentiation between *C. perfringens*, which tested positive and is the most common *Clostridium* sp. that we encounter in clinical specimens (7), and several other *Clostridium* species which tested negative. We emphasize that a negative GDC test cannot be used to rule out a member of the *B. fragilis* group or *C. perfringens* because not all are positive, and further characterization of clinical isolates that resemble these species should be done to provide correct identifications.

In contrast to Jilly et al., we found that many strains of *Fusobacterium* spp. gave positive GDC tests. We do not know why all the *Fusobacterium* spp. previously tested were negative. However, we tested 52 strains, whereas only 23 strains of *Fusobacterium* spp. were tested by Jilly et al., and the differences may relate to sample size. Modifications in the substrate medium composition and current format of the commercial test may also have contributed to differences in the results. Nonetheless, we conclude that the GDC test per se cannot be used to differentiate between *Bacteroides* spp. and *Fusobacterium* spp. For many of the fusobacteria tested, the indicator changed within the substrate to a medium blue. This color was neither as deep a blue as a true positive should be nor as pale as a true negative might be if the inoculum was simply too heavy. This medium blue was considered a weak response. Several strains of *Fusobacterium varium* gave these kinds of equivocal reactions, even on repeat testing, and these were called positive reactions. Wauters and Cornelis found four of eight isolates to be positive for GDC. These isolates were probably *Fusobacterium* spp., though the nomenclature and classification have since changed (8). Colony characteristics, morphology, resistance to kanamycin (e.g., 1,000-μg-disk test), enhanced growth in the presence of 20% bile, hydrolysis of esculin, and production of catalase are some practical additional properties that help differentiate the *B. fragilis* group and *Fusobacterium* spp., as described by Dowell and Lombard (3). However, metabolic-product analysis and some additional tests will be necessary for definitive identification (7).

The GDC-positive, kanamycin-resistant, gram-negative anaerobic rods that show enhanced growth on 20% bile medium are likely to be members of the *B. fragilis* group (7). These bacteria can be differentiated further by using a spot indole test to divide the *B. fragilis* group into indole-negative and indole-positive subgroups (7). For example, *B. fragilis* is by far the most common species in the indole-negative subgroup. Definitive identification could be done as clinically warranted by determining the results of certain carbohydrate fermentation tests (7). In our experience, *Bacteroides eggerthii* and *B. splanchnicus*, although common in feces, are rare in properly collected clinical specimens and thus should not be a frequent diagnostic problem in properly collected specimens. Fermentation of sucrose by species of the most common clinically significant indole-positive *B. fragilis* group, but not by *B. eggerthii* and *B. splanchnicus*, would aid in correct identification.

Of the gram-positive organisms that gave a positive reaction with the GDC test, the clostridia are the most important clinically. Most strains of *C. perfringens*, *C. barati*, and *C. sordellii* were positive for GDC, and occasional strains of *Clostridium difficile* (3 of 12) were positive. Of these four species, *C. perfringens* and *C. sordellii* gave the most consistent and strongest positive reactions, a finding that is in agreement with the observations of others (4, 6). To our knowledge, results of testing *C. barati* and *C. difficile* for GDC have not been reported elsewhere. To differentiate these four *Clostridium* species, microscopic morphologic features would be especially helpful. *C. difficile* and *C. sordellii* form subterminal spores readily, while *C. perfringens* has a distinctive "boxcar" appearance and does not readily form spores in media commonly used in the clinical laboratory. *C. sordellii* is indole positive, whereas *C. difficile* is indole negative; thus, a spot indole test would aid in their further differentiation. *C. barati* resembles *C. perfringens*, but gelatin hydrolysis can differentiate these species (5, 7).

Other anaerobic gram-positive bacilli giving positive re-

sults in the GDC test were *E. limosum* (100%) and *Propionibacterium acnes* (48% of strains tested; Table 1). These organisms have not been positive for GDC in previous studies (4, 6). Though *E. limosum* is somewhat uncommon in some clinical laboratories, it has been considered the cause of septicemia and synovitis in two immunocompromised patients at Indiana University Hospital and may be significant under other clinical circumstances. Therefore, to differentiate these organisms, spot indole and catalase tests and a test for reduction of nitrate can be done. *E. limosum* should show negative results in all these tests. *P. acnes* is usually catalase positive and indole positive and reduces nitrate to nitrite.

The only gram-positive coccus that gave a positive GDC reaction was *P. micros* (53% of strains). Thus, *Peptostreptococcus micros* could be differentiated presumptively when the GDC reaction was positive and the organism showed appropriate morphologic features (i.e., tiny gram-positive cocci producing chains of 10 or more cells or clusters) in gram-stained smears. A negative GDC reaction for a gram-positive coccus would not be helpful. A test for phosphatase production (positive for *P. micros* and negative for *Peptostreptococcus magnus*) may be more reliable than cell size and chain formation because of strain-dependent size variation and morphologic variation of the cocci in various culture media (2).

This was the first evaluation of a commercially available GDC test. To our knowledge, a commercially available GDC test specifically applied as an aid for identification of anaerobic bacteria has not been available in packaged kits designed specifically for anaerobes. We found the test simple, easy to inoculate and interpret, and inexpensive. The test fits the work flow of clinical anaerobic bacteriology in that it can be performed at the same time as Gram reaction and morphology are determined for colonies available in pure culture. There is no requirement for anaerobic conditions or other special atmospheres because incubations are done in ambient air for up to 4 h. Positive reactions should be considered definite, and with some isolates, these reactions were seen after 0.5 h of incubation.

In conclusion, the GDC test, used in conjunction with colony characteristics, microscopic features, Gram reaction, spot indole test, a test for catalase, and a few other procedures, provides a simple and practical addition to the differential methods that can be used for rapid presumptive identification of common clinically encountered anaerobes. Follow-up characterization of isolates with traditional conventional, alternative rapid tests, including those based on the use of chromogenic substrates, and other packaged kits can then be done as needed or desired to permit definitive identification.

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