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API QuadFERM+ with Rapid DNase for Identification of *Neisseria* spp. and *Branhamella catarrhalis*

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The QuadFERM+ system (Analytab Products, Plainview, N.Y.), a 2-h carbohydrate degradation method for the identification of *Neisseria* spp., was evaluated along with a rapid DNase test for confirmation of *Branhamella catarrhalis*. QuadFERM+ identified 100% of 82 *N. gonorrhoeae* and 96% of 54 *N. meningitidis* strains. The two misidentified meningococcal strains were biochemically atypical and were also misidentified by the conventional method. Of 26 *N. lactamica* strains, 25 (96%) were correctly identified. Of 21 *Neisseria* spp., 14 (67%) produced carbohydrate reactions in agreement with the conventional procedure, and 7 strains produced detectable acid in the QuadFERM+ from maltose and sucrose but not glucose. All 9 *N. cinerea* and 30 *B. catarrhalis* strains were asaccharolytic by QuadFERM+. The rapid DNase test was positive for all *B. catarrhalis* strains and negative for all other organisms. Two beta-lactamase-positive *N. gonorrhoeae* strains and 25 (93%) of 27 beta-lactamase-positive *B. catarrhalis* strains were detected by the 2-h acidometric beta-lactamase test on the strip. QuadFERM+ with rapid DNase is a simple and easily interpretable method for identification of these organisms in the clinical laboratory.

Methods for the identification of *Neisseria* spp. include carbohydrate degradation tests (8, 13), chromogenic enzyme substrate tests (6, 7, 16, 17), and combinations of these two approaches (14, 15). Conventional procedures use cystine-tryptic digest agar (CTA) base medium with added carbohydrates and may require prolonged incubation for some isolates before results are available (13). Various modifications of the carbohydrate degradation technique have been developed that allow identification of *Neisseria* spp. within 1 to 4 h (3, 11, 13). Most recently, identification methods with specific chromogenic enzyme substrates have become available for rapid identification of *Neisseria* spp. that grow on selective medium (i.e., *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*) (6, 7, 14, 16, 17). These systems also provide a presumptive identification of *Branhamella catarrhalis*, an organism of recognized clinical importance that can also be occasionally recovered on selective medium (e.g., modified Thayer-Martin [MTM] and Martin-Lewis agars) (4; G. V. Doern, Clin. Microbiol. Newsl. 7:75–78, 1985). This organism is negative in carbohydrate degradation tests, and identification is aided by the demonstration of nitrate reduction and DNase production (4, 13). In addition, these two conventional tests help to differentiate *B. catarrhalis* from another recently described asaccharolytic species, *N. cinerea* (10).

The QuadFERM+ system (Analytab Products, Plainview, N.Y.) is a commercial modification of the rapid carbohydrate degradation technique. In addition to the carbohydrates commonly used to identify *Neisseria* spp., this system includes a penicillin test for the detection of beta-lactamase production by *N. gonorrhoeae* and *B. catarrhalis*. In the present study, QuadFERM+ was evaluated for its ability to provide 2-h identifications of *Neisseria* spp. In addition, a 2-h DNase test (Analytab) (L. H. Greene, P. M. Zel, and J. A. Strong, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C366, p. 389) was evaluated concurrently as an aid in the identification of *B. catarrhalis*.

MATERIALS AND METHODS

Organisms. Organisms used in this study were fresh clinical isolates, stock strains, or recent clinical isolates that had been stored at −70°C in horse serum-Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) (1:1). Clinical isolates were obtained from cultures submitted to the University of Illinois Clinical Microbiology Laboratories and from specimens obtained at the Howard Brown Memorial Clinic, a Chicago-area clinic for sexually transmitted diseases. Organisms (n = 222) included: 82 *N. gonorrhoeae* (isolated from urethra, 35; endocervix, 27; rectum, 15; oropharynx, 2; conjunctiva, 1; stock penicillinase-producing *N. gonorrhoeae*, 2); 54 *N. meningitidis* (throat, 24; rectum, 16; blood; 4; urethra, 4; cerebrospinal fluid, 2; conjunctivae, 2; sputum, 2); 26 *N. lactamica* (throat, 21; cerebrospinal fluid, 2; urethra, 1; rectum, 1; stock strain, 1); 9 *N. cinerea* (stock strains, 4; respiratory tract, 3; wound, 1; cervix, 1); 30 *B. catarrhalis* (respiratory tract, 12; conjunctivae, 8; stock strains, 6; sinus aspirates, 2; blood, 1; wound, 1); and 21 *Neisseria* spp., including 14 *N. subflava* (respiratory tract, 11; blood, 3), 5 *N. mucosa* (respiratory tract, 4; conjunctivae, 1), and 2 *N. sicca* (semen, 1; stock strain, 1). All strains that were stored frozen were subcultured at least three times before QuadFERM+ testing and the conventional test battery.

QuadFERM+ system. The QuadFERM+ system consists of a plastic strip containing several wells; six wells contain dehydrated phosphate buffers and a phenol red indicator. Four of the wells contain glucose, maltose, lactose, or sucrose. The fifth well contains no carbohydrate (negative control). The sixth contains penicillin for the detection of beta-lactamase activity. During this evaluation, a seventh

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well containing phenol red and a DNA substrate was also included in the test battery.

To inoculate the system, a suspension of a pure culture of the organism equivalent to or greater than a no. 3 McFarland standard is prepared in saline from a subculture of the organism growing on chocolate agar, blood agar, or a suitable selective medium. Samples (200 μl) are placed in each of the seven wells, and the wells are sealed with a paper adhesive strip. The test system is incubated at 35°C for 2 h in a non-CO_2 incubator. Reactions are detected by a change in the color of the phenol red indicator; positive reactions are orange or yellow in comparison with the negative control well, and negative tests remain red. The QuadFERM+ system is designed to assist in the identification of *Neisseria* species recovered on either selective or nonselective medium.

Conventional procedures. All gram-negative, oxidase-positive diplococcal isolates were tested for the ability to produce acid from glucose, maltose, fructose, sucrose, and lactose by a 4-h rapid carbohydrate degradation technique (8, 13) and for the ability to hydrolyze *o*-nitrophenyl-β-D-galactopyranoside. These tests and QuadFERM+ were inoculated from an 18- to 24-h subculture of the organism on chocolate agar. Beta-lactamase production was determined by the chromogenic cephalosporin test (Cefinase, BBL) (12). Isolates that grew on MTM agar (BBL) were also tested for production of beta-galactosidase, prolylaminopeptidase, and gamma glutamylaminopeptidase with the Gonochek II system (E. I. du Pont de Nemours & Co. Inc., Wilmington, Del.) (7, 17). Organisms that produced atypical carbohydrate degradation patterns with the rapid carbohydrate degradation method were also tested with conventional CTA-base carbohydrates (BBL) and a modified CTA carbohydrate slant technique (13). Atypical meningococci were also serogrouped with group-specific meningococcal antisera (Welcome Diagnostics, Research Triangle Park, N.C.) by the slide agglutination technique (13). DNase was detected by spot inoculation of organisms on DNase test medium with toluidine blue O (GIBCO Diagnostics, Madison, Wis.) and reading for DNA hydrolysis after 24 and 48 h of incubation at 35°C in 5 to 7% CO_2. Additional tests for some isolates included reduction of nitrate and nitrite, production of iodine-positive polysaccharide from sucrose, and susceptibility to colistin (9, 10, 13). Pigmentation of some isolates was determined by rubbing colonies from chocolate or brain heart infusion agar (BBL) subcultures onto white filter paper.

RESULTS

The conventional identification tests identified all isolates within 4 to 24 h. The rapid carbohydrate degradation method produced easily interpretable carbohydrate reactions for all organisms, except two isolates of *N. meningitidis* and one isolate of *N. subflava*. These meningococci, both blood isolates, were a maltose-negative serogroup Y strain and an asaccharolytic serogroup C strain. Both grew on MTM agar and were gamma-glutamylaminopeptidase positive, prolylaminopeptidase negative, and beta-galactosidase negative. These two meningococcal isolates produced similar results in CTA-based carbohydrate test media. The *N. subflava* strain was glucose negative but maltose and sucrose positive after 4 h. This isolate was positive for glucose, maltose, sucrose, and fructose and negative for lactose in CTA-base carbohydrate tests after 24 h of incubation. All *N. subflava* strains failed to reduce nitrate, reduced nitrite to nitrogen gas, produced iodine-positive polysaccharide from sucrose, and produced smooth, yellowish colonies on brain heart infusion agar. All 30 *B. catarrhalis* and all 9 *N. cinerea* isolates were asaccharolytic after 4 h. In addition, all *B. catarrhalis* isolates were DNase positive after 24 h on toluidine blue O DNase test agar, whereas all other organisms were negative after an additional 24-h incubation. Two of the 54 *N. gonorrhoeae* and 27 of the 30 *B. catarrhalis* were beta-lactamase positive by the chromogenic cephalosporin test.

In most cases, the QuadFERM+ carbohydrate battery performed similarly to the conventional method. The system identified all 52 (100%) *N. gonorrhoeae* strains, with acid production from glucose being the only positive reaction. Among the meningococci, 52 (96%) of the 54 strains that produced acid from both glucose and maltose were identified by QuadFERM+. The asaccharolytic serogroup C strain and the maltose-negative serogroup Y strain produced similar reactions on QuadFERM+. The rapid DNase test was negative for all gonococci and meningococci tested.

Of the 26 *N. lactamica* isolates, 25 (96%) were positive for glucose, maltose, and lactose after 2 h and a single strain was positive for glucose and maltose but negative for lactose after this time. All 9 *N. cinerea* and all 30 *B. catarrhalis* isolates produced no acid in any of the carbohydrate wells during the incubation period. Of the 21 *Neisseria* spp., 14 (67%) produced positive reactions with glucose, maltose, and sucrose and were lactose negative. Seven strains (five *N. subflava* and two *N. mucosa*) produced acid from maltose and sucrose but failed to acidify glucose. One of the seven was the same strain that was glucose negative in the 4-h conventional test system.

The QuadFERM+ acidometric beta-lactamase test detected both beta-lactamase-positive *N. gonorrhoeae* strains and 25 (93%) of the 27 beta-lactamase-positive *B. catarrhalis* strains. Two beta-lactamase-positive *B. catarrhalis* strains were negative by QuadFERM+ after 2 h. All other organisms produced negative beta-lactamase test results on the system.

The rapid QuadFERM+ DNase test was positive for all 30 *B. catarrhalis* isolates tested after 2 h. All the remaining organisms, including the nine asaccharolytic *N. cinerea* strains, were rapid DNase negative.

DISCUSSION

The QuadFERM+ system produced reliable results for most of the isolates tested. All *N. gonorrhoeae* and biochemically typical *N. meningitidis* isolates were correctly identified within 2 h. The atypical meningococci produced similar reactions in both QuadFERM+ and conventional test systems. The single *N. lactamica* strain that was lactose negative by QuadFERM+ produced a slow reaction in the lactose tube of the conventional 4-h carbohydrate battery and in the *o*-nitrophenyl-β-D-galactopyranoside test. The five *N. subflava* and two *N. mucosa* strains that produced positive reactions with maltose and sucrose and negative reactions with glucose required the full 4 h to acidify glucose in the conventional system. There is little chance, however, that these organisms would have been misidentified as gonococci or meningococci by QuadFERM+, since the maltose and sucrose reactions were strongly positive for all seven strains after 2 h. Because some *N. subflava* isolates produce acid from glucose and maltose but not sucrose, colonial morphology and pigmentation should be noted for all isolates tested on the QuadFERM+ system, particularly

when they are recovered from the upper respiratory tract. Saprophytic *Neisseria* isolates that are recovered on nonselective medium should also be tested for the ability to grow on selective media to help differentiate the sucrose-negative *N. subflava* strains from *N. meningitidis*. Because carbohydrate reactions alone are insufficient criteria for identification of saprophytic *Neisseria* spp., additional tests must be performed on occasional clinically significant isolates.

Both carbohydrate degradation tests and chromogenic enzyme substrate tests for identification of *Neisseria* spp. have advantages and disadvantages, and results obtained with either method must be interpreted carefully. Unlike carbohydrate utilization procedures, including the QuadFERM+ system, chromogenic substrate tests are currently restricted to identifying the three *Neisseria* spp. that grow on selective medium. This criterion, however, may not always be reliable for exclusion of saprophytic species. During this study, 5 of the 14 *N. subflava* strains were recovered on MTM medium from oropharyngeal cultures of homosexual men. Similar isolates were also obtained from clinic patients during another evaluation (7). These strains grew luxuriantly on MTM agar on primary isolation and subculture and produced chromogenic substrate test reactions consistent with *N. gonorrhoeae* (i.e., beta-galactosidase negative, gamma-glutamylaminopeptidase negative, and prolylaminopeptidase positive) (7; unpublished data). Acid production from maltose and sucrose clearly differentiated these organisms from gonococci and meningococci in both the conventional carbohydrate system and QuadFERM+. Chromogenic enzyme substrate tests, however, do provide useful information for identifying the pathogenic *Neisseria* spp. with aberrant carbohydrate utilization patterns (e.g., maltose-negative or asaccharolytic meningococci) that would be misidentified by carbohydrate utilization tests. *N. cinerea*, another problem organism, also occasionally grows on selective medium (10) and, like *N. gonorrhoeae*, is prolylaminopeptidase positive, beta-galactosidase negative, and gamma glutamylaminopeptidase negative (1). *N. cinerea* strains are generally asaccharolytic in carbohydrate degradation test systems, including the 4-h conventional procedure and the 2-h QuadFERM+ system in the present study and the 1-h RIM-N system for *Neisseria* identification (11). Some strains of *N. cinerea*, however, have been reported to produce weak positive glucose reactions in some carbohydrate test systems, including the Minitek, RapID-NH, and BACTEC Neisseria differentiation kits (1, 2). *N. cinerea* strains may, therefore, be misidentified by both carbohydrate utilization and enzymatic identification methods, necessitating additional tests (10).

The Quad-FERM+ rapid DNase test performed well as a confirmatory test for *B. catarrhalis*, and complete agreement was observed between this test and the conventional procedure. This test was particularly helpful for differentiating asaccharolytic *N. cinerea* strains from *B. catarrhalis*. Use of this system precludes the need for a DNase test requiring incubation for 24 h or longer.

The QuadFERM+ beta-lactamase test detected both penicillinase-producing *N. gonorrhoeae* and 25 of 27 *B. catarrhalis* strains that were positive by the nitrocefin test. It has been shown that some beta-lactamase-positive *B. catarrhalis* isolates may not produce positive results in rapid acidometric procedures because of the inducible nature of the enzyme (5; Doern, Clin. Microbiol. Newsl.). Futhermore, since the enzyme is cell associated, inoculum size may also affect the rate of penicillin degradation and, hence, penicilloic acid detection (5). This would be a problem especially in non-growth-dependent assay systems like QuadFERM+. Since the inoculum requirement in this system is a no. 3 McFarland or greater, adjustment of the inoculum to a heavier suspension may eliminate false-negative beta-lactamase results without compromising the performance of the other tests.

The QuadFERM+ with rapid DNase is a rapid system that lends itself particularly well to a busy clinical laboratory. Inoculation is fast and simple, and the results are easily interpretable after 2 h. It has the added advantage of incorporating a confirmatory test for *B. catarrhalis*, an organism of recognized significance in certain clinical settings, with the carbohydrate tests routinely used to identify the frequently isolated pathogens *N. gonorrhoeae* and *N. meningitidis*.

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