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Phadebact Monoclonal GC OMNI Test for Confirmation of *Neisseria gonorrhoeae*

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The Phadebact Monoclonal GC OMNI Test (Pharmacia Diagnostics, Piscataway, N.J.) is used for the definitive identification of *Neisseria gonorrhoeae*. In this test, boiled organisms are examined by using a 1-min coagglutination technique. A total of 776 *Neisseria* strains, confirmed to the species level by patterns of acid production from carbohydrates incorporated in cysteine-tryptose agar or morphologically consistent with *Neisseria meningitidis* and fluorescent antibody negative, were tested by the coagglutination technique. Of the 516 isolates of *N. gonorrhoeae*, 8 (1.6%) were negative with the OMNI Test. Of the 260 isolates of *Neisseria* spp. other than *N. gonorrhoeae*, none showed a positive coagglutination reaction. The Phadebact Monoclonal GC OMNI Test provided rapid, accurate identification of *N. gonorrhoeae* (sensitivity, 98.4%; specificity, 100%).

In the past few years, reports of various rapid tests for the culture confirmation of *Neisseria gonorrhoeae* have appeared. However, decreased specificity (1–3, 6), mainly due to cross-reactions with *Neisseria cinerea*, has been a problem with many of the tests. In an earlier study (4), we reported the sensitivity and specificity of the original Phadebact Gonococcus Test to be 94.3 and 100%, respectively. The present study was initiated to determine the sensitivity and specificity of the new Phadebact Monoclonal GC OMNI Test (Pharmacia Diagnostics, Piscataway, N.J.) for the identification of *N. gonorrhoeae*.

MATERIALS AND METHODS

Specimen handling. All specimens were inoculated onto Thayer-Martin medium (GIBCO Diagnostics, Madison, Wis.), incubated overnight in candle extinction jars at 35 to 36°C, and delivered to the Massachusetts Department of Public Health, Center for Laboratories and Communicable Disease Control, the next day. When possible, the cultures were examined after both 24 and 48 h of incubation (5, 7). Oxidase-positive colonies with morphological features typical of *Neisseria* spp. and containing gram-negative diplococci were considered to be presumptively positive for *N. gonorrhoeae* by the criteria of the Centers for Disease Control (5).

Strains. The 786 organisms tested consisted of 776 strains of *Neisseria* spp., 8 *Moraxella* spp., 1 *Staphylococcus* sp., and 1 *Candida* sp. The specimens collected were from 539 anogenital, 238 pharyngeal, and 9 other body sites. *Neisseria* and *Branhamella* distribution by body site cultured is shown in Table 1. All *Neisseria* spp. may be isolated from both anogenital and other body sites. Since *Neisseria* spp. are often recovered from body sites other than anogenital, test specificity is crucial.

All organisms from anogenital sources which demonstrated a positive fluorescent-antibody (FA) reaction and which were morphologically compatible with *N. gonorrhoeae* were considered to be *N. gonorrhoeae*. Organisms from anogenital sources which were FA negative were identified to the species level by the cysteine-tryptose agar (CTA) technique. Pharyngeal cultures which gave a positive FA reaction and were confirmed by CTA testing were considered to be *N. gonorrhoeae*. Pharyngeal cultures which were FA negative on two consecutive 24-h plates and which were morphologically consistent with *Neisseria meningitidis* were considered to be negative for *N. gonorrhoeae*.

FA test. The FA test (5, 7) was performed on presumptively positive organisms by using conjugate manufactured by Difco Laboratories, Detroit, Mich. The quality and working titer of the conjugate were determined prior to use by assay with the following strains: four strains of *N. gonorrhoeae*; three strains of *N. meningitidis*; and one strain each of *Neisseria lactamica*, *Neisseria sicca*, *Neisseria cinerea*, *Neisseria flavescens*, *Branhamella catarrhalis*, and *Enterobacter cloacae*. In accordance with the procedures of the Centers for Disease Control (5), the working dilution of conjugate selected (1:32), along with the surrounding dilutions (1:16 and 1:64), should show sharp endpoints with no cross-reactivity. Thus, the four strains of *N. gonorrhoeae* showed 4+ fluorescence at conjugate dilutions of 1:16, 1:32, and 1:64; *N. meningitidis*, the saprophytic *Neisseria* spp., and *E. cloacae* showed no cross-reactivity. For all subsequent tests, two strains of *N. gonorrhoeae* were used as positive controls, one strain of type B *N. meningitidis* served as the negative control, and one strain of *E. cloacae* was included as the nonspecific-staining control.

Biochemical tests. Pharyngeal organisms giving a positive FA reaction, anogenital organisms giving a negative FA reaction, or any organisms from other sites were identified to the species level by use of CTA with 1% glucose, maltose, sucrose, lactose, mannitol, levulose, and xylose. Inoculation of the carbohydrate-supplemented CTA with control cultures of *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. sicca*, *Neisseria perflava*, and *B. catarrhalis* demonstrated whether each individual sugar had the proper reactivity. Growth on nutrient agar slants at 25 and 35°C was assessed, and an *o*-nitrophenol-beta-D-galactopyranoside hydrolysis reaction was performed. In addition, tests for nitrate and nitrite reduction and for polysaccharide production from 5%

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TABLE 1. Organisms tested and sources

Organism	No. of isolates from:						
	Cervix	Urethra	Pharynx	Anal canal	Sputum	Eye	Hip joint fluid
N. gonorrhoeae	248[a]	237[a]	9	21	0	1	0
N. meningitidis	0	7	207	7	1	0	1
N. lactamica	3	1	16	1	0	0	0
N. cinerea	3	1	1	0	1	1	0
B. catarrhalis	0	0	5	0	0	4	0

[a] Four cervical isolates and four urethral isolates were OMNI negative and FA negative; they were identified as *N. gonorrhoeae* by the CTA technique.

sucrose were performed to identify *N. cinerea* and *B. catarrhalis*.

Coagglutination test. The Phadebact Monoclonal GC OMNI Test kit contains one vial of gonococcal control reagent, one vial of gonococcal OMNI reagent, and 40 disposable slides. The control reagent contains rabbit nonimmune globulin bound to nonviable staphylococci. The OMNI reagent contains mouse monoclonal antibodies IA and IB bound to nonviable staphylococci. The (gonococcal) OMNI reagent is a defined mixture of mouse monoclonal antibodies to different epitopes on a gonococcus-specific membrane protein, protein I.

A few colonies were removed from the primary or subculture plate to make a light suspension in 0.9% saline (pH 7.4), equivalent to a 0.5 McFarland standard, according to the directions of the manufacturer. No attempt was made to ensure pure or viable cultures, since both the FA and OMNI tests may be performed on nonviable cultures. The cotton-plugged test tubes (12 by 75 mm) containing the suspensions were heated in a boiling water bath for 5 min and then cooled to room temperature prior to testing. The boiled colony suspension (1 drop) was then placed on each of two wells on the disposable slide; 1 drop each of control and OMNI reagents was placed adjacent to the suspension. Each suspension and its adjacent reagent drop were thoroughly mixed with a separate applicator stick, and the slide was then rocked for a maximum of 60 s or until agglutination was apparent.

A positive result consisted of an agglutination reaction with the OMNI reagent and no reaction with the control reagent. A negative result was defined as the absence of agglutination in both OMNI and control reagents.

Serogrouping. During this study, we had available the reagents to divide the isolates into two serogroups, WI and WII/III (Pharmacia Diagnostics). Gonococci harboring protein IA are classified as serogroup WI, whereas gonococci containing protein IB belong to serogroup WII/III. Since contact tracing and intense epidemiologic efforts are crucial in the control of penicillinase-producing strains of gonococci, the Monoclonal GC Test was used in addition to the OMNI Test to determine the distribution of serogroups as related to the site of infection and to resistance.

RESULTS

The results of the OMNI Test by specific organism tested are shown in Table 2. All *N. meningitidis*, *N. lactamica*, *N. cinerea*, and *B. catarrhalis* gave negative reactions with both the OMNI and control reagents. Since some laboratories have problems interpreting Gram stains, we included 10 non-*Neisseria* isolates in order to determine the specificity toward these genera. Eight isolates that were negative by both the OMNI Test and FA were identified as *N. gonorrhoeae* by the CTA technique.

The overall serogroup distribution was 19.3% WI and 80.1% WII/III (Table 3). Of the isolates from urogenital sites, 20.3% were WI and 79.0% were WII/III. Of the pharyngeal isolates, all were WII/III; anal canal isolates were 5% WI and 95% WII/III. Three (0.6%) of the urogenital isolates contained both serogroups. Of the isolates belonging to the WI serogroup, 19.6% were beta-lactamase producing; of those belonging to WII/III, only 4% were beta-lactamase producing.

DISCUSSION

Although the serogrouping data were limited to isolates from urogenital sites, they did show a definite correlation between serogroup and penicillinase production. Whereas almost 20% of all isolates belonging to the WI serogroup were penicillinase-producing *N. gonorrhoeae*, only 4% of all WII/III isolates tested were penicillinase producing. These data are in conflict with those of Sandstrom et al. (8). A concentrated look at serovars and their correlations with all antibiotic resistance is needed.

Although we were not able to find any organisms that cross-reacted with the OMNI Test, we are aware that some laboratories have experienced less than 100% specificity. In our laboratory, the saprophytic *Neisseria* spp. gave consistent negative reactions. Since both the OMNI reagent and the fluorescein-labeled conjugate are directed toward the outer membrane of the organism, it is reasonable that eight isolates of *N. gonorrhoeae* were both OMNI and FA negative. The antigen concentration and the pH of the saline used in the preparation of the bacterial suspension are of crucial importance to the sensitivity and specificity of the test. The optimal reaction pH is 7.4; more acidic saline may cause cross-reactivity of *Neisseria* spp. other than *N. gonorrhoeae*, leading to false-positive results. Freshly prepared 0.9% NaCl, pH 7.4, must be used, for repeated exposure to the atmosphere will permit the absorption of carbon dioxide, resulting in a drop in pH. The test suspension should be at a concentration equivalent to a 0.5 McFarland standard. A greater concentration of organisms may cause an antigen overload, which may decrease the specificity of the test.

TABLE 2. Comparison of Phadebact Monoclonal GC OMNI Test with FA and CTA techniques

Organism identified by FA and CTA	No. of OMNI results	
	+	−
N. gonorrhoeae	508	8
N. meningitidis	0	223
N. lactamica	0	21
N. cinerea	0	7
B. catarrhalis	0	9
Non-*Neisseria*[a]	0	10

[a] Includes 8 *Moraxella* spp., 1 *Staphylococcus* sp., and 1 *Candida* sp.

TABLE 3. *N. gonorrhoeae* characterized by serogroup

Serogroup (no. of isolates)	No. of isolates from:					No. of PPNG isolates (%)[a]
	Cervix	Urethra	Pharynx	Anal canal	Eye	
WI (97)	43	53	0	1	0	19 (19.6)
WII/III (402)	198	175	9	19	1	16 (4.0)
Both (3)	2	1	0	0	0	0
Not typed (6)	1	4	0	1	0	0

[a] PPNG, Penicillinase-producing *N. gonorrhoeae*.

All *Neisseria* spp. morphologically compatible with *N. gonorrhoeae* but giving a negative coagglutination reaction should be identified by conventional techniques (FA or CTA or both) or sent to a reference laboratory for identification. *Neisseria* spp. that are not consistent with *N. gonorrhoeae* and that give a negative reaction may be reported as negative for *N. gonorrhoeae* or further identified by CTA. Test results must always be consistent with both gross and microscopic morphological characteristics.

We conclude that the Phadebact Monoclonal GC OMNI Test has a sensitivity of 98.4% and a specificity of 100% and that it is useful for the rapid identification of *N. gonorrhoeae*. As with all new procedures, the results should be correlated with standard confirmatory techniques until both clinicians and laboratorians are confident in the performance and interpretation of the test.

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