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Fluorescent Monoclonal Antibody for Confirmation of *Neisseria gonorrhoeae* Cultures

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We evaluated a monoclonal fluorescent-antibody (FA) reagent (*Neisseria gonorrhoeae* Culture Confirmation Test; Syva Co., Palo Alto, Calif.) for confirmation of *N. gonorrhoeae* isolates obtained from clinics for sexually transmitted diseases in four cities. The FA test was performed in parallel with established confirmation procedures on all organisms growing on 773 primary culture plates of modified Thayer-Martin agar. All *N. gonorrhoeae* isolates reacted with the FA reagent and produced a bright, easily interpretable fluorescence. The FA test correctly identified 533 *N. gonorrhoeae* isolates from 474 patients and did not react with 90 *N. meningitidis* or with 213 non-*Neisseria* isolates. In one city (Baltimore), Gonochek II (Du Pont Co., Wilmington, Del.) failed to identify four *N. gonorrhoeae* isolates reactive with the FA reagent and confirmed as *N. gonorrhoeae* by Phadebact (Pharmacia Inc., Piscataway, N.J.) and acid production from sugars. The FA test was rapid and specific and could be performed directly from primary isolation plates. The test requires 1 h to perform and is applicable to mixed-flora cultures.

Differentiation of *Neisseria gonorrhoeae* from other gram-negative diplococci isolated from mucosal surfaces is important for guiding therapy. Laboratory misidentification of organisms as *N. gonorrhoeae* has potentially serious medical, legal, and social consequences for patients, their partners, and care providers (2). Acid reactions from sugars and nitrate reduction are the standards to which other methods of culture confirmation are compared (7). However, these methods are occasionally compromised by limited or unpredictable growth of *N. gonorrhoeae* in broth or semisolid media (1, 3, 10). More rapid methods for identification based on serological reactions and detection of preformed enzymes have been developed but often require subculture of isolates to provide adequate cellular mass for testing (4, 9). Fluorescent antibodies (FA) for culture confirmation have the advantage of requiring only growth from a primary isolation plate. The high specificity of monoclonal antibodies offers the potential for improvement over available polyclonal fluorescein-conjugated reagents which have been reported to cross-react with *Branhamella catarrhalis* (7).

In this study, we evaluated a new monoclonal FA reagent (*Neisseria gonorrhoeae* Culture Confirmation Test; Syva Co., Palo Alto, Calif.) for confirmation of *N. gonorrhoeae* in cultures of clinical specimens from a variety of mucosal sites. The FA reagent contains monoclonal antibodies prepared against purified outer membrane protein I of *N. gonorrhoeae* and is formulated to react with serovars containing outer membrane proteins IA and IB (Syva Co., personal communication). The description of the development of these antibodies has been published by Tam et al. (11), who found no cross-reactivity with 17 *Neisseria* sp. other than *N. gonorrhoeae*. In this report, isolates from laboratories in four different metropolitan areas (Baltimore, Denver, Indianapolis, and Seattle) were studied to control for variation in the geographical distribution of gonococcal serovars (6). In each laboratory, the FA reagent was compared with the test(s) routinely used in that laboratory for the identification of *N. gonorrhoeae*. In addition, reagent specificity was examined by assessing cross-reactivity with all nongonococcal colonial morphotypes growing on primary culture plates.

MATERIALS AND METHODS

Specimen collection. Consecutive cultures for *N. gonorrhoeae* were obtained by clinicians at clinics for sexually transmitted diseases in four cities: Baltimore, Indianapolis, Denver, and Seattle. In each clinic, modified Thayer-Martin (MTM) agar plates were directly inoculated with swabs obtained from urethral, pharyngeal, rectal, or cervical sites, as indicated by clinic protocols.

Bacterial identification. Laboratory identification of *N. gonorrhoeae* was confirmed by the standard procedures in use by each laboratory as follows: Gonochek II (Du Pont Co., Wilmington, Del.) was used in Baltimore and Indianapolis, QuadFerm+ (Analytab Products, Plainview, N.Y.) was used in Denver, and rapid acid production tests (12) were used in Seattle. All commercial tests were performed according to the instructions of the manufacturer on colonies of oxidase-positive, gram-negative diplococci obtained from primary MTM plates or from chocolate agar subcultures. All colonial morphotypes present on primary MTM plates were identified at least to the genus level.

FA procedure. Bacterial suspensions were prepared by lightly touching five morphologically similar colonies with a bacteriological loop and emulsifying adherent bacteria in 5 μl of deionized water on a clean glass slide. The smears were allowed to air dry and then were gently heat fixed. Lyophilized aliquots of the monoclonal FA reagent were rehydrated

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TABLE 1. Recovery of *N. gonorrhoeae* grouped by anatomical site and location

Location	No. of specimens recovered from:			
	Cervix	Pharynx	Rectum	Urethra
Baltimore, Md.	25	8	4	93
Indianapolis, Ind.	52	4	24	44
Denver, Colo.	30	5	8	83
Seattle, Wash.	55	11	25	62

with diluent provided by the manufacturer. Thirty microliters of FA reagent was placed over each smear and incubated for 15 min at 37°C in a moist chamber. Slides were then gently rinsed with deionized water and allowed to air dry. One drop of the provided mounting fluid was placed on each smear with a glass cover slip. Slides were examined at ×1,000 magnification with fluorescence microscopes. A positive test was defined as the presence of kidney bean-shaped diplococci exhibiting 1+ to 4+ fluorescence.

RESULTS

We evaluated 844 isolates recovered from 674 patients. Isolates were from cervical specimens (205, 24%), pharyngeal specimens (173, 21%), rectal specimens (136, 14%), and urethral specimens (330, 39%). Each study center evaluated similar numbers of isolates (*N. gonorrhoeae* and other flora recovered from MTM agar): 27% of the isolates were collected in Baltimore, whereas 27, 21, and 25% were collected in Indianapolis, Denver, and Seattle, respectively.

Of the isolates, 533 (69%) were *N. gonorrhoeae*. These strains were recovered from 474 patients and were evenly distributed among the four cities (Table 1). All gonococcal isolates reacted with the FA reagent and produced bright, easily interpretable fluorescence. No cross-reactions were observed with *N. meningitidis*, *N. lactamica*, *N. sicca*, *N. subflava*, *B. catarrhalis*, or 204 other isolates growing on MTM (Table 2).

Throughout the study, the FA confirmation test was performed on colonies from primary culture plates and results were generally available within 1 h of starting the test. In contrast, because of mixed cultures or scant growth on primary plates, subculture was often necessary for confirmation of isolates from nonurethral cultures using Gonochek II, QuadFerm+, or acid production tests. In Baltimore, Gonochek II failed to identify four *N. gonorrhoeae* isolates which were reactive by FA and confirmed as *N. gonorrhoeae* using both the Phadebact coagglutination test and acid production from sugars. Three of the four isolates were available for serotyping and auxotyping. Two isolates were cultured from urethral and pharyngeal sites in the same patient; both were proline-requiring auxotrophs of serovar IB-17. The third isolate was a prototrophic IB-3 strain.

Pharyngeal MTM cultures from Baltimore were frequently overgrown with *Capnocytophaga* spp., a group of gliding, gram-negative, fusiform bacteria which require CO_2 for growth and tend to spread as a thin film over the entire plate. The typical appearance of *N. gonorrhoeae* colonies was obscured when this growth occurred, and pure cultures were difficult to obtain, i.e., suspicious growth usually required more than one subculture to produce well-isolated colonies for confirmatory testing. In the Gonochek II system, most *Capnocytophaga* isolates exhibited a δ-glutamylaminopeptidase reaction which resembled that of *N. meningitidis*. *Capnocytophaga* spp. did not react with the FA reagent.

DISCUSSION

This study was designed to evaluate the usefulness of a newly developed monoclonal FA reagent to identify *N. gonorrhoeae* isolated on MTM from patients attending clinics for sexually transmitted diseases. Monoclonal antibodies offer the potential advantage of epitopic specificity. Outer membrane proteins were chosen as the immunogen for antibody production because other workers have shown these proteins to be species specific in agglutination tests (5). Use of monoclonal antibodies in a fluorescence test provides a practical and rapid alternative to confirmatory tests which require subculture. Generally, isolates from primary culture plates could be fully evaluated within 1 h of starting the FA test. With the other confirmatory tests, isolates from mixed cultures sometimes required as much as 48 h for confirmation.

No fluorescence was observed with any other organisms growing on the MTM plates, including 90 *N. meningitidis* isolates and 17 isolates of other gram-negative, oxidase-positive diplococci. Thus, in this study, the FA reagent was 100% specific. Although we did not recover representatives of all *Neisseria* species using MTM agar, our sample represents the strains most commonly encountered in clinic settings for sexually transmitted diseases in four geographically disparate cities. Similarly, none of the other 204 strains of contaminating bacteria or yeasts growing on MTM reacted with the FA reagent. Other investigators have also found that this reagent does not cross-react with other *Neisseria* spp., including 11 strains of *N. cinerea* (J. S. Lewis, J. W. Biddle, M. E. Shepherd, and J. S. Knapp, Abstr. Int. Soc. Sex. Trans. Dis. Res., abstr. no. 90, 1987).

The FA format for a confirmatory test permitted expeditious evaluation of colonies from crowded primary plates without requiring subculture. The FA test could be an advantageous alternative for laboratories using acid production tests, since contamination is the most common problem in the traditional confirmation of *N. gonorrhoeae*. Similarly, laboratories have encountered difficulties with atypical strains of *N. gonorrhoeae* which utilize glucose poorly or with strains of *N. meningitidis* which fail to produce acid from maltose (1, 8). Culture-related problems such as these would be obviated by a reliable, specific FA procedure.

An important additional benefit of the FA test is that it demonstrates the microscopic appearance of isolates, as well as the antibody reactivity. By using oil-immersion microscopy, organisms could be readily visualized as typical kidney bean-shaped diplococci. Thus, microscopic examination adds a level of confidence above that of chromogenic sub-

TABLE 2. Specificity of the monoclonal FA reagent

Organism	No. tested	FA reaction
Neisseria gonorrhoeae	533	2+ to 4+
Neisseria meningitidis	90	–[a]
Neisseria lactamica	4	–
Neisseria sicca	1	–
Neisseria subflava	3	–
Branhamella catarrhalis	9	–
Capnocytophaga spp.	20	–
Gram-negative rods	53	–
Staphylococcus spp.	71	–
Streptococcus spp.	17	–
Gram-positive rods	16	–
Candida spp.	27	–

[a] Symbol: –, negative FA reaction.

strate tests in which preformed enzymes from contaminating bacteria may affect reading. For example, the spreading *Capnocytophaga* organisms, which we encountered in throat cultures from Baltimore, were occasionally difficult to see without careful examination of plates under oblique lighting and produced a yellow color change in the Gonochek II system. If these organisms were inoculated along with an oxidase-positive colony into a Gonochek II tube, the yellow color could be misinterpreted as identifying *N. meningitidis*.

This monoclonal FA reagent may also prove useful for clinical settings for which primary culture is performed at one site, such as a physician's office, and confirmatory testing is performed elsewhere, such as a distant laboratory. Viable *N. gonorrhoeae* are not required for FA reactivity and flooding plates with the oxidase reagent before testing does not affect test interpretation (unpublished data).

Although this study evaluated the use of FA for culture confirmation of isolates from a clinic for sexually transmitted diseases, the specificity of this reagent for strains from nonmucosal sites (blood, joint fluid, spinal fluid) or from children needs to be evaluated. In these situations other species of gram-negative diplococci may be pathogenic; thus, laboratories should confirm the identification of *N. gonorrhoeae* with other methods of speciation.

The cost of the FA reagent may limit its application in some laboratories. Currently, the list price of one 3-ml vial is $275, or approximately $3.25 per test. The manufacturer is offering high-volume pricing which may lower the cost substantially. Decisions on the cost effectiveness of the FA procedure must be weighed against the costs involved in subculture and repetitious testing of strains with variable biochemical reactions.

Nonetheless, we found the *N. gonorrhoeae* fluorescent monoclonal antibody test to be useful in high-volume clinics for sexually transmitted diseases where multiple specimens can be batched for culture confirmation. The test is rapid and 100% specific for *N. gonorrhoeae* isolates recovered from representative urban populations in the United States.

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LITERATURE CITED

1. **Arko, R. J., K. G. Finley-Price, K.-H. Wong, S. R. Johnson, and G. Reising.** 1982. Identification of problem *Neisseria gonorrhoeae* cultures by standard and experimental tests. J. Clin. Microbiol. **15**:435–438.
2. **Dossett, J. H., P. C. Appelbaum, J. S. Knapp, and T. A. Totten.** 1985. Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. J. Clin. Microbiol. **21**:575–577.
3. **Griffin, C. W., III, M. A. Mehaffey, and E. C. Cook.** 1983. Five years of experience with a national external quality control program for the culture and identification of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **18**:1150–1159.
4. **Janda, W. M., K. L. Zigler, and J. J. Bradna.** 1987. API QuadFERM+ with rapid DNase for identification of *Neisseria* spp. and *Branhamella catarrhalis*. J. Clin. Microbiol. **25**: 203–206.
5. **Kellogg, D. S., Jr., K. K. Holmes, and G. A. Hill.** 1976. Cumitech 4, Laboratory diagnosis of gonorrhea. Coordinating ed., S. Marcus and J. C. Sherris. American Society for Microbiology, Washington, D.C.
6. **Knapp, J. S., E. G. Sandström, and K. K. Holmes.** 1985. Overview of epidemiological and clinical applications of auxotype/serovar classification of *Neisseria gonorrhoeae*, p. 6–12. *In* G. K. Schoolnik, G. F. Brooks, S. Falkow, C. E. Frasch, J. S. Knapp, J. A. McCutchan, and S. A. Morse (ed.), The pathogenic *Neisseriae*. American Society for Microbiology, Washington, D.C.
7. **Morello, J. A., W. M. Janda, and M. Bohnhoff.** 1985. *Neisseria* and *Branhamella*, p. 176–192. *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.
8. **Morello, J. A., S. A. Lerner, and M. Bohnhoff.** 1976. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. Infect. Immun. **13**:1510–1516.
9. **Philip, A., and G. C. Garton.** 1985. Comparative evaluation of five commercial systems for the rapid identification of pathogenic *Neisseria* species. J. Clin. Microbiol. **22**:101–104.
10. **Pollack, H. M.** 1976. Evaluation of methods for the rapid identification of *Neisseria gonorrhoeae* in a routine clinical laboratory. J. Clin. Microbiol. **4**:19–21.
11. **Tam, M. R., T. M. Buchanan, E. G. Sandström, K. K. Holmes, J. S. Knapp, A. W. Siadak, and R. C. Nowinski.** 1982. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. Infect. Immun. **36**:1042–1053.
12. **Vera, H. D.** 1948. A simple medium for identification and maintenance of the gonococcus and other bacteria. J. Bacteriol. **55**:531–536.