Fluorescent Monoclonal Antibody for Confirmation of Neisseria gonorrhoeae Cultures

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Received 19 June 1987/Accepted 15 September 1987

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), Gonocheck 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 clinical protocols.

Bacterial identification. Laboratory identification of N. gonorrhoeae was confirmed by the standard procedures in use by each laboratory as follows: Gonocheck 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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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 \( \times 1,000 \) 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 cervicum 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 21, 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 autotyping. Two isolates were cultured from urethral and pharyngeal sites in the same patient; both were proline-requiring autotrophs 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 Gonocheck II system, most Capnocytophaga isolates exhibited a 6-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 clinical 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-
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 Gonocheck II system. If these organisms were inoculated along with an oxidase-positive colony into a Gonocheck 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.

ACKNOWLEDGMENTS

We thank Teri Anderson, Linda Bobo, Della Duncan, Jill Findlay, Charlotte Gaydos, Randi Klatsky, Betty Lou Kramer, and Ann LeMonte for their excellent technical assistance. We are grateful to Cindy Reichart, Linda Sherman, and Tom Riley for their reviews of the manuscript.

LITERATURE CITED


