JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1993, p. 2535–2537
0095-1137/93/092535-03$02.00/0

Vol. 31, No. 9

# Incidence of *Neisseria gonorrhoeae* Isolates Negative by Syva Direct Fluorescent-Antibody Test but Positive by Gen-Probe Accuprobe Test in a Sexually Transmitted Disease Clinic Population

JAMES L. BEEBE,[1]* MICHAEL P. RAU,[1] SYLVIA FLAGEOLLE,[1] BARBARA CALHOON,[2] AND JOAN S. KNAPP[3]

*Division of Laboratories, Colorado Department of Health, Denver, Colorado 80217[1]; University Hospital, Denver, Colorado 80262[2]; and Division of Sexually Transmitted Diseases Laboratory Research, National Center for Infectious Disease, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[3]*

Received 1 February 1993/Returned for modification 22 April 1993/Accepted 2 June 1993

**To determine the accuracy of the Syva (Palo Alto, Calif.) direct fluorescent-antibody (DFA) test in comparison with the Gen-Probe (San Diego, Calif.) Accuprobe culture confirmation test, we tested 395 isolates of *Neisseria gonorrhoeae* from cultures obtained from patients attending a sexually transmitted disease clinic from 1 July 1991 through 30 June 1992. All isolates were tested for DFA reactivity with a polyclonal reagent (Difco Laboratories, Detroit, Mich.) and a monoclonal reagent (Syva, Inc., direct specimen test) and for specific molecular probe reactivity by the Gen-Probe Accuprobe culture confirmation test for *N. gonorrhoeae*. The 395 isolates gave positive results for the Gen-Probe culture confirmation test and the Difco polyclonal direct specimen test. However, 18 (4.6%) of the isolates were negative for *N. gonorrhoeae* by the Syva DFA test. With the exception of six β-lactamase-positive isolates, all isolates that were negative by Syva DFA were sensitive to penicillin, tetracycline, spectinomycin, and ceftriaxone by disk-diffusion susceptibility testing. Auxotyping and serotyping studies indicated that strains negative by Syva DFA consisted of several variants. The frequency of *N. gonorrhoeae* isolates showing negative results by Syva DFA in this patient population ranged from 0 to 11.5%/month. Laboratories using only the Syva DFA test for confirmation of *N. gonorrhoeae* may incur a significant risk of misidentification.**

Rapid identification of *Neisseria gonorrhoeae* has been improved by the introduction of direct fluorescent-antibody (DFA) testing of presumptive *N. gonorrhoeae* isolates in place of the conventional sugar utilization method. However, polyclonal DFA reagents for *N. gonorrhoeae* are recognized to produce false-positive results for other *Neisseria* spp. (1, 4) and false-negative results for *N. gonorrhoeae* isolates (11, 14). In addition, Waitkins and Anderson (15) described penicillinase-producing *N. gonorrhoeae* (PPNG) isolates with negative DFA reaction by a commercial polyclonal conjugate. The introduction of monoclonal reagents was followed by reports of significant improvement in the specificity of the DFA test (6, 17). Although the monoclonal Syva DFA test (Syva, Inc., Palo Alto, Calif.) was initially reported as 100% sensitive, a subsequent report by Walton (16) described two PPNG strains that were negative by this test.

We compared the Syva DFA test with a DNA probe test (Accuprobe; Gen-Probe, San Diego, Calif.) for identification of a consecutive series of *N. gonorrhoeae* isolates from a sexually transmitted disease clinic to determine the incidence of Syva DFA-negative isolates in this population.

The Sexually Transmitted Disease clinic is located within the University Hospital on the campus of the University of Colorado Health Sciences Center. During the study period (1 July 1991 through 30 June 1992), 4,958 patients attended the clinic; 55.6% were male, and 44.4% were female. Racial distribution was 52.0% white, 32.6% black, 12.4% Hispanic, 1.5% Asian, 0.5% Native American, and 1.0% other groups. Patients aged 20 to 39 constituted 67.8% of the population.

Specimens were collected on calcium alginate swabs and immediately inoculated onto Martin-Lewis agar plates. The plates were incubated for 48 h at 35°C in a 5 to 10% $CO_2$-enriched atmosphere. Typical colonies were examined by Gram stain and subjected to oxidase, catalase, and fluorescent antibody testing with a polyclonal fluorescent antibody reagent (Difco Laboratories, Detroit, Mich.). Culture isolates were then referred to the Division of Laboratories, Colorado Department of Health, for confirmation and susceptibility testing. On receipt, isolates were subcultured on chocolate agar plates to ensure purity and viability and subsequently were tested for conventional CTA sugar utilization reactions, reactivity with a monoclonal reagent DFA (Syva, Inc.), and *N. gonorrhoeae* molecular probe reactivity (Accuprobe GC; Gen-Probe) and were subjected to β-lactamase and disk-diffusion susceptibility testing according to National Committee for Clinical Laboratory Standards guidelines (3). Examination of DFA slides was performed with a Zeiss fluorescence microscope equipped with a 50-W mercury lamp and standard fluorescein isothiocyanate filter set. GC agar (BBL Microbiology Systems, Cockeysville, Md.), supplemented with 1% IsoVitaleX in petri dishes (15 by 150 mm), and antibiotic disks (BBL) of penicillin (10 U), spectinomycin (100 μg), tetracycline (30 μg), and ceftriaxone (30 μg) were employed. A quality control strain (F18), obtained from the Centers for Disease Control and Prevention, was used to monitor antibiotic disk performance.

β-Lactamase activity was determined by the chromogenic cephalosporin (Nitrocefin; Glaxo Pharmaceuticals, Ltd.)

* Corresponding author.

TABLE 1. Characteristics of Syva DFA-negative *N. gonorrhoeae* isolates

| Patient no. | Patient characteristic | | | Isolate characteristic | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sex[a] | Age (yr) | Race/ethnicity | Site cultured | Date of collection | Difco DFA result | Gen-Probe result (RLUs[b]) | β-Lactamase result[c] | Auxotype[d] | Serovar |
| S43 | M | 23 | Black | Urethra | 7/29/91 | 4+ | 643,280 | Neg | Proto | NT |
| S55 | M | 21 | Black | Urethra | 8/7/91 | 4+ | 803,754 | Neg | Proto | NT |
| S64 | M | 17 | Black | Urethra | 8/14/91 | 4+ | 725,560 | Neg | Proto | NT |
| S84 | M | 18 | Black | Urethra | 9/4/91 | 4+ | 802,501 | Neg | Proto | NT |
| S113 | M | 29 | Black | Urethra | 9/20/91 | 4+ | 653,930 | Pos | Proto | NT |
| S155 | M | 18 | Black | Urethra | 10/18/91 | 3+ | 864,966 | Pos | Proto | NT |
| S208 | F | 19 | Hispanic | Cervix | 11/22/91 | 3+ | 966,474 | Pos | Proto | NT |
| S213 | M | 32 | Black | Urethra | 12/4/91 | 4+ | 947,021 | Neg | Proto | NT |
| S223 | M | 34 | Black | Urethra | 12/10/91 | 4+ | 957,338 | Neg | Proto | NT |
| S233 | F | 16 | Hispanic | Cervix | 12/21/91 | 4+ | 769,678 | Pos | Proto | NT |
| S246 | M | 39 | Black | Urethra | 1/12/92 | 4+ | 871,149 | Neg | PA | IB-20 |
| S247 | F | 25 | Black | Cervix | 1/12/92 | 4+ | 888,361 | Neg | PA | IB-20 |
| S252 | M | 21 | Black | Urethra | 1/15/92 | 4+ | 773,044 | Neg | Proto | NT |
| S269 | F | 24 | Black | Rectum | 2/5/92 | 4+ | 996,670 | Neg | Proto | IB-24 |
| S271 | F | 39 | Black | Rectum | 2/5/92 | 4+ | 1,000,074 | Neg | PA | IB-20 |
| S295 | F | 22 | Black | Cervix | 2/26/91 | 4+ | 901,720 | Neg | PA | IB-20 |
| S350 | M | 26 | Black | Urethra | 4/24/92 | 3+ | 1,096,759 | Pos | Proto | NT |
| S354 | F | 25 | White | Cervix | 4/24/92 | 3+ | 954,715 | Neg | PA | IB-20 |

[a] M, male; F, female.
[b] RLUs, relative light units.
[c] Neg, negative; Pos, positive.
[d] PA, proline and arginine requirement.

method (9). Fluorescent antibody testing was conducted in accordance with the manufacturer's instructions. The Gen-Probe Accuprobe GC kit was used in accordance with the manufacturer's instructions with a Leader 1 or Leader 50 luminometer. Luminescence of ≥50,000 relative light units was considered positive. Positive and negative controls were run concurrently with each test batch. Auxotypes were determined on the medium of Short et al. (13). Requirements for proline (P), arginine, hypoxanthine, uracil, and methionine were determined. Serotyping was performed as described previously (5).

At the University of Colorado Health Sciences Center Sexually Transmitted Disease Clinic, 4,958 patients were seen during the study period; 86.3% had specimens cultured for *N. gonorrhoeae*. Of specimens cultured, 9.2% were positive, yielding 395 *N. gonorrhoeae* isolates for study. A total of 18 of the 395 isolates (4.6%) were negative by the Syva monoclonal DFA. By Syva DFA, the incidence of negative strains among the clinic population cultured for *N. gonorrhoeae* was 4.2/1,000 and among patients whose *N. gonorrhoeae* culture results were positive, 45.6/1,000. The monthly frequency of recovery of isolates negative by Syva DFA varied from 0 to 11.5% of all isolates tested. Table 1 lists the demographic characteristics of the patients from whom *N. gonorrhoeae* isolates that were negative by Syva DFA were recovered, as well as the results of identification, susceptibility, and typing tests.

All isolates negative by Syva DFA yielded positive results for the Difco polyclonal fluorescent antibody test as well as the Gen-Probe culture confirmation (CC) test and expected sugar fermentations. These isolates were subjected to repeat testing by Syva DFA to confirm the absence of reactivity and produced strong signals by *N. gonorrhoeae* DNA CC test with relative light units in the range of 643,280 to 1,096,759 (average, 867,611) (Table 1). These values were an average of 17.4 times the cutoff value and agreed with values reported by Lewis et al. for a large number of *N. gonorrhoeae* strains in an evaluation of the Gen-Probe *N. gonorrhoeae* CC test (7). The detection version of this DNA probe test has been reported as highly sensitive as well (2, 8, 10).

A total of 11 (61.1%) of the 18 Syva DFA-negative isolates were recovered from predominantly young black males (average age, 25.3 years). All were urethral isolates. The remaining seven (38.9%) isolates were recovered from females (four blacks, two Hispanics, and one white). Three isolates were recovered from cervical swabs; two were recovered from rectal specimens. Five (27.8%) of the isolates were β-lactamase positive and were recovered from three men and two women. Resistance was not detected for any other antibiotic tested (tetracycline, spectinomycin, and ceftriaxone). All 10 isolates recovered from patients in the July to December 1991 period were prototrophic-nontypeable (proto-NT) in auxotype-serovar (A-S) profile although two of these isolates were PPNG. Subsequently, two other A-S profiles, P-arginine–IB-20 and proto–IB-24, were recovered from patients whose specimens were cultured during the January-to-June 1992 period. The transient appearance of isolates negative by Syva DFA was similar to that described for many A-S types (12). Epidemiologic investigation revealed that female patients S208 and S233, from whom PPNG proto-NT was recovered, had in common a male sexual contact whose cultures were negative. Patient S269, from whom a Syva DFA-negative strain was recovered from a rectal culture, also yielded a second *N. gonorrhoeae* isolate from a cervical culture which was positive by the Syva DFA test.

Reliable confirmation of *N. gonorrhoeae* isolates is required to ensure appropriate treatment and disease control measures. When introduced, the Syva monoclonal antibody test was reported as 100% sensitive. No confirmed *N. gonorrhoeae* isolate with a negative Syva DFA result was described in initial reports (6, 17). Subsequent case reports of DFA-negative strains gave no indication of the incidence of these strains (16). We found that a significant percentage

(4.6%) of *N. gonorrhoeae* isolates recovered from sexually transmitted disease clinic patients yielded a negative reaction by the Syva DFA test while exhibiting appropriate reactions with conventional biochemicals, a polyclonal DFA test (Difco), and the Gen-Probe *N. gonorrhoeae* CC test. Since the target epitope for the Syva monoclonal antibody conjugate resides on the principal outer membrane protein, it was assumed that a genetic variant or variants were circulating in the affected population. The A-S data indicate that multiple variants were recovered from clinic patients. The most commonly recovered variant, proto-NT, did not react with the commercial monoclonal reagent or the standard panel of typing reagents employed by the Centers for Disease Control and Prevention for this determination.

To our knowledge, this is the first report of the incidence of Syva DFA-negative isolates. The incidence of these isolates in other regions may be less or greater than that observed in our study. Laboratories considering use of the Syva DFA test as the sole means for confirmation of *N. gonorrhoeae* may incur a significant risk of misidentification and may wish to determine the frequency of *N. gonorrhoeae* isolates which are negative by the Syva DFA test before beginning routine use. We recommend that laboratories which are already using the Syva DFA test for confirmation refer isolates with colonial and microscopic morphology typical of *N. gonorrhoeae* but negative results by the Syva DFA test to another laboratory which uses sugar fermentation or a molecular probe method for confirmation.

No false-negative results were obtained for the Gen-Probe CC test during this study. The use of highly conserved nucleotide sequences as a target for a DNA probe confirmation test may be superior to the use of principal outer membrane protein epitopes as a target for specific monoclonal antibodies for routine laboratory confirmation of *N. gonorrhoeae.*

We thank Beverly Dahan for making available the results of epidemiologic investigations, Tamara Hoxworth for data analysis, and Manhar Parekh for performance of the auxotyping and serotyping studies.

## REFERENCES

1. **Hare, M. J.** 1974. Comparative assessment of microbiological methods for the diagnosis of gonorrhea in women. Br. J. Vener. Dis. **50:**437–441.
2. **Hosein, I. K., A. M. Kaunitz, and S. J. Craft.** 1992. Detection of cervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with deoxyribonucleic acid probe assays in obstetric patients. Am. J. Obstet. Gynecol. **167:**588–591.
3. **Jones, R. N., T. L. Gavan, C. Thornsberry, P. C. Fuchs, E. H. Gerlach, J. S. Knapp, P. Murray, and J. A. Washington II.** 1989. Standardization of disk diffusion and agar dilution susceptibility tests for *Neisseria gonorrhoeae*: interpretive criteria and quality control guidelines for ceftriaxone, penicillin, spectinomycin, and tetracycline. J. Clin. Microbiol. **27:**2758–2766.
4. **Knapp, J. S.** 1988. Historical perspectives and identification of *Neisseria* and related species. Clin. Microbiol. Rev. **1:**415–431.
5. **Knapp, J. S., M. R. Tam, R. C. Nowinski, K. K. Holmes, and E. G. Sandström.** 1984. Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. J. Infect. Dis. **150:**44–48.
6. **Laughon, B. E., J. M. Ehret, T. T. Tanino, B. V. der Pol, H. H. Handsfield, R. B. Jones, F. N. Judson, and E. W. Hook III.** 1987. Fluorescent monoclonal antibody for confirmation of *Neisseria gonorrhoeae* cultures. J. Clin. Microbiol. **25:**2388–2390.
7. **Lewis, J. S., D. Kranig-Brown, and D. A. Taylor.** 1990. DNA probe confirmatory test for *Neisseria gonorrhoeae*. J. Clin. Microbiol. **28:**2349–2350.
8. **Limberger, R. J., R. Beiga, A. Evancoe, L. McCarthy, L. Slivienski, and M. Kirkwood.** 1992. Evaluation of culture and Gen-Probe PACE 2 Assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. J. Clin. Microbiol. **30:**1162–1166.
9. **O'Callahan, C. H.** 1972. Novel method for detection of beta-lactamase by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1:**283–288.
10. **Panke, E. S., L. I. Yang, P. A. Leist, P. Magevney, R. J. Fry, and R. F. Lee.** 1991. Comparison of Gen-Probe DNA probe test and culture for the detection of *Neisseria gonorrhoeae* in endocervical specimens. J. Clin. Microbiol. **29:**883–888.
11. **Pollock, H. M.** 1976. Evaluation of rapid identification of *Neisseria gonorrhoeae* in a routine clinical laboratory. J. Clin. Microbiol. **4:**19–21.
12. **Sarafian, S. K., and J. S. Knapp.** 1989. Molecular epidemiology of gonorrhea. Clin. Microbiol. Rev. **2:**S49–S55.
13. **Short, H. B., V. B. Ploscowe, J. S. Weiss, and F. E. Young.** 1977. Rapid method for auxotyping multiple strains of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **6:**244–248.
14. **Thin, R. N. T.** 1970. Immunofluorescent method for diagnosis of gonorrhea in women. Br. J. Vener. Dis. **46:**27–30.
15. **Waitkins, S. A., and R. D. Anderson.** 1982. Failure of the fluorescent antibody reaction to identify penicillinase-producing gonococci. J. Clin. Pathol. **35:**215–218.
16. **Walton, D. T.** 1989. Fluorescent-antibody-negative penicillinase-producing *Neisseria gonorrhoeae*. J. Clin. Microbiol. **27:**1885–1886.
17. **Welch, W. D., and G. Cartwright.** 1988. Fluorescent monoclonal antibody compared with carbohydrate utilization for rapid identification of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **26:**293–296.