Identification of Problem Neisseria gonorrhoeae Cultures by Standard and Experimental Tests

ROBERT J. ARKO,* KATHRYN G. FINLEY-PRICE, KWEI-HAY WONG, STEVEN R. JOHNSON, AND GILBERT REISING

Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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Standard and experimental tests were used by a reference diagnostic laboratory to determine the identity of 182 "suspected" Neisseria gonorrhoeae isolates submitted by state health departments because of inconclusive laboratory results. More than 97% of these cultures were subsequently identified by a rapid microcarbohydrate test in conjunction with confirmatory immunological procedures. The experimental rapid slide agglutination test using rough-lipopolysaccharide antibody, the Phadebact co-agglutination test, and fluorescent antibody test identified 49.3 to 94.1% of these cultures. Because of frequent problems with carbohydrate utilization, Neisseria meningitidis and Branhamella catarrhalis were the two microorganisms most often confused with N. gonorrhoeae by submitting laboratories.

The identification of problem cultures of Neisseria gonorrhoeae can be difficult in laboratories performing only the standard carbohydrate utilization and fluorescent-antibody (FA) tests for confirmation. The failure of certain N. meningitidis isolates to utilize maltose (9), as well as the delayed glucose utilization by some N. gonorrhoeae strains (4, 7, 13), may lead to errors in identification (9). New rapid tests for confirming and identifying Neisseria isolates as to species are now available and have the potential for improving the proficiency of clinical laboratories. However, false positivity in the Phadebact co-agglutination test (5) with some Neisseria lactamica strains (1) and cross-reactivity in the experimental rough-lipopolysaccharide (R-LPS) and wheat germ agglutination tests (12, 16) with some N. meningitidis strains (8) require caution in interpreting results, especially when testing pharyngeal isolates (10).

The cysteine Trypticase agar medium (CTA; BBL Microbiology Systems, Cockeysville, Md.) to which defined carbohydrates have been added has often been used as a standard procedure for identifying Neisseria as to species and for comparing newer diagnostic tests (15). However, the composition of the CTA medium and the fastidious nature of the test microorganisms have caused problems with this procedure (4, 7, 13). Inconclusive results in the CTA test have been the most frequently cited reason for requesting assistance from the Sexually Transmitted Disease Laboratory at the Centers for Disease Control (CDC) in the identification of problem Neisseria cultures. Other reasons include failure to give the expected FA results, failure to grow on a selective medium such as modified Thayer-Martin (MTM), and agglutination in meningococcal typing sera. The results of our experience in using the newer rapid diagnostic tests in parallel with standard tests to identify problem Neisseria cultures are presented in this report.

MATERIALS AND METHODS

Cultures. Culture specimens were sent to the reference diagnostic laboratory at CDC by state health departments and other federal agencies and were usually accompanied by a brief patient history and previous laboratory results. Specimens were cultured on various types of laboratory media, usually in the form of an agar slant, and mailed in special shipping containers.

Test procedures. Upon receipt in the CDC laboratory, specimens were immediately streaked onto both selective MTM (14) and nonselective gonococcal (GC) base media with 1.0% IsoVitaleX (BBL Microbiology Systems) followed by incubation in a candle extinction jar at 36°C. They were examined with a stereoscopic microscope at 24 or 48 h. Routine Gram stain and oxidase tests were performed. The CTA test (15), rapid microcarbohydrate utilization test (MCT) (17), immunofluorescent staining (GC conjugate; Biologic Products Division, CDC) (11), Phadebact co-agglutination (Pharmacia Fine Chemicals, Piscataway, N.J.) (5), and experimental R-LPS agglutination tests (Diagnostiques OCN, Toronto, Canada) (16) were performed as previously described. Cultures confirmed as N. gonorrhoeae utilized only glucose in one or both carbohydrate tests and gave positive reactions in two or more of the antigen confirming tests. Cultures identified as N. meningitidis were serotyped by slide agglutination with meningococcal typing sera (Biologic Products Division, CDC).
were overgrown as received, are listed inlems encountered
problems on the isolation of N. gonorrhoeae
organisms from patients with pelvic inflammatory
disease. The organism was most frequently
isolated from non-urogenital sites. In a review of
207 specimens, 182 (88.5%) were positive for N.
gonorrhoeae by the modified rapid fermentation
test (MRFT), Bactec, Difco Laboratories, Detroit, Mich.;
Minitek, BBL Microbiology Systems; and MCT, modified rapid
fermentation test, CDC.

RESULTS AND DISCUSSION
The cultures used in this study were preselect-
ed in the sense that they had already been
identified as problem cultures by various state
health department laboratories (Table 1). Some
cultures gave conflicting results in certain bio-
chemical and serological tests. A variety of tests
are often necessary to identify these cultures,
especially those isolated from non-urogenital
sites. Because their sensitivity and specificity
may be less than 100%, a single negative or
positive test result with the CTA, FA, Phade-
bact, or other laboratory procedures may not be
sufficient to confirm or reject a problem culture
as N. gonorrhoeae without additional supporting
evidence. Identification of these cultures by
both immunological and biochemical methods is
highly recommended.

A total of 242 bacterial cultures were submit-
ted during the study period. Of these, 182
(75.2%) arrived at our laboratory in a viable and
untreated condition. No growth was ob-
tained from 37 specimens (15.3%), and 23 (9.5%)
were overgrown with saprophytic microor-
organisms. The relative frequency and types of prob-
lems encountered by the submitting laboratories
are listed in Table 1. Of the 182 viable specimens
received, 141 (77.5%) were subsequently identi-
cated as N. gonorrhoeae (Table 2). The relatively
high number (41 out of 182 or 22.5%) of organ-
isms identified as other than N. gonorrhoeae
were isolated mostly from non-urogenital sites.
Almost half (9 out of 20) of the cultures identified
as Branhamella catarrhalis, Neisseria sicca, or N. lactamica grew adequately on a
selective MTM medium, whereas (6 out of 141)
4.3% of the isolates confirmed as N. gonorrhoeae
failed to grow or grew poorly on MTM.
The high percentage of clinically important B.
catarrhalis strains capable of growing on MTM
(6) may contribute to its being confused with N.
gonorrhoeae.

Biochemically, the failure to obtain the ex-
pected CTA reactions was the problem most
frequently cited by submitting laboratories. We
compared the CTA and MCT procedures for
carbohydrate utilization and found that the MCT
procedure provided highly reliable results. We
were able to obtain by the MCT procedure
correct and reproducible biochemical reactions
with >97% of the cultures tested (Table 3). The
MCT procedure utilizes enzymes formed both
before and during growth of the inoculum in a
medium enriched with Casamino Acids (17). Use
of low levels of carbohydrate also helps to
eliminate false-positive reactions without de-
creasing the sensitivity and specificity of the
MCT procedure.

We should note that, of the N. meningitidis
isolates (9-B, 3-C, 3-Z, 2-nontypable, and 1-each
of serogroups X, Y, Z', and W135), 20 out of 21

| TABLE 1. Frequency of reasons cited for submitting problem N. gonorrhoeae cultures to CDC |
|----------------------------------|----------|----------|
| Inconclusive results or problems with: | Frequency | %        |
| CTA test | 62 | 44.0 |
| Automated or rapid sugar tests (Bactec, Minitek, MRFT)* | 8 | 5.7 |
| FA test | 15 | 10.6 |
| Agglutination in meningococcal typing sera | 6 | 4.3 |
| Failure to grow on selective media (MTM) | 6 | 4.3 |
| Other reasons cited (antimicrobial testing, confirmation of results, identification) | 44 | 31.2 |
| Total N. gonorrhoeae strains tested | 141 | |

* Low incidence of problems may be related to less frequent use. Bactec, Difco Laboratories, Detroit, Mich.; Minitek, BBL Microbiology Systems; MRFT, modified rapid fermentation test, CDC.

| TABLE 2. Identification as to species of culture specimens submitted as problem Neisseria isolates |
|----------------------------------|----------|----------|----------|----------|----------|----------|
| Culture site | N. gonorrhoeae | N. meningitidis | B. catarrhalis | N. sicca | N. flava | N. lactamica |
| Blood | 16 | 2 | 3 | 0 | 0 | 0 |
| Eye | 6 | 0 | 1 | 1 | 0 | 0 |
| Cerebrospinal fluid | 3 | 6 | 0 | 0 | 1 | 0 |
| Joint, wound, or peritoneal exudate | 6 | 1 | 0 | 0 | 0 | 0 |
| Rectum | 2 | 2 | 0 | 0 | 0 | 0 |
| Throat | 12 | 7 | 3 | 1 | 0 | 0 |
| Urogenital | 80 | 2 | 3 | 1 | 0 | 1 |
| Unknown | 16 | 1 | 5 | 0 | 0 | 0 |
| Total | 141 | 21 | 15 | 3 | 1 | 1 |
TABLE 3. Results of tests for *N. gonorrhoeae* and other *Neisseria* organisms identified as problem cultures

<table>
<thead>
<tr>
<th>Test procedure</th>
<th>No. of tests</th>
<th>Tests giving correct reaction*</th>
<th>Time required</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA</td>
<td>82</td>
<td>79.2</td>
<td>6–72 h</td>
</tr>
<tr>
<td>MCT</td>
<td>169</td>
<td>97.6</td>
<td>2–8 h</td>
</tr>
<tr>
<td>FA</td>
<td>188</td>
<td>94.1</td>
<td>1–3 h</td>
</tr>
<tr>
<td>Phadebact slide agglutination test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early 1979 reagents</td>
<td>79</td>
<td>49.3</td>
<td>3–5 min</td>
</tr>
<tr>
<td>Late 1979 reagents</td>
<td>88</td>
<td>94.3</td>
<td></td>
</tr>
<tr>
<td>R-LPS slide agglutination test</td>
<td>142</td>
<td>67.6</td>
<td>3–5 min</td>
</tr>
</tbody>
</table>

*Identification of specimens (correct reaction) was dependent upon results observed in CTA or MCT. For confirmation as *N. gonorrhoeae*, specimen must also be reactive in two or more of the immunological tests (FA, R-LPS, or Phadebact).*

Utilized both glucose and maltose. In situations where the identification of *Neisseria* involves a greater number of meningococci, one may encounter more *N. meningitidis* isolates that fail to utilize maltose, due to the absence of either maltose permease or maltose phosphorylase activity. This can adversely affect the specificity of carbohydrate utilization tests, such as the MCT and CTA procedures.

Variation in serological results obtained with the FA, Phadebact co-agglutination, and R-LPS slide agglutination tests was apparently related to the quality of the reagents used (Table 3). It is apparent that the Phadebact reagents purchased late in 1979 to 1980 were more sensitive and specific than were the earlier lots obtained when the test was first introduced. Because of the diversity of antigens involved in these tests, discrepant results were possibly caused by the quality and quantity of antigens in the test cultures and the respective antibody used in these reagent sera. In preparing the Phadebact antisera, extensive absorption of antigenococcal sera with nongonococcal *Neisseria* is performed to remove cross-reacting antibodies before the antigenococcal immunoglobulin G is coupled to the protein A of heat-killed staphylococci (5). Similar absorptions are made in preparing antibody for most FA conjugates (11). For the R-LPS agglutination test, the common antigenic component R-LPS is purified from *N. gonorrhoeae* for injection into Leghorn hens or roosters to raise antibody. Theoretically, this results in the production of "common" antibody for *N. gonorrhoeae*. It is apparent that the degree of purity of the R-LPS antigen affected the specificity of the test (Tables 3 and 4). It has been reported that nonencapsulated *N. meningitidis* and *N. gonorrhoeae* react with wheat germ agglutinin through β-N-acetyl-D-glucosamine in the LPS of these species (8). Whether such common components are responsible for the cross-reaction in the R-LPS and FA tests has not been determined. However, we observed that the intensity of staining (sensitivity) of the FA procedure for *N. gonorrhoeae* can be affected by the age and subculture of specimens being tested, which may account for some of the variation in results between laboratories.

In addition to routine Gram stain and oxidase tests, colony morphology is often used to aid in identifying *N. gonorrhoeae*. However, the increased prevalence of rough gonococcal types and the submission of cultures that have undergone extensive nonselective transfers on laboratory media may impair the usefulness of colony morphology in identification. Inoculation of rough problem cultures into subcutaneous

TABLE 4. Sensitivity and specificity of tests for identifying *N. gonorrhoeae*

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of tests</th>
<th>% Sensitivity*</th>
<th>% Specificity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTA</td>
<td>68</td>
<td>83.8</td>
<td>98.2</td>
</tr>
<tr>
<td>MCT</td>
<td>142</td>
<td>97.8</td>
<td>99.3</td>
</tr>
<tr>
<td>FA</td>
<td>174</td>
<td>91.9</td>
<td>98.8</td>
</tr>
<tr>
<td>Phadebact slide agglutination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(late 1979–1980 reagents)</td>
<td>65</td>
<td>93.2</td>
<td>98.4</td>
</tr>
<tr>
<td>R-LPS slide agglutination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antiserum lots 12 and 13</td>
<td>42</td>
<td>95.2</td>
<td>88.8</td>
</tr>
</tbody>
</table>

*Number of *N. gonorrhoeae* positive/number of *N. gonorrhoeae* tested.

*Number of positive *N. gonorrhoeae*/total positive tests. Loss in specificity due primarily to cross-activity with *N. meningitidis* or maltose-negative meningococci.
chambers in animals (2) has resulted in the recovery of piliated forms from the chamber fluid and positive identification of the cultures in question (3).

LITERATURE CITED