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Identification of Problem *Neisseria gonorrhoeae* Cultures by Standard and Experimental Tests

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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 gonorrheae* 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).

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)[a]	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	

[a] 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.

RESULTS AND DISCUSSION

The cultures used in this study were preselected 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 biochemical 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, Phadebact, 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 submitted during the study period. Of these, 182 (75.2%) arrived at our laboratory in a viable and uncontaminated condition. No growth was obtained from 37 specimens (15.3%), and 23 (9.5%) were overgrown with saprophytic microorganisms. The relative frequency and types of problems encountered by the submitting laboratories are listed in Table 1. Of the 182 viable specimens received, 141 (77.5%) were subsequently identified as *N. gonorrhoeae* (Table 2). The relatively high number (41 out of 182 or 22.5%) of organisms 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 expected 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 decreasing 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 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

Test procedure	Tests giving correct reaction[a]		
	No. of tests	%	Time required
CTA	82	79.2	6–72 h
MCT	169	97.6	2–8 h
FA	188	94.1	1–3 h
Phadebact slide agglutination test			
Early 1979 reagents	79	49.3	3–5 min
Late 1979 reagents	88	94.3	
R-LPS slide agglutination test antiserum, lots no. 12 and no. 13	142	67.6	3–5 min

[a] 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 antigonococcal sera with nongonococcal *Neisseria* is performed to remove cross-reacting antibodies before the antigonococcal 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 *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*

Test	No. of tests	% Sensitivity[a]	% Specificity[b]
CTA	68	83.8	98.2
MCT	142	97.8	99.3
FA	174	91.9	98.8
Phadebact slide agglutination (late 1979–1980 reagents)	65	93.2	98.4
R-LPS slide agglutination antiserum lot no. 13	42	95.2	88.8

[a] Number of *N. gonorrhoeae* positive/number of *N. gonorrhoeae* tested.

[b] 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).

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