Rapid Confirmatory Identification of *Neisseria gonorrhoeae* with Lectins and Chromogenic Substrates

DAVID M. YAJKO,1* ALBERT CHU,2 AND W. KEITH HADLEY1

Department of Laboratory Medicine, San Francisco General Hospital Medical Center, University of California, San Francisco, California 94110,1 and E-Y Laboratories, San Mateo, California 944012

Received 24 June 1983/Accepted 18 October 1983

A group of five tests utilizing wheat germ and soybean lectins and chromogenic substrates (ortho-nitrophenyl-β-D-galactopyranoside, gamma-glutamyl-β-naphthylamide, and prolyl-β-naphthylamide derivatives) was used as a rapid (30 min) method for the identification of *Neisseria gonorrhoeae*. The rapid method agreed with Minitek test results for all 126 *N. gonorrhoeae* isolates and all 39 nongonococcal isolates tested. Soybean lectin was useful for the identification of rare strains (4 of 126) of *N. gonorrhoeae* which are not agglutinated by wheat germ lectin. The chromogenic substrates differentiate *N. gonorrhoeae* from *Neisseria meningitidis*, *Neisseria lactamica*, and other *Neisseria* species which may grow on Thayer-Martin or other selective media.

The identification of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and other *Neisseria* species in the clinical laboratory is usually accomplished by culture and the performance of carbohydrate degradation tests (17). In the cystine tryptic agar method, incubation and growth of the organism for 24 to 48 h is required before acid production can be detected (11, 13, 17). The reported reliability of the cystine tryptic agar method for the identification of *N. gonorrhoeae* ranges from 73.1 to 98.6% (1, 12, 16). In one study, 21 of 78 isolates of *N. gonorrhoeae* failed to grow in unsupplemented cystine tryptic agar medium (12).

Rapid carbohydrate degradation tests have been devised to reduce the time required for identification after primary culture by eliminating the requirement for growth during the identification test. Some of these tests detect preformed enzymes, whereas others detect a combination of preformed enzymes and enzymes produced during the incubation period (2, 9, 10, 17). Identification of an isolate is usually achieved after 4 to 5 h of incubation. Although carbohydrate degradation reactions are generally reliable for the identification of *Neisseria* species, there are reports of strains of *N. gonorrhoeae* and *N. meningitidis* with aberrant carbohydrate reactions. Maltose-negative *N. meningitidis* strains (8, 14) and asaccharolytic *N. meningitidis* strains (3) have been reported. An aberrant strain of *N. gonorrhoeae* was recently described which produces acid from glucose and maltose (8).

An alternative method to carbohydrate degradation tests in which lectin-specific agglutination is employed for the identification of *N. gonorrhoeae* has been described previously (15). Agglutination of *N. gonorrhoeae* by wheat germ agglutinin (WGA) was shown to be specific for *N. gonorrhoeae* among the strains tested. In addition, four gonococcal isolates which were negative by the fluorescent antibody method were agglutinated by WGA (15). Subsequent studies have shown that non-encapsulated strains of *N. meningitidis* are also agglutinated by WGA (4, 6) and that WGA-nonagglutinable *N. gonorrhoeae* strains exist (4; J. R. Rogers, D. M. Adamson, L. J. Spaulding, and W. L. Blevins, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C13, p. 273).

The use of chromogenic substrates instead of carbohydrate degradation tests for the identification of *N. gonorrhoeae* and *N. meningitidis* was proposed by D’Amato et al. (5). They selected a group of 10 chromogenic substrates which allowed the separation of *N. gonorrhoeae* and *N. meningitidis* from each other and from 17 other species within 4 h after primary isolation on Thayer-Martin agar.

The present study demonstrates that a combination of lectins and chromogenic substrates can be used to obtain an identification of pathogenic *Neisseria* species after a 30-min incubation period.

**MATERIALS AND METHODS**

Isolates. All isolates were clinical specimens from San Francisco General Hospital or the San Francisco City Health Clinic. Sites from which the isolates were obtained included genitourinary tracts, respiratory tracts, wounds, and rectums. Most were isolated on Thayer-Martin agar, but several nonpathogenic *Neisseria* species were isolated on nonselective media. Isolates were either tested immediately after isolation or were frozen for testing at a later date. The identity of pathogenic isolates was determined by the use of Minitek disks (BBL Microbiology Systems, Cockeysville, Md.) in tests performed according to the instructions of the manufacturer. Other isolates were identified by using the Rapid NH system (Innovative Diagnostic Systems Inc., Decatur, Ga.).

Preparation of cell suspension. Bacteria were scraped with a cotton swab from an overnight culture on chocolate agar. The growth was emulsified in 0.8 ml of 0.05 M phosphate-buffered saline, pH 7.2, at a turbidity which exceeded that of a no. 3.0 McFarland Standard.

Reagents and methods. WGA and soybean agglutinin (SBA) (E-Y Laboratories, San Mateo, Calif.) were dissolved at 0.5 mg/ml in 0.05 M phosphate-buffered saline, pH 7.2. One drop of lectin was added to a well in an agglutination test slide (Merco Glass Works, New York, N.Y.). One drop of phosphate-buffered saline was added to another well as a control, and then one drop of bacterial suspension was added to each well. Slides were rotated by hand for 5 min.

Agglutination in the test well was compared with that in the control (buffer) well. If the control well showed autoagglutination, the cell suspension was treated with the antiautoagglutination reagent E-Y 10 (E-Y Laboratories) and then

---

* Corresponding author.
Table 1 shows the number of strains tested and the agreement between the combination of methods described above and the Minitek method for the identification of N. gonorrhoeae, N. meningitidis, N. lactamica, and the other Neisseria species and Branhamella catarrhalis. There was agreement between the two methods in the identification of 161 of 165 (97.6%) isolates tested when only three of the five tests (WGA, ONPG, and GGA) were employed. The four disagreements were with four strains of N. gonorrhoeae which were not agglutinated by WGA.

Table 2 shows the percentage of positive reactions obtained for the WGA, ONPG, and GGA tests. The table includes three additional strains each of the other Neisseria species and B. catarrhalis not included in Table 1. The number of strains which autoagglutinated is shown, as well as the effect of E-Y 10 in removing autoagglutination. Fifteen (12%) N. gonorrhoeae isolates autoagglutinated. E-Y 10 removed autoagglutination in 14 of these 15 strains (93.3%). One strain of N. gonorrhoeae occasionally autoagglutinated even after E-Y 10 treatment. Autoagglutination of N. lactamica was a common characteristic (four of five strains, 80%) and, when present, could not be removed by treatment with E-Y 10. Four strains (40%) of the other Neisseria species autoagglutinated, and like N. lactamica the autoagglutination could not be removed by E-Y 10.

The discovery of four isolates of N. gonorrhoeae which were not agglutinated by wheat germ lectin showed that such strains would be misidentified unless further tests were employed. Table 3 shows the results of supplemental tests which can be used to differentiate WGA-negative N. gonorrhoeae isolates from B. catarrhalis and the other Neisseria species. All four WGA-negative N. gonorrhoeae isolates were agglutinated by SBA, as were 30 additional N. gonorrhoeae strains, whereas none of 10 other Neisseria species tested was agglutinated by SBA. PA activity was not detectable in four B. catarrhalis strains under the conditions of these experiments. This result differentiates B. catarrhalis from N. gonorrhoeae and from the other Neisseria species tested. In addition, none of the B. catarrhalis strains tested here were able to grow on Thayer-Martin agar, in contrast to N. gonorrhoeae (Table 2).

By using two lectin agglutination tests (WGA and SBA) and three chromogenic substrate tests (ONPG, GGA, and PA), we were able to differentiate N. gonorrhoeae from all other Neisseria and B. catarrhalis strains tested and obtain 100% agreement with the Minitek method.

**DISCUSSION**

The combination of lectin agglutinations and chromogenic substrate tests described here yields rapid, accurate identification of the Neisseria species which grow on the selective media used by most clinical laboratories. The method does not require bacterial growth and does not involve carbohydrate degradation reactions.

The most frequently cited reasons for submitting problem cultures of N. gonorrhoeae to the Centers for Disease Control Sexually Transmitted Disease Laboratory are problems with the cystine tryptic agar test and rapid sugar tests (1). Difficulties with carbohydrate tests accounted for 49.7% of the reasons cited for submission of cultures to the Centers for Disease Control in a recent report (1). Inconclusive results or problems with the fluorescent antibody test accounted for 10.6%, whereas agglutination in meningococcal typing sera and failure to grow on selective media each accounted for 4.3% of the reasons cited (1).

The presence of the enzyme GGA in N. meningitidis differentiates this organism from N. gonorrhoeae (7). In a study of atypical Neisseria strains, Hoke and Vedros (8) showed that a maltose-negative strain of N. meningitidis remained positive for GGA activity. Conversely, an N.
gonorrhoeae isolate which produced acid from glucose and maltose (and possibly sucrose) remained negative for GGA activity. Thus, the genes for maltose degradation and GGA activity are apparently not closely linked genetically, and isolates with an aberrant maltose reaction may be identified by testing for GGA activity.

The commonly used rapid carbohydrate degradation methods for identifying Neisseria species require a 4- to 5-h incubation period (10, 13, 17). Yong and Prytula (17) identified within 4 h all 377 strains of N. gonorrhoeae tested by using a rapid microcarbohydrate method. Within 2 h, 51% of the strains were identified, whereas 14% were identified after 1 h of incubation. In the present report, all 165 strains of Neisseria species were identified after a 30-min incubation period.

In a test of the use of WGA for the identification of N. gonorrhoeae, Rogers et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1982) found 6 (4.4%) isolates which were not agglutinated by WGA and 15 (11.1%) which were uninterpretable because of autoagglutination. In our study of 126 N. gonorrhoeae isolates, we found 4 (3.2%) which were not agglutinated by WGA. All four of these strains were agglutinated by SBA. In agreement with the results of Rogers et al., 15 of 126 (11.9%) of the gonococcal isolates in our study autoagglutinated. However, the antiagglutination reagent E-Y 10 removed autoagglutination in 14 (93%) of these strains.

When three tests (WGA, ONPG, and GGA) were used for the identification of N. gonorrhoeae, there was 97.6% agreement with the Minitek method. When two additional tests were employed (SBA and PA), there was 100% agreement with Minitek. The use of lectins plus chromogenic substrates offers a rapid and accurate method which does not depend upon growth or carbohydrate degradation for the identification of N. gonorrhoeae. The method is easily adaptable to the clinical laboratory.

### LITERATURE CITED


