Comparison of a Slide Coagglutination Technique with the Minitek System for Confirmation of *Neisseria gonorrhoeae*

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The Phadebact gonococcus test was performed on 314 bacterial isolates to compare the coagglutination technique with the Minitek system for the confirmation of *Neisseria gonorrhoeae*. Direct testing, using colonies of *N. gonorrhoeae* mixed with the Phadebact gonococcus test reagents, produced noninterpretable results in many cases. However, an alternative method, described by the manufacturer, of heating several colonies in distilled water for 20 min before testing alleviated most interpretation difficulties. Complete agreement was achieved with both the Phadebact and Minitek tests for all 236 isolates of *N. gonorrhoeae*. Of the 78 nongonococcal isolates tested with Phadebact, two isolates gave false-positive results, whereas one isolate was reported as noninterpretable. The Phadebact gonococcus test appears to be a simple and rapid alternative to confirmatory biochemical procedures for *N. gonorrhoeae*.

Laboratory confirmation of *Neisseria gonorrhoeae* is primarily based on characteristic sugar utilization patterns. These are normally performed by using cystine-Trypticase agar medium or more recently by the Minitek system (BBL Microbiology Systems, Cockeysville, Md.) (16, 17, 20). Both methods are expensive, time consuming, and can lend themselves to erroneous reactions due to the use of small inocula. Immunofluorescent staining techniques can be used in some well-equipped laboratories as a tool for rapid and sensitive identification of *N. gonorrhoeae* in urogenital specimens (8, 9, 12, 13, 18). However, gonococcal-like colonies from non-urogenital sites must be confirmed by other procedures due to cross-reactions with other organisms (4, 5, 14, 18). Alternative methods, such as enzymatic profiles (3) and microcarbohydrate utilization techniques (23), have been developed for the rapid identification of *N. gonorrhoeae*. These procedures require large inocula and a minimum of 4 h of incubation for proper test results. A staphylococcal coagglutination technique has been developed to provide a more rapid and simple testing procedure for the confirmation of *N. gonorrhoeae* (7). The coagglutination technique, with the Phadebact gonococcus test (Pharmacia Diagnostics, Piscataway, N.J.), was compared with the Minitek *Neisseria* identification system as a confirmatory procedure.

MATERIALS AND METHODS
Organisms. A total of 314 bacterial isolates were obtained from the Forsyth County Health Dept., Winston-Salem, N.C., the North Carolina Department of Human Resources, Raleigh, and the North Carolina Baptist Hospital, Winston-Salem. The nongonococcal isolates included organisms that exhibit colony morphology, Gram reaction, or oxidase production similar to that of *N. gonorrhoeae*.

Isolation and maintenance. Both selective (Thayer-Martin) and nonselective (chocolate) media were used for isolation (6). Some organisms were isolated from body sites other than urogenital areas. Isolated organisms were maintained on chocolate agar in 5 to 8% CO₂ at 37°C. After testing, each isolate was frozen in a fetal calf serum–Columbia broth (BBL) solution (50% vol/vol) at −70°C for future reference.

Identification. All organisms were Gram stained and then tested for the production of oxidase by the Kovacs technique (21). All *Neisseria* isolates were speciated by the Minitek system; however, final identification of organisms other than *Neisseria* isolates was performed by using conventional biochemical methods (11, 19, 22, 24). All isolates were evaluated by the Phadebact gonococcus test and the Minitek system.

Minitek system. Minitek identification was performed by the instructions of the manufacturer. Paper disks impregnated with various substrates were used for biochemical determinations. Each disk was inoculated with 0.05 ml of a turbid suspension (corresponding to a McFarland no. 9 standard) of each test isolate prepared in Minitek *Neisseria* broth. After 18 to 24 h of aerobic incubation in a humidor at 37°C, the following test determinations were made: acid production from dextrose, maltose, and sucrose; hydrolysis of o-nitrophenyl-β-D-galactopyranoside; and reduction of nitrate.

Coagglutination test. The Phadebact gonococcus test kit contains two test reagents: (i) the gonococcus
reagent, consisting of heat-killed staphylococci coated with purified anti-gonococcal antibodies, and (ii) the control reagent, consisting of the heat-killed staphylococci coated with nongonococcal antibodies. Several colonies of a test isolate were either tested directly on a microscope slide with the Phadebact reagents, or suspended in 0.5 ml of distilled water and heated to 80 to 100°C for 20 min. The organism suspension was added to separate portions of the coagglutination reagents and rotated manually, with all reactions determined macroscopically within 2 min. Dropper vials supplied by the manufacturer delivered quantities of test reagent which were thought to be in excess of amounts necessary for definitive coagglutination results. Therefore, to conserve reagents while maintaining satisfactory coagglutination reactions, 10-μl amounts of test reagents were used in all test determinations. The reactions were graded only for the production of agglutination and not evaluated for the strength of agglutination as described by the manufacturer. Agglutination reactions of gonococcal isolates were variable with heavier suspensions producing more rapid agglutination than lighter suspensions. Agglutination occurring only with the gonococcal reagent, when coupled with appropriate colony morphology, Gram reaction, and oxidase test results, indicated the presence of *N. gonorrhoeae*. Lack of agglutination with the gonococcus reagent or control reagent suggested that the organism was not *N. gonorrhoeae*. Organisms agglutinating with both reagents represented noninterpretable results.

RESULTS

An overall agreement of 99% was achieved between the Phadebact and Minitek systems with 314 bacterial isolates. Complete agreement was seen in all 236 isolates of *N. gonorrhoeae* (Table 1). However, with the 78 nongonococcal organisms, one isolate of *N. subflava* and one isolate of *Moraxella osloensis* gave false-positive results with Phadebact. In addition, an isolate of *N. sicca* produced a noninterpretable result with Phadebact.

DISCUSSION

The instructions of the manufacturer suggest direct slide agglutination testing with fresh clinical cultures. Our initial experiments with this procedure produced many noninterpretable results. Menck (15) tested tonsillo-pharyngeal specimens by Phadebact and found noninterpretable reactions which he termed as "pseudo coagglutination". He retested those isolates in the presence of trypsin to reduce the pseudo coagglutination. However, there were still some isolates that consistently exhibited noninterpretable reactions. Our results using this procedure on a few isolates produced similar pseudo coagglutination. Barnham and Glynn (1) reported poor results in distinguishing *Neisseria* species. They performed their studies by using a prototype reagent and encountered autoagglutination similar to the pseudo coagglutination described by Menck (15). Consequently, we tested all isolates by the heat treatment procedure, as previously described, to alleviate most interpretation difficulties.

Definitive results were produced in all but one case by using a heat-treated organism suspension. This isolate of *N. sicca* produced a reaction described by previous authors as "fine granules not involving reagent staphylococci and readily distinguishable from true coagglutination" (7). We concluded that reactions of this nature were noninterpretable and would require identification by another test procedure when using Phadebact for routine confirmation of *N. gonorrhoeae*.

Isolates of nonpathogenic *Neisseria* species, *N. meningitidis*, *Moraxella* species, *Haemophilus influenzae*, and *Pseudomonas aeruginosa* were tested by Phadebact because of oxidase production, Gram reaction, or colony morphology similar to *N. gonorrhoeae* (2, 11, 18, 19, 24). Furthermore, each of these is known to produce cross-reactions with unabsorbed antigonococcal antibody (7). False-positive cross-reactions were produced with the Phadebact antigonococcal reagent for only two isolates. One isolate of *N. subflava* and one isolate of *M. osloensis* produced false-positive agglutination indistinguishable from *N. gonorrhoeae* isolates. Each isolate was retested and evaluated for reaction strength with quantities of test reagents delivered by dropper vials supplied by the manufacturers. The reaction strength upon retesting was comparable to reaction strengths of isolates of *N. gonorrhoeae*. The identity of these isolates was confirmed by the Special Bacteriology Unit.

**Table 1. Results of testing 314 bacterial isolates with the Phadebact gonococcus coagglutination procedure**

<table>
<thead>
<tr>
<th>Organisms*</th>
<th>No. of isolates</th>
<th>No. of positive tests with Phadebact</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>236</td>
<td>236</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td><em>N. sicca</em></td>
<td>21</td>
<td>1*</td>
</tr>
<tr>
<td><em>N. flavae</em></td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>N. subflava</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Branaheella catarrhalis</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>Moraxella sp.</em></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>A. calcoaceticus var. anitratus</em></td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td><em>A. calcoaceticus var. luoffii</em></td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>TM-1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>G. haemolysans</em></td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* All organisms were identified by Minitek or conventional tests.
* One isolate produced a noninterpretable result.
of the Center of Disease Control, Atlanta, Ga. TM-1 and Gemella haemolyans (formerly N. haemolyans) isolates were included as organisms that are frequently encountered in the pharynx and other mucosal membranes and demonstrate Gram reactions similar to those of N. gonorrhoeae (10, 19, 22). In addition, TM-1 isolates have cultural characteristics, colony morphology, oxidase production, and biochemical reactions similar to those for N. gonorrhoeae (10, 19). Acinetobacter species may mimic N. gonorrhoeae upon Gram reaction or possess similar cultural characteristics (18, 19). Phadebact reactions for these nongonococcal isolates were negative.

All 236 isolates of N. gonorrhoeae were in complete agreement with both Phadebact and Minitek test procedures. Both fresh clinical cultures as well as stock isolates of N. gonorrhoeae produced positive coagglutination without discerning differences in reactions. No cross-reactions were obtained with the N. meningitidis isolates as seen with immunofluorescent staining techniques (13, 15). Furthermore, this confirmatory test can be performed from primary isolation media, without additional subculture, in which isolation and quantity of suspected colonies is sufficient. The approximate cost per test of the Minitek is $1.80, as compared with $1.36 using the Phadebact system. If the Phadebact test is performed with only 10 μl, the cost per test is reduced to $0.28, thereby providing a substantial cost saving in time as well as materials. We think that the Phadebact gonococcus test is an economical, rapid, and simple procedure for the confirmatory identification of N. gonorrhoeae which can be performed in even modestly equipped laboratories.

LITERATURE CITED