Evaluation of the Phadebact Test for Identification of Neisseria gonorrhoeae

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The Phadebact Gonococcus Test (Pharmacia Diagnostics, Piscataway, N.J.) is used for the identification of Neisseria gonorrhoeae. In this test, boiled organisms are examined by using a 3-min coagglutination technique. A total of 313 isolates of Neisseria confirmed by the fluorescent-antibody technique or identified to the species level by the cysteine-tryptose agar utilization technique, were tested by the coagglutination technique. Of 229 isolates of N. gonorrhoeae, 13 (5.7%) were negative by the coagglutination technique on the first testing, 4 (1.7%) of which remained negative on subsequent testing after subculture. Of the 84 isolates of Neisseria other than N. gonorrhoeae, none showed a positive coagglutination reading. The Phadebact Gonococcus Test provides rapid, accurate identification for most isolates of N. gonorrhoeae. As with any new technique, standard procedures (fluorescent-antibody or cysteine-tryptose agar analysis or both) should be performed along with the new technique until laboratory workers are comfortable with the performance and interpretation of the test. We must, however, emphasize that it is very important to follow carefully the procedures outlined on the package insert.

In the past few years, reports of a new coagglutination test for the identification of Neisseria gonorrhoeae have appeared. There have been contradictory results (1–4, 6) about the utility of the test, but almost all of these reports used procedures which altered those recommended in the package insert.

Since a new product, the Phadebact Gonococcus Test, will in some laboratories replace the fluorescent-antibody or biochemical utilization technique or both, this study was initiated to determine whether the product is reliable when the recommended protocol is followed.

MATERIALS AND METHODS

Specimens for culture were inoculated immediately onto Thayer-Martin medium, incubated overnight in candle extinction jars at 35 to 36°C, and delivered to the Massachusetts Department of Public Health State Laboratory Institute the next day. When possible, the cultures were examined after 24 and 48 h of incubation (5, 7). Oxidase-positive colonies with morphological features typical of Neisseria and containing gram-negative diplococci were considered to be presumptively positive for N. gonorrhoeae by the criteria of the Centers for Disease Control (CDC, Atlanta, Ga.) (7). The fluorescent-antibody (FA) test (5, 7) was performed on all presumptively positive organisms with conjugate manufactured by Difco Laboratories (Detroit, Mich.). Before use, the quality of the conjugate was determined by use of the following: four strains of N. gonorrhoeae; two strains of Neisseria meningitidis; and one strain each of Neisseria lactamica, Neisseria perflava, Neisseria flavescens, Branhamella catarrhalis, Staphylococcus epidermidis, Escherichia coli, and Enterobacter cloacae. In accordance with CDC procedures (7), the working dilution selected (1:32), along with the surrounding dilutions (1:16 and 1:64), showed clear-cut results with no cross-reactivity. Thus, the four strains of N. gonorrhoeae showed +4+ fluorescence at dilutions of 1:16, 1:32, and 1:64. N. meningitidis, the saprophytic Neisseria, and the other bacteria showed no cross-reactivity. With each test, two strains of N. gonorrhoeae were used as positive controls; one strain of N. meningitidis served as a negative control, and one strain of Enterobacter cloacae was included as the nonspecific, staining control.

If necessary, the organisms were identified to the species level by use of cysteine-tryptose agar (CTA) with 1% glucose, maltose, sucrose, lactose, mannitol, levulose, and xylose. N. gonorrhoeae, N. meningitidis, N. lactamica, N. sicca, N. perflava, and B. catarrhalis provided quality control for these sugars. Thus, each individual sugar was tested for proper reactivity. Growth on nutrient agar slants at 25 and 35°C was assessed, and an o-nitrophenylβ-D-galacto-pyranoside hydrolysis reaction was performed.

Organisms from an anogenital source which demonstrated a positive FA reaction were considered to be N. gonorrhoeae. Anogenital cultures which were neg-
ative by FA analysis were identified to the species level by the CTA technique. Pharyngeal cultures which gave a positive FA reaction were confirmed by CTA testing. Pharyngeal cultures which were morphologically consistent with *N. meningitidis* and showed two consecutive (from 24-h plates on consecutive days) negative FA reactions were considered to be negative for *N. gonorrhoeae*. Anogenital cultures showing a positive FA and negative Phadebact reaction were confirmed as *N. gonorrhoeae* by the CTA technique. Cultures with insufficient growth or which had been incubated for 48 h or more when first examined were subcultured onto half-plates of Thayer-Martin medium and incubated for 24 h in a CO2 incubator before being retested.

The Phadebact Gonococcus Test kit contains two vials each of gonococcal reagent and control reagent. The gonococcal reagent is composed of immunoglobulin G antigonococcal antibodies (raised in rabbits and absorbed to remove cross-reacting antibodies) coupled to the protein A of heat-killed staphylococci. The control reagent is composed of immunoglobulin G from nonimmunized rabbits, coupled to the protein A of heat-killed staphylococci.

The growth was removed from the culture medium with a sterile cotton-tipped swab and emulsified in 0.5 ml of distilled water in a test tube (10 by 75 mm). The tubes were put into a boiling-water bath for 5 min. The gonococcal and control reagents were brought to room temperature before being used.

A glass slide (52 by 227 mm) was subdivided into 30-mm sections with a wax pencil. One drop of the gonococcal reagent and 1 drop of the control reagent were placed alternately on the slide. With a Pasteur pipette, one drop of the boiled colony suspension was added to the gonococcal reagent, and one drop was added to the control reagent on the slide. Each suspension was thoroughly mixed with an applicator stick. The slide was rocked for 3 min by being tilted 45° every 2 s. Reactions were observed with indirect lighting against a dark background. At 3 min, agglutination reactions in the gonococcal and control reagents were graded on a scale of 0 to 4+ and recorded. Isolates that were positive by the FA test and negative by the Phadebact test were subcultured onto Thayer-Martin medium, incubated for 24 h in a CO2 incubator, and retested.

A positive result consisted of a significantly stronger reaction in the gonococcal reagent compared with that in the control reagent. All isolates which gave positive reactions in the gonococcal reagent showed very minimal or no reaction in the control reagent. A negative result was defined by a lack of reaction in the gonococcal reagent with a negative reaction in the control reagent. An uninterpretable result consisted of reactions of equal strength in both the gonococcal and control reagents.

### RESULTS

A total of 313 *Neisseria* isolates were tested with the Phadebact coagglutination test. Of these isolates, 229 were confirmed as being *N. gonorrhoeae* by the FA technique; 83 isolates were identified to the species level by CTA utilization tests as, or were FA negative and morphologically consistent with *N. meningitidis*. One isolate was identified as *N. sicca*.

Table 1 shows the sources of the cultures classified by clinic type. Clinics for homosexuals accounted for the majority of pharyngeal and anal canal cultures. Cultures are routinely taken only from the oropharynx and anal canal of the all-male bathhouse clients unless a urethral culture is specifically requested. Clinics treating sexually transmitted diseases, neighborhood health centers, and other laboratories that submitted cultures for confirmation supplied isolates from both men and women.

Of the 229 isolates of *N. gonorrhoeae*, 212 reacted positively on the first Phadebact testing. Thirteen cultures were negative on primary testing but became positive on a retest with a fresh subculture. Six of these cultures had insufficient growth on the first testing. Four additional cultures remained negative upon subsequent testing. Identification as *N. gonorrhoeae* by the CTA technique was confirmed on all Phadebact-negative–FA-positive cultures.

The Phadebact coagglutination and FA results are compared by source in Table 2. It is significant that there were no isolates which were FA negative and Phadebact positive. Of 107 urethral isolates, all from males, 103 were positive by both FA and Phadebact analyses. Three isolates of *N. gonorrhoeae* were nonreactive to the Phadebact technique but FA positive. These three cultures were from males at the clinic for

### TABLE 1. Classification of cultures by clinic type

<table>
<thead>
<tr>
<th>Type of clinic</th>
<th>Urethra</th>
<th>Cervix</th>
<th>Anal canal</th>
<th>Pharynx</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinic for homosexual men</td>
<td>19</td>
<td>0</td>
<td>17</td>
<td>65</td>
<td>101</td>
</tr>
<tr>
<td>Bathhouses (homosexual men)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Sexually transmitted disease clinics</td>
<td>60</td>
<td>35</td>
<td>7</td>
<td>5</td>
<td>107</td>
</tr>
<tr>
<td>Health centers</td>
<td>18</td>
<td>35</td>
<td>1</td>
<td>4</td>
<td>58</td>
</tr>
<tr>
<td>Confirmations</td>
<td>10</td>
<td>22</td>
<td>0</td>
<td>2</td>
<td>34</td>
</tr>
</tbody>
</table>
TABLE 2. Comparison of Phadebact coagglutination with FA results

<table>
<thead>
<tr>
<th>Source</th>
<th>FA positive/ Phadebact positive</th>
<th>FA positive/ Phadebact negative</th>
<th>FA negative/ Phadebact positive</th>
<th>FA negative/ Phadebact negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethra</td>
<td>103</td>
<td>3</td>
<td>0</td>
<td>1°</td>
</tr>
<tr>
<td>Cervix</td>
<td>88</td>
<td>1</td>
<td>0</td>
<td>3°</td>
</tr>
<tr>
<td>Anal canal</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3°</td>
</tr>
<tr>
<td>Pharynx</td>
<td>9</td>
<td>1°</td>
<td>0</td>
<td>76°</td>
</tr>
</tbody>
</table>

a. *N. meningitidis.

b. Two isolates were *N. meningitidis*; one was *N. sicca.

c. *N. meningitidis* (FA 2 to 3+).

homosexuals; two of the men were examined on the same day, and the third was examined 7 days later. Epidemiologically, there appeared to be no connection among the three men. The only isolate negative by both methods was *N. meningitidis*. A total of 88 cervical cultures were positive by both methods, 1 culture was FA positive and Phadebact negative, and 3 cultures were negative by both methods. Of the 28 anal canal cultures, 25 were positive by both methods, none was positive by FA analysis and negative by the Phadebact test, and 3 *N. meningitidis* isolates were negative by the Phadebact test. The 86 pharyngeal cultures yielded nine isolates positive by both methods. One culture gave a 3+ FA result initially and a 2+ FA result subsequently and was determined to be *N. meningitidis* by the CTA technique. This isolate remained negative by the Phadebact test. Seventy-six cultures, negative by both the FA and Phadebact tests, were morphologically consistent with, or identified by, CTA analysis as *N. meningitidis*.

Although grading the degree of coagglutination was not recommended by the manufacturer, we gave positive reactions a grade of 1+ to 4+ to determine the ease of interpreting the results (Table 3). Two of us (M.S.H. and J.R.K.) read the tests with the following grading criteria: a 4+ reaction showed large clumps; a 3+ reaction showed slightly less heavy clumping as compared with the 4+ reaction; a 2+ reaction showed moderate clumping with medium-sized aggregates; and a 1+ reaction showed a much finer clumping activity, but definitely had greater activity than the smooth texture of a nonreactive culture. Almost 80% of the positive reactions were of 3+ to 4+ reactivity. About 15% were somewhat less reactive (of 2+ reactivity), and 4.4% gave a 1+ reaction. Four isolates showed no coagglutination.

**DISCUSSION**

Considering that 13 anogenital isolates were negative on the first testing, for whatever reasons, the overall sensitivity of the test was 92.6%. It is our policy, and we hope it to be the policy of every laboratorian, that when final results do not confirm presumptive diagnoses, further testing is performed. For example, every anogenital isolate which is FA negative is automatically identified to the species level by biochemicals. In the case of the Phadebact test, an anogenital isolate giving a negative test result for *N. gonorrhoeae* should be reexamined with a fresh 24-h subculture. If the second test remains negative, then identification to the species level by biochemicals or the FA test or both should be performed. If these procedures are not available, a subculture should be forwarded to a reference laboratory for identification. Other investigators (1, 2) have reported false-positive results, especially in pharyngeal cultures; we did not obtain any false-positive results from any of the four sources tested. We conclude that the Phadebact Gonococcus Test has a high sensitivity and specificity and that it is useful for the rapid identification of *N. gonorrhoeae*.

As with all new procedures, results should be correlated with standard confirmatory techniques until the laboratory workers are confident in the performance and interpretation of the test. It is very important to follow carefully the procedures outlined on the package insert.

**LITERATURE CITED**

2. Hampton, K. D., R. A. Stallings, and B. L. Wasilaukas. 1979. Comparison of a slide coagglutination technique with