Evaluation of Rapid Carbohydrate Degradation Tests for Identification of Pathogenic Neisseria

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A total of 156 clinical isolates were tested by the modified rapid fermentation test, the BACTEC Neisseria differentiation kit, and the cystine-Trypticase agar method. The modified rapid fermentation test and BACTEC methods accurately identified at least 95% of 101 strains of N. gonorrhoeae tested and at least 91% of 45 strains of N. meningitidis tested within 4 h. Overall, the cystine-Trypticase agar method was the most accurate (97%) but required as long as 48 h of incubation. The data presented appear to show that rapid carbohydrate degradation tests can provide reliable and specific identification results.

Due to the increasing numbers of reported gonococcal infections, the increasing recognition of extragenital gonococcal infections, and the implication of Neisseria meningitidis in urogenital infections and neonatal ophthalmia (3, 9, 11, 14, 16), the development and use of rapid confirmatory tests for the identification of pathogenic Neisseria have assumed considerable importance. An added complication is that other "saprophytic" Neisseria species have been implicated as the etiologic agents in infections of the compromised host (20, 27).

Presently, there are three methods used in the identification of Neisseria species in the laboratory. Culture with subsequent carbohydrate degradation tests of isolates is now often used. Some laboratories identify isolates of N. gonorrhoeae with a fluorescent-antibody technique; however, there have been reports of cross-reactions between N. gonorrhoeae and non-gonococcal neisseriae when this method had been employed (24, 25). Finally, agglutination procedures have been adapted to identification schemes for N. meningitidis and N. gonorrhoeae. With N. meningitidis, however, cross-reactions among strains of Neisseria species may occur in specific meningococcal grouping sera, posing problems in accurate species identification (6, 8, 23). Recently, a staphylococcal coagglutination technique has been developed for the identification of N. gonorrhoeae (7). This method claims rapid confirmatory identification with 2 min. Evaluations of coagglutination have provided conflicting results (2, 13), indicating that further testing and possibly improvement may be warranted. It is thus useful for clinical and epidemiological purposes to confirm the identity of pathogenic Neisseria with carbohydrate degradation tests.

Carbohydrate degradation by Neisseria has been studied for many years to develop better methods of demonstrating biochemical reactions. Many techniques employing various agar bases, growth factors, percentages of agar, inoculum sizes, amounts of media, and methods of inoculation have been reported in the literature (4, 10, 12, 18, 22, 26, 28). These reactions have been shown in agar plates, agar slants, semisolid media, and liquids.

The purpose of this study was to compare three procedures: the modified rapid fermentation test (MRFT), essentially as described by Brown (4), the semisolid cystine-Trypticase agar (CTA) technique, and the BACTEC Neisseria differentiation kit.

MATERIALS AND METHODS

The 156 clinical isolates used in this study consisted of 101 strains of N. gonorrhoeae, 45 strains of N. meningitidis, 4 strains of N. lactamica, 2 strains of N. subflava, 1 strain of N. sicca, 2 strains of Branhamella catarrhalis, and 1 strain of CDC group II F which grew on modified Thayer-Martin medium. Except for seven strains of N. meningitidis and three strains of N. lactamica obtained from the Center for Disease Control (courtesy of R. E. Weaver), all of the strains were from the Minnesota Department of Health and the University of Minnesota Clinical Microbiology Laboratory. In addition, all strains were selected randomly and were coded so that their identities remained unknown until all data were analyzed. Inocula for the three methods were taken from the same pure culture plate (chocolate agar) which had been incubated at 35 to 37°C in a candle extinction jar for 18 to 24 h. A Gram-stained smear and oxidase test were performed on each of the cultures, as was a smear stained with gonococcal fluorescein-labeled antisemrum (Difco).

For the MRFT, a buffered-salt solution (pH 7.0 to 7.1) was prepared and stored at 4 to 6°C. Concentrated solutions of carbohydrates (dextrose, maltose, and sucrose [Difco Laboratories, Detroit, Mich.]) were stored
at 4 to 6°C and added to the buffer solution only when the test was performed with the organism suspension. The method of Brown (4) was modified in the following ways. First, the amount of phenol red used in the buffered-salt solution was doubled, allowing for easier interpretation of reactions. Second, because of the possible contamination of maltose with dextrose, a 10%, rather than 20%, solution of maltose was used.

For the CTA technique, 4.3 g of CTA (BBL Microbiology Systems, Cockeysville, Md.) was mixed in 150 ml of distilled water, and the pH of the solution was carefully adjusted to 7.6 with 0.1 or 1 N NaOH. The solution was heated until the agar completely dissolved and then autoclaved at 121°C for 15 min. For each 50 ml of base (cooled to approximately 56°C), 2.5 ml of a 20% filter-sterilized carbohydrate solution (dextrose, maltose, and sucrose [Difco]) was added, and the final solution was dispensed in 2-ml amounts into screw-capped tubes (13 by 100 mm). These tubes were stored at 4 to 10°C. A loopful of pure culture of the test organism was inoculated into the top one-third of the tube. Tubes were then capped tightly and incubated in ambient air (not CO₂) at 35 to 37°C for 24 to 48 h. Carbohydrate degradation caused a change of indicator (phenol red) from red to yellow.

The BACTEC Neisseria differentiation kit (Johnston Laboratories, Inc., Cockeysville, Md.) consists of three vials containing ¹⁴C-labeled dextrose, maltose, and fructose, respectively. Upon metabolism of these carbohydrates, there is a release of ¹⁴CO₂ into the supernatant gas. The BACTEC instrument analyzes this gas for radioactivity and, if a threshold level is exceeded, indicates metabolism of that carbohydrate. With this kit, 0.3-ml samples of a heavy suspension in a broth provided by the manufacturer were inoculated into the three vials. In addition, an ONPG (O-nitrophenyl-β-D-galactopyranoside) test strip was inoculated and incubated at 35 to 37°C. The carbohydrate vials were incubated at 35 to 37°C for 3 h and inserted into a BACTEC instrument (model 460) in which the supernatant gas was aspirated and assayed for its content of ¹⁴CO₂. A reading above a threshold level of 20 indicated metabolism of the substrate and was interpreted as a positive reaction. The ONPG test strip was observed for the appearance of a yellow color, indicating β-galactosidase activity.

RESULTS

All isolates were oxidase-positive, gram-negative diplococci. All but two strains of N. gonorrhoeae, otherwise identified on the basis of growth characteristics and biochemical reactions, stained with gonococcal fluorescein-labeled antisera. There were six non-gonococcal strains (five N. meningitidis, one N. lactamica), identified on the basis of growth characteristics and biochemical reactions, which stained with the gonococcal fluorescein-labeled antisera. The results of the carbohydrate degradation tests for all 156 clinical isolates by the MRFT, BACTEC, and CTA methods are shown in Table 1. All three methods were highly effective in the identification of N. gonorrhoeae and N. meningitidis. Identification of three additional Neisseria species (N. lactamica, N. subflava, N. sicca), Branhamella catarrhalis, and CDC group II F was accurately made by MRFT and CTA; BACTEC failed to identify one of four strains of N. lactamica. The MRFT identified 96% of organisms after 1 to 4 h of incubation, the BACTEC radiometric method identified 94% of organisms after 3 h of incubation, and the CTA technique identified 97% of organisms after 24 to 48 h of incubation. Although the majority of positive CTA reactions (138) were recorded after 24 h of incubation, some of these may have been positive after 6 h if readings had been taken at this time.

DISCUSSION

Three methods for identifying Neisseria based upon the production of acid from various carbohydrates have been studied. Our investigation confirmed the reliability of the rapid carbohydrate degradation technique. In his evaluation of the Kellogg and Turner (17) rapid fermentation test, Brown found the methodology to be unsatisfactory (4). By using Brown’s modifications of the method, as well as by increasing the amount of phenol red indicator and decreasing the concentration of maltose, we found that the MRFT was rapid, easy to read, and reproducible. Earlier studies with the MRFT have also shown similar results (5, 21). The MRFT measures preformed enzymes and thus has the advantage of being independent of growth. In contrast, a positive reaction in the CTA technique depends on adequate microbial growth, which may take as long as 48 h, thus eliminating the possibility of early identification. The agents for the MRFT are easily prepared and can be stored for several months at 4 to 6°C, although care must be taken in adjusting the pH of the buffered-salt solution as well as in preparing the concentrated carbohydrate solutions. Furthermore, the use of disposable tubes and small amounts of medium makes this method not only rapid, but also economical.

Our study has also shown that a commercially available system, the BACTEC Neisseria differentiation kit, provides satisfactory and rapid results. We were unable to confirm the frequency (45%) of high readings in BACTEC for fructose reported by Applebaum and Lawrence (1). As with the MRFT, the BACTEC kit has the advantage of not requiring growth of the test organism in the vials. Further, there is no subjectivity in the interpretation of color changes because BACTEC provides a numerical output. The disadvantages of this method are the requirements for an expensive instrument and a heavy initial inoculum.
Problems with CTA carbohydrate medium have been reported for many years. These problems appear to be both biochemical and technical in origin. Catlin has reported that Neisseria strains which metabolize dextrose do so primarily by an oxidative pathway rather than by fermentation (6). The practical importance of understanding the difference between fermentative and oxidative metabolism has been emphasized by Hugh and Leifson (15) and by Shtibel and Toma (25). Although much of the literature has referred to the fermentative activity of neisseriae, these species metabolize dextrose by strictly aerobic means, namely the Entner-Doudoroff and pentose-phosphate pathways (19), and therefore produce only a small amount of acid. In addition, neisseriae are slowly growing and fastidious microorganisms which require enriched media and controlled growing conditions. For these reasons, several technical details must be rigidly observed to obtain accurate and reliable results with the CTA carbohydrate tests. Extreme care must be taken in preparing the medium and adjusting its pH, as well as in standardizing its inoculation, incubation, and interpretation. By following these criteria, the CTA method was the most accurate one used (97%) in this study, although the test required 24 to 48 h of incubation. Similar results were published by Shtibel and Toma (25).

In conclusion, rapid carbohydrate degradation tests were found to be reliable confirmatory identification procedures. The MRFT accurately identified 97% of gonococci, 93% of meningococci, and 100% of miscellaneous Neisseria and related species within 4 h. The BACTEC method accurately identified 95% of gonococci, 91% of meningococci, and 90% of miscellaneous Neisseria and related species within 3 h. Overall, the CTA method was the most accurate, identifying 96% of gonococci and 100% of other Neisseria species, but the method required as long as 48 h of incubation.

**Table 1. Comparison of results of carbohydrate degradation in the MRFT, BACTEC, and CTA methods**

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains tested</th>
<th>Cumulative no. (%) of strains showing positive reaction(s) after incubation for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>101</td>
<td>37 (37)</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>45</td>
<td>11 (24)</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td>N. subflava</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>N. sicca</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>B. catarrhalis*</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>CDC group II F*</td>
<td>1</td>
<td>—</td>
</tr>
</tbody>
</table>

*Not determined.

* B. catarrhalis and CDC group II F do not metabolize the carbohydrates used in this study.

**ACKNOWLEDGMENTS**

We thank Johnston Laboratories, Inc., for providing the BACTEC Neisseria differentiation kits.

**LITERATURE CITED**

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