Clinical Evaluation of the Rapid Carbohydrate Degradation Microtube Method for Identification of *Neisseria* Species

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The rapid carbohydrate degradation (Carr Microbiologicals, Wichita, Kans.) microtube method is a new test system designed for the identification of *Neisseria* spp. The system consists of four microtubes containing different carbohydrates in a peptone-Bitone basal medium. This method was evaluated for accuracy and speed in identifying species of *Neisseria*. Of the 386 clinical isolates used in this study, 98.4% were correctly identified to species level in 4 h with the rapid carbohydrate degradation system; parallel testing of the same isolates with conventional cystine-tryptic agar resulted in 96.1% accuracy in 48 h.

*Neisseria* species are identified in most diagnostic microbiology laboratories by using carbohydrate degradation methods. Conventional methods require 24 to 48 h of incubation for completion of the test (15). A number of rapid methods which employ various agar bases, growth factors, sugar concentrations, inoculum concentrations, inoculum sizes, filter paper disks, radiometry, gas chromatography, spectrophotometry, etc., and which use solid, semisolid, or liquid media have been reported in the literature (2, 4–7, 11, 12, 16–19, 22, 23, 25–27).

Some inherent disadvantages exist with several of these methods. For example, radiometric methods (7, 24) require the purchase of expensive equipment and the use of ¹⁴C-labeled substrates. Gas-liquid chromatography (17) involves not only extraction procedures, but also the purchase of gas chromatography equipment. The identification of bacteria by genetic transformation (4) requires techniques, such as the preparation of wild-type deoxyribonucleic acid, which are not feasible in many diagnostic laboratories.

Methods involving CO₂ release from fermentable carbohydrates (23) require the use of spectrophotometry and preliminary preparation methodology which may not be practical in many clinical laboratories. The New York City fermentation media require horse blood and the preparation of a yeast dialysate. The Minitek system (BBL Microbiology Systems, Cockeysville, Md.) uses the manufacturer's equipment and has shown a sensitivity of around 92% within 4 h (2, 16).

In this paper we report on the evaluation of a commercially available rapid carbohydrate degradation (RCD) microtube system that does not require any prior preparation or purchase of special equipment and yields accurate results in 0.5 to 4.0 h. We used 386 strains of gonococci, meningococci, *Neisseria lactamica*, *Neisseria sicca*, *Neisseria subflava*, *Neisseria flavescens*, and *Brahmanella catarrhalis*. The method uses preformed enzymes as well as those synthesized by bacteria during growth in an enriched medium.

**MATERIALS AND METHODS**

A total of 386 strains of bacteria consisting of *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, *N. sicca*, *N. subflava*, *N. flavescens*, and *B. catarrhalis* were used in this study. Six of the strains were reference cultures obtained from the American Type Culture Collection and the Oklahoma State Health Department, and 380 were isolates from clinical specimens and were obtained from the microbiology laboratories of Oklahoma Memorial Hospital and Clinics and the Oklahoma State Health Department. The organisms were identified on the basis of morphology, Gram reaction, oxidase test, carbohydrate degradation, fluorescent-antibody staining, and coagglutination procedures (15). Stock cultures were maintained at −76°C in Trypticase (BBL Microbiology Systems) soy broth containing 15% (vol/vol) glycerol. When needed, these cultures were thawed at room temperature and subcultured on chocolate agar plates. The plates were incubated in a CO₂ incubator (6 to 8% CO₂) at 35°C for 18 to 24 h. These cultures were used as coded unknowns in the determination of carbohydrate degradation.

**Test system.** The RCD system (Carr Microbiologicals, Wichita, Kans.) consists of borosilicate microtubes (6 by 50 mm) containing the basal medium and a carbohydrate. The microtube is supported in an upright position by 2 ml of solidified 1.5% agar in a 13-by 100-mm screw-capped tube (Fig. 1). The basal medium contains (wt/vol): peptone, 1%; Bitone, 1%;
beef heart digest, 0.8%; sodium chloride, 0.5%; corn starch, 0.1%; agar, 1.5%; and phenol red, 0.003%, in demineralized water. The final pH is 7.5 ± 0.2. The system consists of four tubes, each containing a carbohydrate. The final concentration of glucose, sucrose, or lactose is 2.0%, and the final concentration of maltose is 0.5%. An additional tube containing fructose (2.0%) can also be included if desired. The media are stable at 4°C for up to 12 weeks. A 3-mm loopful of organisms from an overnight (18 to 24 h) culture on chocolate agar or Thayer-Martin medium is thoroughly mixed in the upper one-fourth of the microtube containing the carbohydrate medium. The tube system is incubated at 35°C and examined at 30-min intervals. A positive reaction is indicated by a yellow color in the inoculated portion of the medium, whereas a negative reaction causes no change or a deepening of the red color.

Parallel tests with cystine-tryptic agar (CTA) were performed for comparison. CTA was prepared from dehydrated material according to the manufacturer’s directions (Difco Laboratories, Detroit, Mich.). Filter-sterilized solutions of carbohydrates were added to the sterile CTA (cooled to approximately 52°C) to achieve a final concentration of 1%. A loopful of pure culture of the organism was inoculated into the top one-third of the tube and incubated at 35°C for 24 to 48 h. Carbohydrate degradation was indicated by a color change of the indicator from red to yellow.

RESULTS

A total of 382 isolates of Neisseria species and 4 Brahmanella catarrhalis isolates were used to compare the degradation of glucose, maltose, sucrose, and lactose by the RCD method and the CTA method. Of the 375 isolates of N. gonorrhoeae, N. meningitidis, N. lactamica, N. sicca, and N. subflava, 87 (23.2%) strains gave expected reactions within 30 min, 65% in 1 h, 76% in 2 h, and 98.4% within 4 h by the RCD method (Table 1). On the other hand, only 8.5% of the isolates gave expected results in the CTA media in 4 h, with 89% yielding reactions in 24 h and 96% in 48 h. Only 1 isolate of gonococcus failed to produce acid from glucose in the RCD method, whereas 14 isolates failed to degrade glucose in the CTA method. One isolate of N. sicca gave a maltose-negative reaction in the CTA method. All the other isolates gave expected reactions in both the RCD and CTA methods, with gonococci using the glucose only; meningococci using the glucose and maltose; N. lactamica using the glucose, maltose, and lactose; N. sicca using the glucose, maltose, lactose, and sucrose; and N. subflava producing acid from the glucose, maltose, and sucrose. None of the 11 strains of N. flavescens and B. catarrhalis produced acid from any of the carbohydrates in either method.

A comparison of the accuracy of carbohydrate degradation by the RCD and CTA methods is given in Table 2. The RCD method was highly effective, identifying 98.4% of the isolates within 4 h. Five of the isolates which were negative at 4 h yielded positive results within 24 h. The CTA method required 24 to 48 h for the identification of 96.1% of the isolates.

DISCUSSION

The increase in the number of gonococcus isolates obtained from nongenital sites and of meningococcus and N. lactamica isolates obtained from genital sites and the ability of other species of Neisseria to cause opportunistic infections in debilitated patients necessitate the rapid and accurate identification of these organisms. The laboratory procedures presently employed for identifying these gram-negative, oxidase-positive diplococci to the species level involve cultural characteristics, fluorescent-antibody techniques, coagglutination, and carbohydrate degradation (1, 2, 5-7, 11, 12, 14-16, 18, 19, 22, 23, 25-27). The immunofluorescent techniques are commonly used in the identification of gonococci. However, there have been reports of cross-reactivity between gonococci and other members of Neisseria (20, 22). Agglutination and coagglutination methods are also prone to cross-
TABLE 1. Comparison of carbohydrate degradations in the RCD and CTA methods

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>Cumulative no. showing acid reaction at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RCD</td>
</tr>
<tr>
<td>N. gonorrhoeae</td>
<td>226</td>
<td>61</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>64</td>
<td>12</td>
</tr>
<tr>
<td>N. lactamica</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>N. sicca</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>N. subflava</td>
<td>29</td>
<td>2</td>
</tr>
</tbody>
</table>

* —, Not determined.

TABLE 2. Accuracy of carbohydrate degradations in the RCD and CTA methods

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of strains (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
</tr>
<tr>
<td>RCD&quot;</td>
<td>386</td>
</tr>
<tr>
<td>CTA&quot;</td>
<td>386</td>
</tr>
</tbody>
</table>

* Expected reactions took place within 4 h.
" Expected reactions took place in 24 to 48 h.

reactivity among the species and thus warrant further evaluations and improvements (3, 9, 10, 13).

Other methods used for the identification of Neisseria spp. include use of enzymatic profiles, wheat germ agglutinins, CO₂ evolution from carbohydrates, electron capture gas chromatography to detect acetoin, and genetic transformation (4, 8, 17, 21, 23). Most of these procedures are seldom used in clinical diagnostic laboratories. The most commonly used method is carbohydrate degradation reactions in CTA. The CTA medium not only requires 24 to 48 h of incubation, but also has been found to be inadequate in certain cases owing to the failure of occasional strains of gonococci to produce acid from glucose (6, 11, 16, 19, 25). Consequently, numerous methods have been reported in the literature which are claimed to be both sensitive and rapid (5, 11, 12, 16, 19, 24, 26).

One of the commonly used rapid methods is that of Kellogg and Turner (14), which does not require growth of the bacteria. However, Young et al. (27) report that false-positive reactions and a light inoculum cause slow or incomplete changes with glucose. Similarly, purity of maltose has been found to be important (16).

With the growing workload in most clinical microbiology laboratories, it is desirable to use methods that are not only rapid but also sensitive and specific. The RCD method evaluated herein uses preformed enzymes, thereby giving the expected results within 4 h. Thus, the results can be obtained the same day if the culture is inoculated at the beginning of the day. The RCD method did not give false-positive reactions with maltose or false-negative reactions with glucose, it does not require purchase of expensive equipment, it costs around 80¢ for a set of four to five tubes, and it uses familiar bacteriological techniques. It was found to be rapid and reliable, identifying over 98% of isolates within 4 h.

ACKNOWLEDGMENTS

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LITERATURE CITED

Carbohydrate degradation by Neisseria