

## Comparison of Three Methods for Identification of Pathogenic *Neisseria* Species

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A radiometric procedure was compared with the Minitek and Cystine Trypticase Agar sugar degradation methods for identification of 113 *Neisseria* species (58 *Neisseria meningitidis*, 51 *Neisseria gonorrhoeae*, 2 *Neisseria lactamica*, 2 *Neisseria sicca*). Identification of meningococci and gonococci was confirmed by agglutination and fluorescent antibody techniques, respectively. The Minitek method identified 97% of meningococci, 92% of gonococci, and 100% of other *Neisseria* after 4 h of incubation. The radiometric (Bactec) procedure identified 100% of gonococci and 100% of miscellaneous *Neisseria* after 3 h, but problems were encountered with meningococci: 45% of the later strains yielded index values for fructose between 20 and 28 (recommended negative cut-off point, <20), with strongly positive (>100) glucose and maltose and negative *o*-nitrophenyl- $\beta$ -D-galactopyranoside reactions in all 58 strains. The Cystine Trypticase Agar method identified 91% of meningococci, 90% of gonococci, and 100% of other *Neisseria* after 24 to 48 h. Prolongation of the Cystine Trypticase Agar incubation period led to abnormal lactose/sucrose reactions in some meningococci and gonococci. Radiometric and Minitek systems are more accurate and convenient than Cystine Trypticase Agar techniques, but, on the basis of these results, radiometric fructose sensitivity levels for meningococci need reevaluation.

*Neisseria* species are being increasingly isolated from clinical specimens in routine diagnostic microbiology laboratories (16). Gonococci may be present in specimens outside the genital tract (6, 7), and meningococci may be cultured from several atypical sites (2, 8, 11). In addition, other *Neisseria* species may cause opportunistic infection in debilitated patients (14, 17, 18).

Methods usually employed for identification of *Neisseria* species include sugar degradation in Cystine Trypticase Agar (CTA) and modified NYC (New York City) media (5, 16) and fluorescent antibody testing and agglutination procedures (11, 16). Each of these methods has disadvantages. The CTA method is fairly insensitive and requires incubation for up to 72 h before reactions can be read (16). The NYC procedure, while being more accurate and permitting more rapid interpretation (incubation 8 to 20 h), necessitates quite complex plate preparation techniques (5). Direct fluorescent antibody methods are at present only used routinely in the identification of gonococci; commercially available fluorescent antibody reagents are not completely specific between genera, but will usually distinguish between *Neisseria meningitidis* and *Neisseria gonorrhoeae* (10). Agglutinating sera are only available for meningococci, but some strains of other *Neisseria* species, such as

*N. gonorrhoeae* and *Neisseria (Branhamella) catarrhalis*, may agglutinate in meningococcal grouping sera, posing problems in species identification (1).

The Minitek system (BBL Microbiology Systems) has recently been introduced for identification of various bacteria. Although this method has been found useful in identification of *Enterobacteriaceae*, little has been published on the use of this method in *Neisseria* species identification (13, 15).

An automated radiometric method for identifying *Neisseria* species has recently been made available. This method, which may be used with the conventional Bactec blood culture apparatus, is based on utilization of  $^{14}\text{C}$ -labeled maltose, glucose, fructose, and *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG). Previously published data suggest that this method is potentially a rapid, accurate, and reliable method for identification of *N. gonorrhoeae* (16).

The present study compares the efficacy of radiometric, Minitek, and CTA sugar degradation methods in the identification of 113 *Neisseria* species from clinical specimens.

### MATERIALS AND METHODS

Clinical isolates of *N. meningitidis* were kindly provided by Harry A. Feldman, State University of New

York, Syracuse, N. Y., and *N. gonorrhoeae* strains were provided by Clyde Thornsberry, Center for Disease Control, Atlanta, Ga. Other *Neisseria* species were obtained from the Philadelphia Department of Health Bacteriology Laboratory. *Neisseria* were cultured throughout the study on modified Thayer-Martin plates (BBL Microbiology Systems). Cultures were regularly tested for purity by Gram staining and subculture. Methods pertaining to CTA sugar degradation (glucose, maltose, lactose, sucrose) were those endorsed in the *Manual of Clinical Microbiology* (9), with deletion of fructose. Gonococcal fluorescent antibody-staining antiserum as well as meningococcal agglutinating sera were purchased from Difco Laboratories, Detroit, Mich.; techniques were as described by the manufacturer. Oxidase activity was determined with Pathotec strips (General Diagnostics, Morris Plains, N. J.).

The Minitek method (BBL Microbiology Systems) was utilized by preparing a dense suspension of organisms (MacFarland no. 9 standard) in the special *Neisseria* broth supplied by the manufacturer, with growth taken from purity plates. Samples of 50  $\mu$ l were inoculated into individual wells, disks (glucose, maltose, sucrose, ONPG) were dispensed, and plates were incubated in a Minitek humidior at 37°C for 4 h, in an atmosphere without added CO<sub>2</sub>. Mineral oil disk overlay was not used.

Bactec *Neisseria* Differentiation Kits were purchased from Johnston Laboratories, Inc., Cockeysville, Md. Samples (0.3 ml) of a dense suspension of organisms taken from plates into vials of special broth were inoculated into vials containing glucose, maltose, and fructose labeled with <sup>14</sup>C. An additional tube of inoculum was incubated with an ONPG disk. After incubation for 3 h at 37°C, vials were examined in a Bactec model 460 instrument (Johnston Laboratories); the ONPG disk was observed for color change. A recording of  $\geq 20$  on the radiometric detection instrument was interpreted as a positive reaction. All positive cultures were checked for purity by Gram staining.

## RESULTS

All organisms were oxidase-positive, gram-negative diplococci. Identifications of all gonococci and meningococci were confirmed by direct fluorescent antibody and slide agglutination tests, respectively.

Results comparing the efficacy of radiometric, Minitek, and CTA sugar degradation procedures in the identification of 113 clinically isolated *Neisseria* species are presented in Tables 1 and 2. As can be seen from Table 1, the radiometric method was highly effective in the case of gonococci, identifying 100% of these strains within 3 h. Minitek identification required 4 h of incubation and identified 47/51 (92.2%) of organisms, whereas CTA sugar degradation tests identified 46/51 (90.2%) after 24 to 48 h of incubation. Prolongation of CTA incubation periods to 72 h resulted in three false-positive reactions (1 sucrose- and lactose-positive; 2 sucrose-positive) without increasing test sensitivity.

TABLE 1. Comparison of radiometric, Minitek, and CTA sugar degradation methods for identification of 51 gonococcal strains

Method	No. of strains giving correct reactions	No. of strains giving incorrect reactions
Radiometric	51	
Minitek	47	4
CTA	46	5

TABLE 2. Comparison of radiometric, Minitek, and CTA sugar degradation methods for identification of 58 meningococci

Method	No. of strains giving correct reactions	No. of strains giving incorrect reactions
Radiometric	32	26
Minitek	56	2
CTA	53	5

Meningococcal identification was less clear-cut (Table 2). Although 100% of strains were glucose- and maltose-positive (both >100) and ONPG-negative with the Bactec method, test sensitivity was hampered by the relatively high incidence of meningococcal strains (26/58) giving radiometric index values for fructose of  $\geq 20$ , with a range of 20 to 28. The Minitek method identified 56/58 (96.6%) of meningococci after 4 h. Conventional CTA methods identified 53/58 (91.4%) of strains after 24 to 48 h; prolongation of incubation periods by an extra 24 h resulted in five false-positive reactions (three lactose- and sucrose-positive; 2 sucrose-positive) without an increase in test sensitivity.

Identification of four miscellaneous *Neisseria* species (two *Neisseria lactamica*, two *Neisseria sicca*) was excellent; all organisms were identified by radiometric, Minitek, and CTA methods.

## DISCUSSION

There is no practical single method absolutely determining the "true" identification of individual isolates of *Neisseria* species. Thus, when identifications by two methods disagree, it is difficult to be positive as to which is truly correct. Notwithstanding these problems, we believe that the results presented in this study yield meaningful information with respect to rapid methods of identification of *Neisseria* species from clinical specimens.

Data presented here show that the radiometric and Minitek methods have substantial advantages over conventional CTA sugar degradation techniques in identification of gonococci and miscellaneous *Neisseria* strains. Overall, the radiometric method showed the greatest sensi-

tivity, with the added advantage of very short (3-h) incubation periods. In laboratories that do not possess Bactec instrumentation, Minitek is an acceptable alternative for identification of these organisms.

Problems were encountered in the radiometric identification of meningococci, in that 45% of strains yielded fructose values of  $\geq 20$  (recommended negative cut-off point,  $< 20$ ). Although positive glucose and maltose values were all well above 100, strict acceptance of a fructose value of  $\geq 20$  as positive would have led to erroneous identification in 26/58 of meningococcal strains. In the light of these findings, radiometric fructose sensitivity levels for meningococci need reevaluation before this method can be recommended for routine identification of these organisms. Until this is the case, Minitek seems to be the most rapid and reliable technique for identifying meningococci.

Earlier problems related to suspension media/inoculum size in the *Neisseria* Minitek method (13, 15) have largely been obviated by commercial availability of special *Neisseria* suspension broth. Heavy inocula in the latter medium yielded satisfactory results. It should be emphasized that only clear-cut color changes from red to yellow/orange should be interpreted as positive; equivocal color changes are read as negative.

Another rapid method for *Neisseria* identification is that of Cox et al. (3), which utilizes  $^{14}\text{C}$ -labeled glucose and maltose as substrates in a liquid scintillation spectrometer procedure. This method seems comparable to the Bactec method with regard to accuracy, and has the added advantage of a shorter incubation time (30 min); lack of data on fructose and/or sucrose values in the scintillation method precludes information as to its specificity.

Other reported methods for *Neisseria* species identification include electron-capture gas-liquid chromatography of metabolites in a chemically defined medium (12) and enzymatic profile techniques (4). Both of these methods are still in the experimental stage.

In summary, radiometric and Minitek methods are more accurate and yield more rapid results in species identification of *Neisseria* strains than conventional CTA sugar degradation tests. At the present time, the Bactec method seems optimal for identification of gonococci, and the Minitek technique seems best for meningococcal species identification. If problems related to fructose sensitivity levels of meningococci with the radiometric method can be resolved, this would appear to be the method of choice in laboratories equipped with Bactec blood culture instrumentation. For laboratories

that do not have this equipment, the Minitek method offers an acceptable alternative for identification of gonococci as well as meningococci.

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