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Comparison of a Radiometric Procedure with Conventional Methods for Identification of *Neisseria*

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A radiometric procedure was compared with the conventional cystine tryptic agar (CTA) sugar fermentation method for identification of *Neisseria* species. Four different ATCC cultures of *Neisseria* were identified by both procedures with identical results. The only difference noted was that the radiometric procedure required 3 h for completion, whereas the conventional CTA sugar method required overnight incubation. The radiometric procedure was also compared with the fluorescent antibody (FA) and CTA methods for identification of *Neisseria gonorrhoeae*. The organisms examined were gram-negative, oxidase-positive diplococci isolated from 49 clinical specimens sent to the laboratory for bacteriological analysis. Results obtained by both CTA and FA procedures were comparable. However, the radioisotope method appeared to be superior to the other two methods in that only one isolate identified as positive by both the CTA and FA methods was not identified radiometrically, whereas four isolates positive by the radiometric method were not identified by the other two procedures. Thus, a total of seven more positive identifications were made radiometrically than by either of the two other methods. All positive identifications were confirmed by a reference laboratory. These results indicate that the radiometric procedure is more rapid and reliable as compared with both the CTA and direct FA methods and, thus, may serve as a valuable addition to the methodology available for diagnostic microbiology.

Isolation of *Neisseria* species from a variety of different clinical specimens is becoming a more common occurrence in diagnostic microbiology laboratories. The presence of the gonococcus in specimens other than those obtained from the genital area is no longer a rarity, and, furthermore, meningococci have reportedly been isolated from urinary tract specimens, further complicating isolation and/or identification (4, 11). Thus, rapid and accurate identification of species of *Neisseria* isolated from clinical specimens has become quite important for diagnostic microbiology. The usual procedure employed for identifying these gram-negative diplococci as to species involves their fermentative reactions in cystine tryptic agar (CTA) to which carbohydrates have been added (5). Another procedure being used more frequently is the direct fluorescent antibody (FA) technique (6, 9). However, both of these methods for identifying *Neisseria* as to species have some disadvantages. The CTA base procedure requires growth of the organism for at least 18 to 24 h and in some instances up to 72 h before fermentation patterns can be determined. Some of the more fastidious strains may not grow sufficiently even after 72 h of incubation for definitive results to be obtained. Furthermore, there are reports that prolonged incubation may cause nonspecific fermentation patterns (3). A major disadvantage of the FA procedure is the lack of available antisera for many species of *Neisseria*, as well as some cross-reactions between strains of gonococci and meningococci (10).

A radiometric method for identifying *Neisseria* as to species has recently become available to those laboratories equipped with the Bactec apparatus. This method permits analyses of carbohydrate utilization by resting cell suspensions of the test organism over a 3-h period. It does not require growth of the bacteria but does require a heavy initial inoculum to obtain useful results. The assay is based on utilization of ^{14}C-labeled glucose, maltose, or fructose and *o*-nitrophenyl-β-D-galactopyranoside (ONPG) activity. Because this method has the potential for being a rapid, reliable, and accurate test for identifying *Neisseria* as to species, a comparison was made in this laboratory between the radiometric procedure and the standard fermentation and FA methods. Approximately 50 clinical isolates of oxidase-positive, gram-negative diplococci ob-

tained from a variety of clinical specimens sent to this laboratory for isolation and identification of bacterial pathogens were examined.

MATERIALS AND METHODS

All media and reagents used in this study were purchased from commercial sources. Those strains of *Neisseria* not isolated from clinical specimens were purchased from the American Type Culture Collection, Rockville, Md., and are so designated. The procedures used for culture and identification as to species of *Neisseria* by CTA sugar fermentation were those recommended in the *Manual of Clinical Microbiology* (5). Confirmation of organisms identified as *Neisseria gonorrhoeae* was performed by FA staining with a kit purchased from Difco Laboratories, Detroit, Mich. The technique employed was exactly as described by the manufacturer. Cytochrome oxidase activity of the organisms studied was determined with Pathotec CO strips purchased from General Diagnostics, Morris Plains, N.J.

The Bactec Neisseria Differentiation Kit, model ND-1 purchased from Johnston Laboratories, Inc., Cockeysville, Md., was used for radiometric identification of *Neisseria* species. The procedure involved a 3-h incubation of a heavy inoculum taken from a chocolate agar purity plate into a vial containing either glucose, maltose, or fructose labeled with ^{14}C. An additional tube of inoculum was incubated with an ONPG disk. After incubation at 37°C the vials were examined in a Bactec model 225 instrument (Johnston Laboratories), and the ONPG disk was observed for a color change. A recording of 15 or greater on the radiometric detection instrument was taken as a positive reaction. This procedure permits identification of *N. gonorrhoeae, N. meningitidis,* and *N. lactamica.* Other gram-negative, oxidase-positive diplococci were referred to as *Neisseria* species. All cultures were checked for viability by subculture onto chocolate agar plates after incubation in CTA or radiometric medium. In every case subcultures in Transgro medium were sent to the Philadelphia Department of Health Laboratory for confirmation of *N. gonorrhoeae* and to the Pennsylvania Department of Health Bacteriology Laboratory for confirmation of other species of *Neisseria.* These laboratories employed CTA sugar and FA procedures for identification of these organisms.

RESULTS

Results of comparison of CTA sugar and radiometric procedures for identification of four different species of *Neisseria* other than *N. gonorrhoeae* is presented in Table 1. The data show that the results were comparable for these ATCC cultures of *Neisseria.* The only difference noted was that the radiometric identification could be completed at least 21 h sooner than that based on CTA sugar fermentation. Because a commercial source of reliable FA reagents for all of these species is not yet available, this procedure was not included in this comparison.

The number and type of clinical specimens from which gram-negative, oxidase-positive diplococci used in these studies were as follows: blood, 3; cervix, 25; penis, 3; urethra, 3; and vagina, 15. All but three of these clinical specimens were obtained from the genitourinary tract. The exceptions were blood cultures. Bacterial detection in blood cultures is routinely performed radiometrically in this laboratory.

There were 49 gram-negative, oxidase-positive diplococci isolated from the above noted clinical specimens. Two of these isolates, both from blood cultures, were similarly identified by both the CTA sugar and radiolabeled substrate procedures. The isolates were not identified as *N. gonorrhoeae* by the methods employed in this study. One isolate was identified as *N. perflava,* and the other was identified as *N. meningitidis.* Both identifications were confirmed by the Pennsylvania Department of Health Bacteriology Laboratory. The remaining 47 specimens were examined for the presence of *N. gonorrhoeae* by all three procedures. As is evident in Table 2, the radiometric procedure appeared to be more efficient than either of the other two procedures for identification of this organism. These data also indicate that the CTA and FA procedures appear to be equal in their ability to identify *N. gonorrhoeae.* However, seven isolates identified as *N. gonorrhoeae* by the radio-

TABLE 1. *Differentiation of selected Neisseria species by the CTA sugar fermentation and radiometric procedures*

Organism[a] examined	ATTC no.	Identification method							
		CTA[b]				Bactec[c]			ONPG disk
		Glucose	Maltose	Lactose	Sucrose	Glucose	Maltose	Fructose	
N. flavescens	13120	−	−	−	−	−	−	−	−
N. meningitidis	13077	+	+	−	−	+	+	−	−
N. subflava	10555	+	+	−	+	+	+	+	−
N. lactamica	23970	+	+	+	−	+	+	−	+

[a] All organisms tested were gram-negative, oxidase-positive diplococci.
[b] Positive reaction indicated by color change after 1 to 3 days of incubation at 37°C.
[c] Positive reaction indicated by "growth index" of 15 or greater after 3 h of incubation at 37°C.

TABLE 2. *Comparison of radiometric, CTA sugar fermentation, and FA methods for identification of N. gonorrhoeae*[a]

Method	No. positive[b]	No. negative
CTA	35	12
FA	35	12
Radiometric	42	5

[a] All organisms tested were gram-negative, oxidase-positive diplococci.

[b] Definition of positive reactions same as in Table 1.

metric procedure were negative by the FA and CTA methods, a difference of approximately 15%.

The total number and percentage of isolates identified as gonococci by each procedure is presented in Table 3. Approximately 64% (30/47) were identified by all three procedures. Four isolates were negative by all of the techniques employed. These four organisms could not be further identified as *N. gonorrhoeae* by the radiometric procedure alone and not by the other methods. These identifications were all confirmed by the Philadelphia Department of Public Health Laboratory of Bacteriology. It is of interest to note that 17 of 49 cultures sent to the city or state laboratories for confirmation failed to survive mailing on Transgro medium. There was only one identification by CTA and FA that was not confirmed by the radiometric method. None of the isolates was identified only by the CTA or FA procedures.

DISCUSSION

The data obtained in this study show that the radiometric procedure for identifying *Neisseria* species by the Bactec Neisseria Differentiation Kit is a useful method for the routine diagnostic laboratory that is currently using the Bactec apparatus for detecting bacteria in blood cultures. The data indicate that this method can identify isolates of *N. gonorrhoeae* which are missed by the CTA sugar and FA procedures. Identification of *Neisseria* by the radioisotopic procedure is much more rapid than that achieved by CTA sugar fermentation. The latter method requires 1 to 3 days of incubation, whereas the former is completed within 3 h. Although FA procedures are also rapid, the advantage of the isotopic method is that all pathogenic *Neisseria* can be identified radiometrically, while at present antisera for FA are available commercially only for *N. gonorrhoeae*. However, it should be noted that one disadvantage of the radioisotopic method, aside from the need for the Bactec apparatus, is the requirement for a heavy inoculum of a bacterial isolate for the required reactions to occur.

There are other methods available for the rapid identification of *Neisseria* species. All utilize sugar fermentation for identification as to species. The procedure of Kellogg and Turner (6) later modified by Brown (1) employs 4% (wt/vol) final concentration of glucose, maltose, sucrose, or lactose in a small volume of balanced salts solution with a heavy inoculum of bacteria. Utilization of the sugars is allowed to proceed for up to 4 h at 37°C. The authors claim that reactions can be read reliably in as early as 15 to 30 min. Morse and Bartenstein (8) adopted this procedure to the sugar-impregnated paper disks of the Minitek system. Still another rapid method is that of Cox et al. (2), which employs ^{14}C-labeled glucose and maltose as substrates. The authors count $^{14}CO_2$ trapped on NaOH-impregnated filter paper strips after 30 min of incubation at 37°C. This procedure also requires a heavy inoculum and a liquid scintillation spectrometer. These methods appear to be equal to the radiometric procedure that we are reporting. However, we have tried the Minitek method in our laboratory with only limited success.

It is of interest that the procedures employed in this study did not identify an excessive number of *N. meningitidis* isolates. This indicates that the maltose-containing medium used in this study was not contaminated with glucose, a situation that would result in faulty identification. There was only one isolate identified as *N. meningitidis*. This identification was by both the CTA sugar fermentation and radiometric procedure. The identification was confirmed by the Pennsylvania Department of Health Bacteriology Laboratory.

The results of this study indicate that the radiometric procedure is more reliable for identification of *N. gonorrhoeae* than the other two methods utilized. The Bactec procedure failed to identify only one isolate, while the CTA and FA procedures failed in each of four cases. Thus,

TABLE 3. *Analysis of results of comparison of three different methods for identifying N. gonorrhoeae*

Method utilized			Total no.	% of total
Radiometric	CTA	FA		
+	+	+	30	63.8
+	−	−	4	8.5
+	−	+	4	8.5
+	+	−	4	8.5
−	+	+	1	2.1
−	+	−	0	0
−	−	+	0	0
−	−	−	4	8.5

there were seven more positive identifications of *Neisseria* by the radioisotopic procedure than with the other two methods. Therefore, it appears that the radiometric procedure for differentiation of *Neisseria* species may be considered not only a simple and rapid method for use in clinical microbiology, but also reliable. It should be noted, however, that the relatively high cost of the fully automated Bactec 225 apparatus used in this study (i.e., $36,000.00) would probably preclude the purchase of this equipment just for this procedure. On the other hand, those laboratories which already utilize this equipment for blood culture assays could undoubtedly perform this radiometric procedure for *Neisseria* without additional cost, except for the radiolabeled media, at a cost of about $1.00 per bottle, which is not excessively higher than the cost of individual tubes of fermentation medium bought from commercial suppliers.

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