

Rapid Fermentation Confirmation of *Neisseria gonorrhoeae*

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A rapid sugar fermentation procedure has been developed for the confirmation of *Neisseria gonorrhoeae* from either primary isolation media or purification media. A lightly buffered salt solution with pH indicator and sugars was heavily inoculated with presumptively positive growth. Proper fermentation patterns are obtained in 1 to 4 h with no interference from inhibited contaminants or variation in results due to differing growth requirements of gonococcal strains.

Practical species differentiation in the genus *Neisseria* has relied primarily on the fermentation patterns developed by the growth of pure cultures in the presence of glucose, maltose, fructose, and sucrose. Four factors have a bearing on the effective use of this simple yet important procedure for the confirmation of species identity. First, the culture has to be pure; otherwise, erroneous fermentation patterns result. Second, erroneous fermentation patterns can be obtained if insufficient inocula are used or if the fermentation medium is inadequately inoculated. Third, a fermentation medium composition different from that of the maintenance medium (1) may cause slow pattern development. Fourth, some cultures of the more fastidious *Neisseria* have been shown to require additional growth factors for proper fermentation patterns (9).

Obtaining a pure culture of *Neisseria gonorrhoeae* has been expedited by the introduction of Thayer-Martin (TM) medium which has provided the basis for the presumptive laboratory identification of *N. gonorrhoeae* (6). However, because of the bacteriostatic character of TM medium, 34 h is required before purification and confirmation are complete. So lengthy a confirmation time has been of little value with a rapidly transmitted, short-incubation disease like gonorrhea. Laboratory confirmation of *N. gonorrhoeae* would be improved by the development of a rapid fermentation procedure such as has been used with other bacteria (2, 3). This paper presents the application of a rapid fermentation procedure (1 to 2 h) to the confirmation of *N. gonorrhoeae* by using cells taken either directly from presumptively positive growth on TM medium or from

secondary isolation plates. The results reported here did not depend upon growth in the presence of the carbohydrates, and bacteriostatically inhibited contaminants had little or no effect on the results.

MATERIALS AND METHODS

N. gonorrhoeae isolated on Thayer-Martin (TM) medium were acquired from the Fulton County Health Department, Atlanta, Georgia. Each isolate was presumptively identified as *N. gonorrhoeae* when it grew on TM medium and formed oxidase-positive, morphologically typical colonies composed of gram-negative diplococci. Fluorescent-antibody procedures (8), colonial morphology (4), and growth fermentation patterns (7) corroborated the identification of the isolates as *N. gonorrhoeae*. *N. meningitidis* isolates were acquired from male patients at the Naval Air Station in Atlanta, Georgia (5). All neisserial strains, regardless of source, were examined for oxidase activity, cell morphology, Gram stain character, and proper growth fermentation patterns in cysteine-Trypticase-agar medium. In addition, *N. meningitidis* strains were serotyped by the Special Bacteriology Unit, Bacteriology Branch, Laboratory Division, Center for Disease Control. Representatives of other neisserial species were either isolated and identified in the Venereal Disease Research Section or acquired from the American Type Culture Collection. All neisserial species were cultivated after isolation on GC Medium Base with a defined supplement (8).

A buffer-salt solution was prepared to establish a reproducible initial indicator color and to resist pH changes due to fermentable contaminants in the carbohydrates. The solution consisted of dipotassium hydrogen phosphate (0.04 g), potassium dihydrogen phosphate (0.01 g), potassium chloride (0.8 g), phenol red (0.2 ml of 1% aqueous solution), and distilled water (100 ml). The buffer solution was dispensed into plastic tubes (12 by 75 mm) with snap-on caps

(Falcon catalog no. 3054) in 0.50-ml amounts and frozen ready for use in numbers corresponding to the number of tests to be performed at one time.

Concentrated solutions of glucose, maltose, and fructose (20%) were made up in distilled water. These carbohydrate concentrates were placed in 2-oz sterile-plastic dropping bottles (Wheaton catalog no. ES-2) for convenience, and each bottle was labeled with a distinctive color. The fermentation tube cap (12 by 75 mm plastic tube) for each carbohydrate was marked with the same color as the bottle. Although sterility has not been a problem, these solutions were stored at -25 C to avoid possible chemical or microbial changes. If sterility of the fresh carbohydrate is desired, the solutions may be sterilized by membrane filtration and bottled and stored as described.

To set up fermentation patterns, we labeled a tube containing 0.25 ml of the buffer-salt solution for each culture to be tested. A smooth suspension of selected, typical gonococcal growth from each culture was made in this buffer-salt solution by using a 2-mm diameter loop to scrape up from the plate a ball of growth approximately 2 mm in diameter. One full drop (or 0.05 ml) of each suspension was placed in each of three tubes already containing 0.5 ml of the buffer-salt solution and a drop (or 0.05 ml) of the appropriate carbohydrate. A cell suspension of each culture was prepared, and carbohydrate tubes were inoculated before proceeding to the next culture. The caps of all tubes were snapped shut, and they were shaken vigorously and incubated in a water bath at 35 to 36 C for 1 to 4 h. Incubation was slightly more effective in a water bath than in a dry-air incubator, and the tube caps either could be closed loosely or snapped shut.

RESULTS AND DISCUSSION

Cells from 220 primary isolates on TM medium, presumptively identified as *N. gonorrhoeae*, fermented properly with this procedure (Table 1). Only four isolates required

TABLE 1. Rapid fermentation patterns of isolates from Thayer-Martin medium

Organisms	Isolates tested (no.)	Glucose	Maltose	Fructose
<i>N. gonorrhoeae</i>	220	+	-	-
<i>N. meningitidis</i>				
Group B	8	+	+	-
Group C	5	+	+	-
Slaterus X	3	+	+	-
Slaterus Y	5	+	+	-
Slaterus Z	1	+	+	-
Untypable	7	+	+	-
Rough	7	+	+	-
<i>N. lactamicus</i>	7	+	+	-

^a Tubes were incubated a maximum of 4 h in a 35 to 36 C water bath.

TABLE 2. Rapid fermentation patterns of other species in the *Neisseria*^a

Organism	Isolates tested (no.)	Glucose	Maltose	Fructose
<i>N. flava</i>	3	+	+	+
<i>N. perflava</i>	5	+	+	+
<i>N. subflava</i>	3	+	+	-
<i>N. sicca</i>	3	+	+	+
<i>N. flavescens</i>	3	-	-	-
<i>N. catarrhalis</i>	4	-	-	-

^a Tubes were incubated a maximum of 4 h in 35 to 36 C water bath.

TABLE 3. Characteristics of rapid and conventional fermentation procedures

Rapid	Conventional
Can use cells from primary isolation plates	Should not use cells from primary isolation plates
1 to 4 h of incubation	18 to 42 h of incubation
Reactions obtained independently of growth capability	Reactions dependent upon adequate growth
Reagents compact, stable, and readily maintained	Reagents bulky with limited shelf life

TABLE 4. Typical growth fermentation reactions of *Neisseria* species^a

Organism	Glucose	Maltose	Fructose	Sucrose	Lactose	Manitol
<i>N. gonorrhoeae</i>	+	-	-	-	-	-
<i>N. meningitidis</i>	+	+	-	-	-	-
<i>N. lactamicus</i>	+	+	-	-	+	-
<i>N. subflava</i>	+	+	-	-	-	-
<i>N. flava</i>	+	+	+	-	-	-
<i>N. perflava</i>	+	+	+	+	-	-
<i>N. sicca</i>	+	+	+	+	-	-
<i>N. flavescens</i>	-	-	-	-	-	-
<i>N. catarrhalis</i>	-	-	-	-	-	-

^aThis information was extracted from chapter XII (Gonorrhea) in Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections, 5th ed., 1970, American Public Health Association and current literature on *Neisseria lactamicus*.

more than 1 to 2 h for completion of the fermentation (4 h), and most of the other 216 isolates could be read in 30 min. Representatives of *N. meningitidis* serotypes and *N. lactamicus* fermented properly and as quickly as *N. gonorrhoeae* isolates (Table 2). With either *N. gonorrhoeae*, *N. meningitidis*, or *N. lactamicus*, the fructose tubes were definitely orange to pink in color and not bright yellow as

when fermentation had occurred with either glucose or maltose. As shown in Table 2, members of several other neisserial species were also found to give proper fermentation patterns.

The color and texture of suspensions of neisserial strains and species varied too much for comparison with each other. As a consequence the set of carbohydrate tubes for each strain or species was read without reference to any other set. Within each set, the "negatives" were either pink or orange-pink, and the "positives" were yellow. When less than the stipulated amount of inoculum was used, the result was occasionally a yellowish-orange positive; however, this positive could be satisfactorily distinguished from the frankly orange negatives. A more distinct difference could be obtained by prolonging the incubation period to 4 to 6 h. The pigmented, nonpathogenic *Neisseria* which, because of their pigment, usually gave orange negatives also produced definite yellow positives. The fermentation tubes were not read the following day, because insignificant initial contamination could develop to a level that would cause erroneous results. No evidence was seen of alteration of sugar solutions by freezing and thawing, nor did autoclaving the buffer solution affect the test results.

This procedure was developed as a confirmation procedure for presumptively positive primary isolates of *N. gonorrhoeae* on TM medium or for the first transfer growth from such media. Confirmation of the culture was available within 1 to 4 h when primary isolates were used and within 30 h when the first transfer growth was used. Use of growth from the primary isolation on TM medium depended upon the amount of presumptively positive growth and the number and distribution of contaminating colonies. In cases where growth on primary isolation plates was insufficient, a secondary isolation plate of TM medium made fermentation testing possible the next morning. Neither inhibited contaminants nor gonococcal growth capabilities were factors in obtaining reproducible fermentation patterns with gonococcal isolates. A comparison of conventional and rapid fermentation procedures is given in Table 3.

The three carbohydrates should be checked with known control cultures before exposing them to unknown cultures. Some lots of maltose contained excessive amounts of contaminating, readily fermentable substances. For example, only one of four lots of maltose from one commercial source was satisfactory.

Such a situation would be a problem for any fermentation procedure.

The selection of glucose, maltose, and fructose and the omission of mannitol, lactose, and sucrose were based on the following information derived from the known fermentative capabilities of *Neisseria* species (Table 4). Glucose fermentation alone would characterize an isolate as being *N. gonorrhoeae*, whereas the additional fermentation of maltose would indicate some other member of the genus *Neisseria*. Fructose was included primarily as an internal control of negative reaction and secondarily as an indicator of the presence of a species other than *N. meningitidis*. Mannitol is not fermented by any of the *Neisseria* and provides no useful distinctions. Lactose was fermented only by *N. lactamicus*, which could be distinguished from *N. gonorrhoeae* by maltose fermentation. Sucrose served to distinguish between *N. flava*, *N. perflava*, and *N. sicca*, each of which also fermented maltose. The fermentative activities illustrated in Table 2 show that the procedure can be applied to six other members of the *Neisseria* as well as to *N. meningitidis*, *N. lactamicus*, and *N. gonorrhoeae*. Formal classification of other members of the *Neisseria*, which would have required additional strains and criteria, was not within the scope of this study.

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