Modified Oxidation-Fermentation Medium for Detection of Acid Production from Carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*

JOAN S. KNAPP* AND KING K. HOLMES

Neisseria Reference Laboratory, Department of Medicine, University of Washington, Seattle, Washington 98195-2145

Received 13 December 1982/Accepted 1 April 1983

A modified oxidation-fermentation medium was developed as a practical medium for highly sensitive and specific detection of acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. A total of 756 strains representing 17 *Neisseria* spp. and *Branhamella catarrhalis* were tested in this medium, in which the protein concentration was reduced relative to the carbohydrate concentration, phenol red was substituted for bromthymol blue at a low concentration, and the initial pH was adjusted to 7.2. Sugar utilization patterns were consistent with published results and with other cultural and biochemical characteristics for these species. The reactions obtained using this medium were qualitatively better and more reproducible than those obtained in cystine-Trypticase agar (BBL Microbiology Systems, Cockeysville, Md.) medium.

Cystine-Trypticase agar (CTA; BBL Microbiology Systems, Cockeysville, Md.) has been considered unsuitable for detecting acid production from carbohydrates by *Neisseria* spp. for many years (9, 14). In Seattle and Chicago (13) much difficulty has been experienced in detecting acid production from glucose by fastidious strains of *Neisseria gonorrhoeae*. We approached the problem initially by heavily inoculating 1.0 ml (instead of 2.5 ml) of the carbohydrate-containing CTA medium. With this approach, glucose utilization by fastidious strains of *N. gonorrhoeae* was demonstrable more consistently, but often several plates of fastidious gonococci were required to provide sufficient inoculum.

Oxidative organisms such as the *Neisseria* spp. produce less acid than do fermentative organisms. In addition, *Neisseria* spp. produce ammonia from peptone (6), so that any acid produced from carbohydrate may be partially or completely neutralized if the concentration of peptone is high. The oxidation-fermentation medium developed by Hugh and Leifson (12) contains a lower concentration of peptone relative to the concentration of carbohydrate than is found in CTA medium. In preliminary studies, we found that gonococci failed to give an acid reaction in this medium. We have therefore developed a modified oxidation-fermentation (MOF) medium to detect carbohydrate utilization by *Neisseria* spp. and *Branhamella catarr-*

halis. This MOF medium has proven more sensitive than conventional CTA medium for detection of carbohydrate utilization by these species.

MATERIALS AND METHODS

Strains. The strains used in this study are listed in Table 1. They include the taxonomic type strains, laboratory strains, and primary isolates of pathogenic and commensal species from human subjects and rabbits. Strains of *N. gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* were isolated on Thayer-Martin medium from consecutive patients attending a Sexually Transmitted Diseases clinic. The strains were purified on chocolate blood agar, and their identities were confirmed by Gram stain, oxidase reaction, and carbohydrate utilization tests. Tests of taxonomic type strains, laboratory strains, and selected *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* strains were performed both in CTA medium and MOF medium. Tests of freshly isolated commensal *Neisseria* spp. were tested in MOF medium. Strains of commensal species were isolated on sheep blood bilayer plates and purified on this medium, and their identities were confirmed as for the pathogenic species. Additional biochemical tests performed to confirm their identity included reduction of nitrate and production of polysaccharide from sucrose. Laboratory strains were cultured on supplemented GC base medium (Difco Laboratories, Detroit, Mich. [19]) and grown at 36°C in a CO₂-enriched atmosphere. Strains were stored at -70°C.

Nitrate reduction tests were performed in nitrate broth (Difco) containing potassium nitrate at a final

concentration of 0.1% and supplemented with 1% heat-inactivated horse serum. Tests for nitrate reduction were made daily for up to 5 days. Zinc powder was added to detect residual nitrate in each negative test reaction.

Production of polysaccharide from sucrose was detected on heart infusion agar medium containing 5% sucrose which was streak inoculated with the test strain. Plates were incubated for 48 h at 36°C and tested with a drop of Lugol iodine (Gram strain reagent diluted 1:4); a positive reaction was recorded if a dark blue color developed immediately (3).

Preparation and use of MOF medium. The basal medium contains 0.2% proteose peptone no. 3 (Difco), 0.5% NaCl, 0.03% dipotassium hydrogen phosphate, 0.03% agar, and 0.25 ml of 0.017% phenol red solution per liter. These ingredients were dissolved and sterilized at 121°C for 15 min. Filter-sterilized carbohydrate solutions were added to a final concentration of 1% (vol/vol), the pH was adjusted aseptically to 7.2, and the medium was dispensed in 2.5-ml volumes in 12- by 75-mm sterile tubes (Falcon Plastics, Oxnard, Calif.). The basal medium may be stored at room temperature for at least 2 months. The complete medium may be stored at 4°C for several months and at room temperature for several weeks provided desiccation does not occur. Room temperature storage before inoculation is desirable to detect any contaminated medium. Best results were obtained when a heavy inoculum was removed from a pure subculture with a single wipe of a cotton applicator, which was then stabbed with the MOF medium along the side of the tube and then rotated against the side of the tube as it was withdrawn to express a very dense inoculum. Distribution of the inoculum throughout the tube interfered with the accurate assessment of acid production. Growth from one streak plate of gonococcal culture on supplemented GC base medium was sufficient to inoculate six carbohydrate tests. Only when gonococci had lysed so that growth was adherent and difficult to harvest was additional inoculum desirable or a longer incubation time required. For other species, growth from secondary and subsequent streaks of a streak plate culture was sufficient to inoculate six tests. Tests were incubated at 36°C without supplementary CO₂. When glucose-utilizing organisms were harvested from supplemented GC base medium (which contains glucose) and inoculated into MOF medium without glucose, slight acidification of the MOF medium sometimes occurred due to acid production from the glucose carried with the inoculum. This reaction often persisted for about 2 h, after which the permanent reaction developed. Some alkalization of the medium surface occurred when acid was not produced. Reactions by strong acid producers could be read after 4 h, and most reactions were visible after 8 h, but incubation for 24 h was required to detect reactions by weak acid-producing strains. A semiquantitative measure of the amount of acid produced was made by comparing tests with phenol red color standards (LaMotte, Chestertown, Md.).

Preparation and use of CTA medium and modified CTA medium. CTA medium (pH 7.3) was prepared from commercial CTA medium. A medium with the same composition as CTA medium but containing a peptone concentration of 0.2% (in contrast to the normal 2%) was compared with the MOF medium.

Those media were inoculated and incubated as for the MOF medium.

RESULTS

Comparison between CTA and MOF media for detection of acid production by taxonomic type strains and laboratory strains. The taxonomic type strains, excluding *Neisseria cuniculi*, and 37 laboratory strains were tested concurrently in CTA and MOF media containing glucose, maltose, sucrose, mannitol, and lactose. Concurrently, strains were tested for polysaccharide production and nitrate reduction. The acid reaction patterns obtained for these strains in MOF medium agreed with those reported in the literature (Table 2). All acid reactions in MOF and CTA media were compared with the phenol red color standards to make a semiquantitative estimate of the acid detected in these media. For reactions in MOF medium all strains produced sufficient acid to lower the pH to ≤6.8, whereas acid reactions in CTA media did not lower the pH below 7.0, except for maltose and sucrose reactions by *Neisseria perflava*, *N. sicca*, and *N. mucosa*. The reactions for tests in MOF medium are incorporated in Table 4.

Comparison between CTA and MOF media for detection of acid production by clinical isolates of *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*. Forty-four strains of *N. gonorrhoeae*, 17 strains of *N. meningitidis*, and 4 strains of *N. lactamica* were isolated from 44 consecutive patients attending an STD clinic, cultured on Thayer-Martin medium, purified on chocolate blood agar, and tested in both CTA and MOF media containing glucose, maltose, sucrose, or lactose. Twelve (35%) of 34 strains of *N. gonorrhoeae* failed to produce detectable acid from glucose in CTA medium, whereas only 2 (6%) of 34 strains produced sufficient acid to acidify the medium to pH 6.8 or lower when compared with phenol red color standards. The remaining 20 (58%) of 34 strains acidified the medium to pH 7.0. In contrast, all strains produced detectable acid in MOF-glucose medium, and 21 (61.8%) of 34 strains acidified the medium to pH 6.8 or lower. The remaining strains acidified the medium to pH 7.0. Acid reactions in CTA-glucose medium were limited to the top portion of the tube, whereas acidification in MOF-glucose was uniform throughout the medium.

Only 4 (23.5%) of 17 strains of *N. meningitidis* acidified CTA-glucose and CTA-maltose media to pH 6.8 or lower compared with 16 (94.1%) of 17 strains in MOF-glucose and MOF-maltose media. Acidification of CTA-glucose medium was somewhat weaker than that of CTA-maltose medium, but was equally strong in MOF-glucose and MOF-maltose media.

We noted that when the completed CTA tests were stored at room temperature for several days, the CTA-glucose medium became neutral; the reactions in MOF-glucose medium were stable. This is consistent with the observations of Berger (5).

Variability between strength of acidification of media containing glucose, maltose, or lactose by *N. lactamica* strains was not observed. None of the four strains lowered the pH of the CTA media below 7.0, whereas all MOF media were acidified to pH 6.8 or lower; all reactions were stable when stored.

Comparison between MOF medium and CTA medium containing 0.2% peptone. The modified CTA medium with 0.2% peptone (0.2 CTA) was compared with MOF medium containing glucose, maltose, sucrose, fructose, mannitol, or lactose. The reactions are listed in Table 3. Reactions for *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* were identical in 0.2 CTA media and MOF media. However, acid reactions were obtained in MOF media for other commensal strains, whereas reactions with these commensals were not detected in 0.2 CTA-maltose, and 0.2 CTA-sucrose. Consequently, 0.2% CTA medium was not further investigated.

Detection of acid production from carbohydrates in MOF medium by *Neisseria* spp. and *B. catarrhalis*. A total of 756 strains of *Neisseria* spp. and *Branhamella catarrhalis* were tested in

MOF medium containing glucose, maltose, sucrose, fructose, mannitol, or lactose. All freshly isolated strains were tested in this phase of the study. The results are summarized in Table 4. Reactions in these media were consistent with those reported in the literature. To confirm their identities, strains were tested concurrently for their ability to reduce nitrate, produce polysaccharide from sucrose, and produce beta-galactosidase.

Use of MOF medium to detect sugar utilization by other "problem" *Neisseria* spp. MOF medium was not directly compared with CTA medium, except for the strains of *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica* and the type strains for other species. However, we have used MOF medium to detect utilization of maltose by two strains of "maltose-negative" meningococci from another reference laboratory. Of six strains identified as *Neisseria subflava* on the basis of CTA testing in another laboratory and again in our laboratory, four were reclassified as *N. mucosa* and two were reclassified as *N. perflava*.

DISCUSSION

Tests to determine the production of acid from carbohydrates including glucose, maltose, sucrose, fructose, mannitol, and lactose have been central in the classification of *Neisseria* spp. Early researchers (10, 20, 21) devised classifica-

TABLE 1. Strains tested in MOF medium for detection of acid production by *Neisseria* spp. and *B. catarrhalis*^a

Species	No. of strains	Type strain (NRL)	Source (reference)
<i>N. animalis</i>	3	30000 (NCTC 10212)	NV (2)
<i>N. canis</i>	3	30001 (ATCC 14687)	NV (2)
<i>N. caviae</i>	2	30002 (ATCC 14659)	NV (1)
<i>N. cinerea</i>	5	30003 (ATCC 14685)	NV; NRL (3)
<i>N. cuniculi</i>	6		NRL (6)
<i>N. denitrificans</i>	1	30005 (ATCC 14686)	
<i>N. elongata</i>	2	30006 (ATCC 25295)	
<i>N. elongata</i> (var. <i>glycolytica</i>)		30007 (ATCC 29315)	
<i>N. flava</i>	14	30008 (Berger 10d)	NV (5), NRL (8)
<i>N. flavescens</i>	1	30009 (ATCC 13120)	
<i>N. gonorrhoeae</i>	427	30010 (ATCC 19424)	NRL (426)
<i>N. lactamica</i>	12	30011 (ATCC 23970)	NRL (11)
<i>N. meningitidis</i>	132	30012 (ATCC 13077)	NRL (131)
<i>N. mucosa</i>	17	30013 (ATCC 19696)	NRL (16)
<i>N. ovis</i>	3	30014 (NCTC 11227)	NV (2)
<i>N. perflava</i>	98	30015 (ATCC 10555)	NRL (97) ^b
<i>N. sicca</i>	1	30016 (Berger 6b)	
<i>N. subflava</i>	5	30017 (Berger h231)	NRL (4)
<i>B. catarrhalis</i>	23	30018 (ATCC 25238)	SAM (4), PP (13), NRL (5)

^a Abbreviations: NTCC, National Collection of Type Cultures, London, England; NV, N. Vedros, School of Public Health, University of California, Berkeley; ATCC, American Type Culture Collection, Rockville, Md.; NRL, *Neisseria* Reference Laboratory, freshly isolated strains; SAM, S. A. Morse, Department of Microbiology and Immunology, University of Oregon Health Sciences Center, Portland; PP, Peter Piot, Instituut voor Tropische Geneeskunde "Prins Leopold", Antwerp, Belgium.

^b Differentiation between *N. perflava* and *N. sicca* was not made for freshly isolated strains.

TABLE 2. Selected biochemical characteristics for differentiation between *Neisseria* spp. and *B. catarrhalis*^a

Species ^b	Acid from ^c :						Nitrate reduction	Polysaccharide from sucrose
	G	M	S	F	Ma	L		
<i>N. animalis</i>	—	—	+	—	—	—	—	+
<i>N. canis</i>	—	—	—	—	—	—	+	—
<i>N. caviae</i>	—	—	—	—	—	—	+	—
<i>N. cinerea</i>	—	—	—	—	—	—	—	—
<i>N. cuniculi</i>	—	—	—	—	—	—	—	—
<i>N. denitrificans</i>	+	—	+	+	+	—	—	+
<i>N. elongata</i> (var. <i>glycolytica</i>)	—	—	—	—	—	—	—	—
<i>N. flava</i>	+	+	—	+	—	—	—	—
<i>N. flavescens</i>	—	—	—	—	—	—	—	+
<i>N. gonorrhoeae</i>	+	—	—	—	—	—	—	—
<i>N. lactamica</i> ^d	+	+	—	—	—	+	—	—
<i>N. meningitidis</i>	+	+	—	—	—	—	—	—
<i>N. mucosa</i>	+	+	+	+	—	—	+	+
<i>N. ovis</i>	—	—	—	—	—	—	+	+
<i>N. perflava</i>	+	+	+	+	—	—	—	+
<i>N. sicca</i>	+	+	+	+	—	—	—	+
<i>N. subflava</i>	+	+	—	—	—	—	—	—
<i>B. catarrhalis</i>	—	—	—	—	—	—	+	—

^a Data are compiled from Reyn (15) and Berger (6).

^b Of these species, *N. cinerea*, *N. elongata* (var. *glycolytica*), *N. flava*, *N. flavescens*, *N. gonorrhoeae*, *N. lactamica*, *N. meningitidis*, *N. mucosa*, *N. perflava*, *N. subflava*, and *B. catarrhalis* are isolated from humans; the other species are of animal origin.

^c Abbreviations: G, glucose; M, maltose; S, sucrose; F, fructose; Ma, mannitol; L, lactose.

^d Only *N. lactamica* produced beta-galactosidase.

tions based on cultural characteristics and acid production from carbohydrates. Acid production tests were performed either on litmus ascitic agar or in serum peptone sugars containing 5% inactivated horse serum with Andrade indicator. Elser and Huntoon (10) described three chromogenic groups, I, II, and III, which were identified as *Neisseria perflava*, *N. flava*, and *N. subflava*, respectively, and produced acid from carbohydrates as shown in Table 2. They noted that acid reactions from some carbohydrates was not detectable for up to 7 days. When studying the chromogenic strains, Wilson (21) found that detectable acid was produced from glucose and maltose only after 2 to 3 days and from sucrose only after 4 to 6 days. Results for some tests were not reproducible. Wilson concluded that several subspecies might be recognized within the specie *N. pharyngis*. Wilson and Smith (20) incubated tests for 14 days and did not comment on the time required for the detection of acid reactions from different carbohydrates. These workers also found that the results were not consistent for tests performed concurrently in agar and broth media. On the basis of these results, and the failure to observe consistent cultural characteristics (colonial morphology and bacterial cell size and arrangement) within each group, Wilson and Smith concluded that "fermentation tests do not afford a reliable means of distinguishing between the different members of the group of gram-negative cocci" and suggested "that instead of dividing them up

into a number of species, *catarrhalis*, *flavus*, *cinereus*, *mucosus*, *siccus*, they should be grouped under the broad term *Neisseria pharyngis*." It should be noted, however, that none of the early classifications included the nitrate reduction or polysaccharide production tests, which have been so useful in more recent classifications.

CTA medium was developed by Vera (16) for practical purposes since the medium could be autoclaved and consequently was prepared more conveniently than either ascitic fluid- or serum-containing media used routinely at that time. This medium proved satisfactory not only for culture of *N. gonorrhoeae* but also as a medium suitable for detection of acid production from carbohydrates. It was intended that this medium should support growth of strains, and all 35 strains of *N. gonorrhoeae* produced acid reactions from glucose within 24 h, obviously a vast improvement of the earlier methods. CTA medium became the medium of choice for detection of acid production of *Neisseria* spp. in the United States.

The oxidation-fermentation medium developed by Hugh and Leifson (12) permits detection of acid production by oxidative organisms, which produce less acid than do fermentative organisms, and by organisms such as *Neisseria* spp., which produce ammonia from peptone, neutralizing some or all of the acid produced in peptone-containing media. In our study, the initial pH was adjusted to 7.2, and phenol red

TABLE 3. Comparison between MOF and modified CTA media for detection of acid production from carbohydrates by selected *Neisseria* spp.

Species	Acid produced in ^a :							
	MOF				0.2 CTA			
	G	M	S	L	G	M	S	L
<i>N. flava</i> NRL 7350	+	+	—	—	+	—	—	—
<i>N. mucosa</i> NRL 7345	+	+	+	—	+	—	—	—
<i>N. subflava</i> NRL 6284	+	+	—	—	+	—	—	—
<i>N. sicca</i> NRL 7349	+	+	+	—	—	—	—	—
<i>N. lactamica</i> NRL 16405	+	+	—	+	+	+	—	+
<i>N. gonorrhoeae</i> NRL 6689	+	—	—	—	+	—	—	—
<i>N. meningitidis</i> NRL 7327	+	+	—	—	+	+	—	—

^a Sugar abbreviations as in Table 2.

(pK, 7.9) was substituted for bromthymol blue (pK, 7.0). Phenol red was used at a fixed concentration of 0.0425 mg/liter rather than at the concentration used in CTA medium (17 mg/liter), because the higher concentration inhibited the acid reaction, perhaps due to its buffering capacity; when reduced to the intensity approximating that of the phenol red color standard, acid reactions for the taxonomic type strains were distinct and consistent.

We were faced with a dilemma when evaluating MOF medium for speciating *Neisseria* spp., since the standard CTA medium with which the MOF medium would be compared was itself considered unreliable for detecting acid production by some *Neisseria* spp. (4, 9, 14). For this reason we tested MOF medium in three phases. We compared the acid reaction patterns in MOF medium for the taxonomic type strains and the named laboratory strains provided by other laboratories with the reaction patterns reported in the literature (Table 2). The sensitivity of the MOF medium was demonstrated by comparison with reactions obtained concurrently in CTA medium. By comparison with the LaMotte phenol red color standards we determined that when acid was produced, the MOF medium was acidified to $\leq$ pH 6.8, but that CTA medium was not acidified lower than pH 7.0. It was shown that reactions obtained in MOF medium were accurate and more distinct than those obtained in CTA medium.

The second phase of the evaluation was to confirm the sensitivity of the MOF medium for determination of acid production from glucose by *N. gonorrhoeae*, *N. meningitidis*, and *N. lactamica*. This was done for three reasons. First, it has been shown that CTA medium has given unreliable results for *N. gonorrhoeae* strains (9, 14), but since the acid reaction pattern for this species is well accepted we could demonstrate the sensitivity of MOF medium relative to CTA medium. Second, evaluation of freshly isolated strains was desirable since it has been shown that acid production from glucose (and fructose) is more difficult to detect in freshly

isolated strains. Third, it has been demonstrated that reactions for some carbohydrate tests are not stable (5), and we wished to evaluate MOF medium in this respect. We demonstrated accurate and sensitive detection of acid reactions for all strains which were tested from the first subculture of primary isolates and further that all color reactions were stable, even when media were stored for several days, whereas color reactions in CTA medium were not stable for some carbohydrates.

The third phase of the evaluation of MOF medium was to determine the acid reaction patterns for the putative saccharolytic *Neisseria* spp., *N. mucosa*, *N. sicca*, *N. perflava*, *N. flava*, and *N. subflava*. We faced a dilemma when considering these species since their taxonomic position has not been conclusively defined. These species were initially described as *Diplococcus mucosus* (18a), *D. pharyngis siccus* (18a), chromogenic group I (10), chromogenic group II (10), and chromogenic group III (10), respectively. (Although von Lingelsheim described *D. pharyngis flavus* groups I, II, and III, he did not determine acid production from fructose.) Acid reactions were determined for all of these species (except *N. mucosa*) by Elser and Huntoon (10); the reactions are those listed in Table 2. *N. mucosa* strains were not recognized by either Elser and Huntoon or later workers since they did not use the nitrate reduction test, which permits differentiation between *N. mucosa* and the other saccharolytic species. This species was not recognized and described again until 1959 (17, 18). The species *N. sicca*, *N. perflava*, *N. flava*, and *N. subflava* were maintained in editions of *Bergey's Manual of Determinative Bacteriology* as discrete species between 1923 and 1957. *N. sicca* strains were distinguished from *N. perflava* strains solely on the basis of colonial morphology since both species produced acid from glucose, maltose, sucrose, and fructose.

At the time of the studies of Berger and co-workers (2, 4, 5, 7, 8), the recognized species of chromogenic saccharolytic neisseriae included

TABLE 4. Acid production from carbohydrates in MOF medium by *Neisseria* spp. and *B. catarrhalis*

Species	No. tested	Acid (%) from ^a :						Nitrate reduction	Polysaccharide from sucrose
		G	M	S	F	Ma	L		
<i>N. animalis</i>	3	100	0	100	100	0	0	—	+
<i>N. canis</i>	3	0	0	0	0	0	0	+	—
<i>N. caviae</i>	2	0	0	0	0	0	0	+	—
<i>N. cinerea</i>	5	0	0	0	0	0	0	—	—
<i>N. cuniculi</i>	6	0	0	0	0	0	0	—	—
<i>N. denitrificans</i>	1	100	0	100	100	100	0	—	+
<i>N. elongata</i>	2	0	0	0	0	0	0	—	—
<i>N. flava</i>	14	100	100	0	100	0	0	—	—
<i>N. flavescens</i>	1	0	0	0	0	0	0	—	+
<i>N. gonorrhoeae</i>	427	100	0	0	0	0	0	—	—
<i>N. lactamica</i> ^b	12	100	100	0	0	0	100	—	—
<i>N. meningitidis</i>	132	100	100	0	0	0	0	—	—
<i>N. mucosa</i>	17	100	100	100	100	0	0	+	+
<i>N. ovis</i>	3	0	0	0	0	0	0	+	—
<i>N. perflava</i> *	98	100	100	100	100	0	0	—	+
<i>N. sicca</i>	1	100	100	100	100	0	0	—	+
<i>N. subflava</i>	5	100	100	0	0	0	0	—	—
<i>B. catarrhalis</i>	23	0	0	0	0	0	0	+	—

^a Sugar abbreviations as in Table 2.^b Beta-galactosidase was produced only by strains of *N. lactamica*.^c *N. perflava* strains included in this category may include strains of *N. sicca* which cannot be differentiated by biochemical characteristics.

only *N. sicca*, *N. perflava*, *N. flava*, and *N. subflava*. Berger and Wulf (8) described the characterization of human nasopharynx *Neisseria* spp. by cultural characteristics and acid production tests. They found that *N. sicca* and *N. perflava* produced consistent, distinct acid reactions in maltose and sucrose and weaker reactions from glucose and fructose on litmus-ascitic agar incubated for 48 h at 37°C. Sucrose-negative strains (*N. flava* and *N. subflava*) also produced weak reactions in fructose and weak or no reactions in glucose; prolonged incubation did not improve the reactions. *N. sicca* and *N. perflava* acidified maltose broth medium to pH 5.03 to 5.04 within 3 days, whereas *N. flava* and *N. subflava* acidified the medium only to pH 5.63 and pH 5.23, respectively, after incubation for 7 days, thus indicating that strains of the latter species produced less acid than did strains of the former. Sucrose-positive human *Neisseria* spp. produced polysaccharide, whereas sucrose-negative species did not (3). Berger further found that strains of *N. sicca* produced smooth and glistening colonies of pigmented and nonpigmented types, in contrast to the grey, somewhat dry colonial characteristics which were the sole basis for their differentiation from *N. perflava* (15). Berger therefore suggested that differentiation between these species was not possible. Berger suggested that the sucrose-positive, polysaccharide-positive *N. perflava* (*N. sicca*) strains could be readily distinguished from the sucrose-negative, polysaccharide-negative species, *N. flava* and *N. subflava*, but that the latter could not be differentiated on the basis of fruc-

tose utilization because of the unreliability of this test. He therefore suggested that *N. flava* and *N. subflava* be combined under the name *N. subflava* and that the fructose-positive strains be recognized as *N. subflava* var. *flava*.

Subsequently, on the basis of the work of Wilson (21), Wilson and Smith, Berger (20), and Berger and Brunhoeber (7), Reyn (15) grouped the species *N. perflava*, *N. flava*, and *N. subflava* within the species *N. subflava* and retained the species *N. sicca* with an amendment of the colony description to include pigmented and nonpigmented, smooth, glistening colonies.

We have followed the classification suggested by Berger and combined *N. perflava* and *N. sicca* strains since we have found no reliable method for differentiating between them. We did not observe any strain that produced grey, dry, crumbling colonies in the sucrose-positive, nitrate-negative group. In contrast to Berger, we have separated the species *N. flava* and *N. subflava* since the use of MOF medium permitted reproducibly consistent, distinct detection of acid production from glucose and fructose.

We did not use CTA medium, because it has been considered unsuitable as a reference standard for detecting acid production from fructose and glucose by the sucrose-negative species (4). We did test concurrently for polysaccharide production to support our separation of the polysaccharide-producing *N. sicca*-*N. perflava* and *N. mucosa* strains from the polysaccharide-negative *N. flava* and *N. subflava* strains. Nitrate reduction further differentiated *N. mucosa* from *N. sicca*-*N. perflava*.

The use of MOF medium does not permit rapid detection of sugar utilization, although reactions by strong acid producers such as *N. meningitidis*, *N. lactamica*, *N. perflava*, *N. sicca*, and *N. mucosa* could be read accurately at 6 to 8 h. We obtained unequivocal results for all species. These reactions were consistent upon repeated testing and were consistent with other differential biochemical characteristics for these species.

The results obtained in this study contrast with those obtained by Berger (2), who modified the oxidation-fermentation medium by substituting phenol red for bromthymol blue, lowering the initial pH to 7.1, and adding heat-inactivated horse serum to a final concentration of 5% (vol/vol), and phenol red at a final concentration of 6.25 mg/liter. Berger was unable to detect acid production from glucose by one of two strains of *N. gonorrhoeae* or by one of two strains of *N. meningitidis*, and he abandoned further investigation of this medium. In early investigations in our laboratory, we also attempted to stimulate growth of gonococci in MOF medium by the addition of heat-inactivated horse serum without success. Subsequently, we decided upon the use of a heavy inoculum sufficient to produce acid without requiring growth (although some strains of both pathogenic and commensal species do grow at the surface of MOF medium).

In agreement also with the observations of Berger, we found that fructose is slightly unstable and that MOF-fructose medium acidified slightly when stored, but results in this medium have been unequivocal, and we have experienced no difficulty in differentiating fructose-positive *N. flava* from fructose-negative *N. subflava*.

MOF medium offers several practical advantages. Both the medium base, and the complete carbohydrate-containing media may be stored at room temperature for up to 2 months, provided desiccation does not occur. Media may be inoculated as required; no additional preparation of reagents is required at the time of inoculation. MOF medium has been used in our laboratory since 1978 for routine species identification of *Neisseria* spp. isolated on Thayer-Martin medium without evidence of aberrant results when compared with the results of other biochemical and serological tests.

MOF medium is proposed as an alternative medium for accurate detection of acid production from carbohydrates by *Neisseria* spp. and *B. catarrhalis* in clinical laboratories and for taxonomic studies.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant AI-12191 from the National Institutes of Health.

LITERATURE CITED

1. Baron, E. S., and A. K. Saz. 1976. Effects of types of media on the production of acid from glucose by so-called glucose-negative strains of *Neisseria gonorrhoeae*. J. Clin. Microbiol. 3:330-333.
2. Berger, U. 1960. Über den Kohlenhydrate-Stoffwechsel von *Neisseria* und *Gemella*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 180:147-149.
3. Berger, U. 1961. Polysaccharidbildung durch saprophytische *Neisserien*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. 181:345-348.
4. Berger, U. 1961. Zur Verwertung von Monosacchariden durch saprophytischen *Neisserien*. Arch. Hyg. (Berlin) 145:190-195.
5. Berger, U. 1961. Zur Variabilität der Zuckervergarungen durch *Neisserien*. Arch. Hyg. (Berlin) 145:296-301.
6. Berger, U. 1963. Die anspruchlosen *Neisserien*. Ergeb. Mikrobiol. Immunol. Exp. Ther. 36:99-167.
7. Berger, U., and H. Brunhoeber. 1961. *Neisseria flava* (Bergey et al. 1923): Art oder Varietät? Z. Hyg. Infektskr. 148:39-44.
8. Berger, U., and B. Wulf. 1961. Untersuchungen an saprophytischen *Neisserien*. Z. Hyg. 147:257-268.
9. Brown, W. J. 1975. A comparison of three fermentation methods for the confirmation of *Neisseria gonorrhoeae*. Health Lab. Sci. 13:54-58.
10. Elser, W. J., and F. M. Huntoon. 1909. Studies on meningitis. J. Med. Res. 20:371-541.
11. Hehre, E. F., and D. M. Hamilton. 1946. Bacterial synthesis of an amylopectinlike polysaccharide from sucrose. J. Biol. Chem. 166:77-78.
12. Hugh, R. E., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-26.
13. Morello, J. A., S. A. Lerner, and M. Bohnhoff. 1976. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. Infect. Immun. 13:1510-1516.
14. Pollock, H. M. 1976. Evaluation of methods for the rapid identification of *Neisseria gonorrhoeae* in a routine clinical laboratory. J. Clin. Microbiol. 4:19-21.
15. Reyn, A. 1974. Genus I. *Neisseria* Trevisan, p. 428-432. In R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
16. Vera, H. D. 1948. A simple medium for identification and maintenance of the gonococcus and other bacteria. J. Bacteriol. 55:531-536.
17. Véron, M., P. Thibault, and L. Second. 1959. *Neisseria mucosa* (*Diplococcus mucosus* Lingelsheim). I. Description bactériologique et étude du pouvoir pathogène. Ann. Inst. Pasteur 97:497-500.
18. Véron, M., P. Thibault, and L. Second. 1961. *Neisseria mucosa* (*Diplococcus mucosus* Lingelsheim). II. Étude antigénique et classification. Ann. Inst. Pasteur 100:166-179.
- 18a. von Lingelsheim, W. 1906. Die bakteriologischen Arbeiten der Kgl. hygienischen Station zu Beuthen O.-Schl. Während der Genickstarreepidemie in Oberschlesien im Winter 1904/05. Klin. Jahrb. 15:373-489.
19. White, L. A., and D. S. Kellogg, Jr. 1965. *Neisseria gonorrhoeae* identification in direct smears by a fluorescent antibody-counterstain method. Appl. Microbiol. 13:171-174.
20. Wilson, G. S., and M. M. Smith. 1928. Observations on the gram-negative cocci of the nasopharynx, with a description of *Neisseria pharyngis*. J. Pathol. Bacteriol. 31:597-608.
21. Wilson, S. P. 1928. An investigation of certain gram-negative cocci met within the nasopharynx, with special reference to their classification. J. Pathol. Bacteriol. 31:477-492.