

NOTES

Simplified Sugar Fermentation Plate Technique for Identification of *Neisseria gonorrhoeae*

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A simplified sugar fermentation technique incorporating (i) a glucose and (ii) a maltose, lactose, and sucrose plate was developed for confirming large numbers of *Neisseria gonorrhoeae* cultures.

A simplified sugar fermentation method to confirm the presence of *Neisseria gonorrhoeae* has long been needed as a routine procedure in the public health laboratory.

The carbohydrate fermentation test in general use for many years consists of fermentation patterns of the *Neisseria* tested against glucose, maltose, lactose, and sucrose, each in separate test tubes, in a semisolid cysteine-Trypticase agar (CTA) base (3). This expensive and time-consuming technique is not always entirely satisfactory in the screening of large numbers of cultures because the results are occasionally difficult to interpret due to nonspecific changes in the indicator, particularly with maltose (1, 2).

We obtained excellent results in a study which made use of two fermentation plates, one a triple sugar agar plate containing 0.5% each of maltose, lactose, and sucrose, and a single sugar agar plate containing 1.0% glucose. The medium base used in our study was CTA (BBL), modified to contain a total of 1.78% agar (BBL) and 1.0% vancomycin-colistimethate-nystatin (VCN) inhibitor (BBL). A phenol red indicator is included in the CTA base medium. The VCN inhibitor and the Seitz-filtered sugar solutions were added aseptically to the sterilized base medium at 50 C and then poured into petri plates (100 by 15 mm). Storage of the plates at a temperature of 6 C proved satisfactory for as long as 2 weeks.

Adding the Thayer-Martin VCN antibiotic mixture (4) allowed direct inoculation from the original culture plate. In most cases this eliminated the need for subculture or purification. Four to eight cultures were tested per sugar plate, which reduced preparation time and cost and accelerated definitive laboratory reports.

By using this technique, 279 of 288 presump-

tive positive cultures showed the typical fermentation pattern. Nine (3%) failed to grow on the sugar plates but grew on Martin-Lester medium which had been inoculated at the same time.

Parallel fermentation of 29 *N. gonorrhoeae* isolates, 5 *N. meningitidis* isolates, and 2 *N. lactamicus* isolates was conducted on sugar plates and on conventional CTA tube sugars without VCN inhibitors (Table 1). *N. meningitidis* and *N. lactamicus* isolates showed correct reactions on both plate and tube. Three gonococcus isolates failed to grow on the carbohydrate plates but did grow on Martin-Lester plates and were confirmed on CTA sugar tube media. In the presence of the VCN inhibitor, these strains apparently require additional growth factors.

On the glucose plate, a definite color change from pink to bright yellow was observed in the area surrounding growth of *N. gonorrhoeae*. The triple sugar plate showed a pink growth. *N. meningitidis* and *N. lactamicus* showed bright yellow fermentation on both the glucose and

TABLE 1. Comparison of sugar fermentation reactions of *Neisseria* on sugar plates versus CTA tube technique

Organisms	No. isolates tested	Isolates confirmed on	
		Plates	CTA tubes
<i>N. gonorrhoeae</i>	29	26 ^a	29
<i>N. meningitidis</i>	5	5	5
<i>N. lactamicus</i>	2	2	2

^a Three of nine isolates which did not grow on fermentation plates were included in this comparison.

triple sugar plates. No nonspecific indicator changes or weak fermentation reactions were found on any of the fermentation plates. The uninoculated portions of the plates served as a control to compare the color changes.

In preliminary studies all cultures which grew on the sugar plates showed typical carbohydrate fermentation patterns on both the glucose and combined maltose, lactose, and sucrose plates after incubation at 35 C in a candle jar. A heavy inoculum from a 24- to 48-h actively growing primary culture was necessary to give satisfactory results in 18 to 24 h on the sugar plates.

LITERATURE CITED

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