

# A RAPID CARBOHYDRATE UTILIZATION TEST FOR THE IDENTIFICATION OF PATHOGENIC NEISSERIA

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## SYNOPSIS

A paper carbohydrate utilization test (PAPCUT) for identifying pathogenic *Neisseria* is described. It is performed on a reagent-impregnated filter paper, and the results are read in 2 hours. The test is comparable to the conventional cystine-Trypticase agar method in identifying the gonococcus and meningococcus. In view of its simplicity, rapidity and low cost, it is suitable for use by routine laboratories.

## INTRODUCTION

In recent years, a number of studies have been described (1, 2, 3, 4, 5, 6, 7, 8, 9) for the confirmation of pathogenic *Neisseria* through the use of rapid methods for detecting biochemical reactions. Compared to the widely used cystine-Trypticase agar (CTA) method, (9) these techniques have several advantages. Test results are known within a few hours, and there is less problem with contamination. However, some of the tests are costly and tedious to perform. This report presents a rapid method for detecting carbohydrate utilization, in which the organisms are inoculated on pieces of reagent-impregnated filter paper, and the results are read after 2 hours of incubation.

## MATERIALS AND METHODS

### Test organisms

These were isolated from nasopharyngeal and anogenital specimens using modified Thayer-Martin medium. (10) Preliminary identification was by colonial morphology, oxidase reaction and Gram smear. The strains were subcultured for 18 - 24 hours on modified Thayer-Martin medium or chocolate agar, and then tested. All strains were tested in parallel for carbohydrate utilization with the CTA method (9) or the paper carbohydrate utilization test (PAPCUT). Test results on the CTA system were read after 24 and 48 hours.

**Medium preparation**

The composition of the completed medium was as follows:

Bacto Proteose Peptone No 3 (Difco)	0.5 g
L-cysteine HCl (Sigma)	0.013 g
Bromocresol purple (Reachim)	0.1 g
KH <sub>2</sub> PO <sub>4</sub>	0.049 g
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O	0.159 g
Carbohydrate (nil for control)	2.5 g
Distilled water	50 ml

The medium was prepared by adding Bacto Proteose Peptone No 3 (Difco) 2.5 g, and bromocresol purple (Reachim) 0.5 g to 250 ml of 0.025 molar phosphate buffer, pH 7.2. The medium was then briefly heated, and divided into 50 ml aliquots. Then 2.5 g of carbohydrate (dextrose, maltose, sucrose, lactose) was added to the respective aliquot. The maltose was from British Drug House, while the other carbohydrates were from Sigma Chemical Co. No carbohydrate was added to the control. The reagents were boiled for about a minute, and while they were cooling 0.5 ml of L-cysteine HCl (Sigma) solution (26 mg/ml) was added into each aliquot. The reagents were then divided into 5 ml lots, and frozen at -20°C. When in use, they were kept at 4°C.

**PAPCUT test**

The test was performed by dropping the respective carbohydrate or control reagents, from sterile Pasteur pipettes, on pieces of Whatman No 1 filter paper, measuring 1 x 4 cm, placed in Petri dishes. Sufficient amount of the reagents were added to just saturate the pieces of filter paper. Using a bacteriological loop, 3 to 4 colonies were picked up from each culture and smeared on the surface of one piece of reagent-impregnated filter paper. The loop was then flamed, and the procedure repeated with another piece of filter paper. Three to four strains could be tested on each piece of paper, separated approximately 1 cm from

each other. The Petri dishes, with their covers on, were then placed in a plastic box together with a piece of moistened cotton wool. The box was covered and incubated at 37°C for 2 hours. Carbohydrate utilization was indicated by the presence of a yellow zone around the inoculum. The paper remained purple if there was no utilization.

**RESULTS**

Figure 1 shows the typical reactions obtained with two gonococcal isolates (one was a penicillinase-producer) and one meningococcal strain on glucose, maltose, sucrose and control strips. There was no difficulty in distinguishing positive from negative reactions with most of the strains tested. A few strains gave weak positive reactions which were seen as greenish discolourations beneath the inocula. Occasionally a negative reaction gave a faint purple pallor beneath the inoculum due to excess of moisture

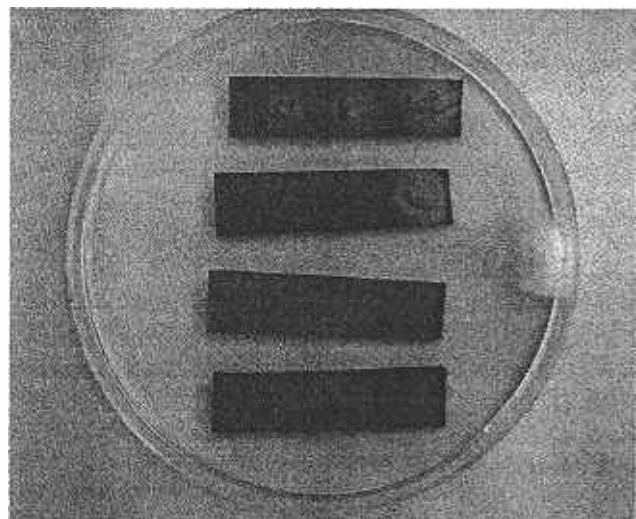


Figure 1: Paper carbohydrate utilization test. A positive reaction is indicated by a yellow zone. Test organisms from the left are: gonococcus, penicillinase-producing gonococcus and meningococcus. Test strips from above down contain: glucose, maltose, sucrose and no carbohydrate (control). A piece of moistened cotton wool is on the right.

**TABLE 1. Carbohydrate utilization by Neisseria species using the CTA and PAPCUT methods.**

Species	No.	Methods	No. strains positive				
			Glucose	Maltose	Sucrose	Lactose	Control
N. gonorrhoeae	103	CTA	102	0	0	0	0
		PAPCUT	103	0	0	0	0
N. meningitidis	14	CTA	14	14	0	0	0
		PAPCUT	13	14	0	0	0
N. lactamica	28	CTA	28	28	0	28	0
		PAPCUT	28	28	0	28	0
N. sicca	4	CTA	4	4	4	0	0
		PAPCUT	2	4	4	0	0

CTA : cystine-Trypticase agar  
 PAPCUT : paper carbohydrate test

from the inoculum which had diluted the pH indicator. It was, however, not difficult to distinguish this from a weak positive reaction.

All 103 gonococcal isolates (11 were penicillinase-producers) showed glucose utilization by PAPCUT (Table 1). One strain was negative with the CTA method. This strain was later shown to be a gonococcus by the immunofluorescent method. Of the 14 meningococcal strains one did not show glucose utilization by PAPCUT though maltose utilization was demonstrated. However, on repeating the test from a subculture a positive glucose result was obtained. All the *N. lactamica* gave concordant results with the two methods. Two of the 4 *N. sicca* did not show glucose utilization with the paper method, though maltose and sucrose were utilised.

## DISCUSSION

Many of the rapid methods for detecting biochemical reactions by bacteria depend on the presence of preformed enzymes (1, 2, 4, 5, 8) and growth of the bacteria is not a requirement. In the earlier methods (1, 2, 4, 6) the test organisms were suspended in unenriched media. It was later found (8) that enrichment of the medium improved the sensitivity of the system, and the test could then be performed in a shorter time.

In our filter paper method, we originally used an unenriched medium and found that it was sufficient to identify approximately 90% of the gonococci and meningococci. However, the addition of peptone and cysteine was necessary for a few strains which gave weak glucose reactions. The enriched medium was therefore adopted for the study. It is unlikely that even with the enrichment significant growth could have taken place within the short incubation time to influence the results. The improved sensitivity is probably due to activation of the enzyme systems, thereby increasing the metabolism of the bacteria. With this system, all the pathogenic *Neisseria* gave the correct biochemical reactions, except for one strain of meningococcus which gave an initial negative reaction for glucose. The reason for the variable glucose reactions *N. sicca* is probably due to the lack of preformed enzymes within the bacteria. Despite this drawback, such strains may be suspected from the pattern of positive reactions with the non-glucose carbohydrates.

To obtain reproducible results the *Neisseria* strains should be grown on modified Thayer-Martin medium or chocolate agar for no longer than 24 hours. If the incubation time exceeds this, a few strains will give negative glucose results. This is probably due to a reduction in the amount of preformed enzymes within the bacteria after having reached maximum growth.

The inoculum should be heavy, and well spread out on the filter paper to ensure maximum contact with the reagents. Over saturation of the filter paper will reduce the sensitivity of the system. Likewise, dehydration of the filter paper should be avoided by keeping to a minimum its exposure time to the atmosphere during inoculation. During incubation a high humidity must be maintained within the box. The reagents may be kept at  $-20^{\circ}\text{C}$  for 3 months, and at  $4^{\circ}\text{C}$  for 1 month. Using standard bacteriological techniques, contamination of the reagents has not been a problem.

Because of its simplicity, rapidity and low cost the paper carbohydrate utilization test is suitable for use by routine microbiology laboratories to identify pathogenic *Neisseria*. A modification of the method (without the peptone and cysteine) has been used by us to detect carbohydrate utilization by less fastidious bacteria. The results of these studies will be published later.

## ACKNOWLEDGEMENT

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