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A Simple Carbohydrate Fermentation Test for Identification of the Pathogenic *Neisseria*

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The carbohydrate fermentation test in cystine-Trypticase agar-tubed medium was compared with the Minitek system with carbohydrate-impregnated paper disks in Müeller-Hinton broth for identification of *Neisseria gonorrhoeae* and *N. meningitidis*. There was 100% agreement between the methods for confirmation of *N. meningitidis*. The paper disk method confirmed 98% of the *N. gonorrhoeae* isolates; the cystine-Trypticase agar method confirmed 96%. Reactions with the paper disk method could be read in 4 h.

Recently the Minitek (Bioquest) system became available for use in the identification of the *Enterobacteriaceae* and the anaerobes. The flexibility of the system allows for its adaption for identification of organisms other than the enterics and anaerobes. Our interest in the system was in its use in the identification of pathogenic *Neisseria*.

Carbohydrate fermentation reactions are generally used to confirm an isolate as *N. gonorrhoeae* or *N. meningitidis*. The test in general use utilizes glucose, maltose, sucrose, and lactose in separate tubes of semisolid cystine-Trypticase agar (CTA) base (2). This is an expensive, time-consuming technique and not always satisfactory because of the frequent difficulty in reading the results. The test requires 18 to 24 h to read and may require 72 h before a final reading can be given. Therefore, any technique which would be more reliable and give results more rapidly would be an improvement.

The Minitek system consists of 12-well rectangular, disposable plastic plates and covers; vials of Trypticase-peptone broth for making the inoculum; an automatic pipetter with disposable tips; a humidor to provide a moist environment during incubation and cartridges of substrates with appropriate indicators impregnated in 6-mm paper disks. The substrates used in this study included: dextrose-nitrate, maltose, sucrose and lactose disks with phenol-red indicator.

In this study 550 clinical isolates of *N. gonorrhoeae*, 16 isolates of *N. meningitidis* and 3 strains of *N. lactamica* were tested. The carbohydrate fermentation reactions were determined using the conventional tube method with CTA base medium to which dextrose, maltose or sucrose was added; lactose was included when *N. meningitidis* or *N. lactamica* was tested. The final sugar concentration was 1%. The tubes were inoculated with 0.1 ml of a Müeller-Hinton broth suspension of the test culture and were incubated at 35 C for 18 to 24 h before reading. Tubes with negative reactions at the first reading were held an additional 24 h. When the CTA reactions remained negative, fluorescent antibody tests were performed on isolates which had been presumptively identified as *N. gonorrhoeae* on the basis of colonial morphology, Gram stain, and oxidase reactions.

Fermentation reactions using the paper disk method were determined by suspending the isolate in 1.0 ml of Müeller-Hinton broth to a turbidity approximately equal to that of a MacFarland no. 5 nephelometer standard. A 0.05-ml amount of the suspension was inoculated into the wells of the plastic plate which contained the appropriate substrate disks. Sterile mineral oil (0.01 ml) was added to each well according to the manufacturer's directions for fermentation reactions. The plates were placed in the humidor and incubated at 35 C for 18 to 24 h.

Table 1 gives the results of the study. Of the 550 isolates of *N. gonorrhoeae*, 529 (96%) were confirmed in 18 to 24 h by the CTA tube method, 6 required 48 h, and the remaining 15 were confirmed by fluorescent antibody. 543 (98%) of the isolates were confirmed in 18 to 24 h by the paper disk method; the remaining seven were among those which had to be confirmed by fluorescent antibody. There was 100% agreement between the CTA and paper disk methods with the *N. meningitidis* and *N. lactamica* isolates tested. The reactions with

TABLE 1. *Comparison of carbohydrate fermentation reactions of Neisseria with Minitek disk versus CTA tube method*

Organisms	No. of isolates	Isolates confirmed (18 to 24 h)	
		CTA tubes	Minitek disk
Neisseria gonorrhoeae	550	529[a]	543 (98%)
N. meningitidis	16	16	16 (100%)
N. lactamica	3	3	3 (100%)

[a] Fifteen isolates were confirmed by fluorescent antibody; 6 isolates were CTA positive in 48 h.

the paper disks were clear-cut and stable.

The CTA method requires an incubation period to allow for growth of the organism. The paper disk method is dependent on the presence of preformed enzymes; therefore, this method was evaluated as a rapid method for determination of the fermentation reactions. Fifty strains of *N. gonorrhoeae* and the 16 stains of *N. meningitidis* were tested, observing the times after inoculation when the reactions could be read. Clear-cut reactions were obvious within 4 h; however, this was inoculum dependent. Suspensions of the organisms equal to a no. 4 or no. 5 MacFarland nephelometer standard were required for definitive reactions in 4 h.

Three media were compared as a suspending medium for making the inoculum: the inoculum broth available from the manufacturer, balanced salt solution described by Brown (1), and Müeller-Hinton broth. Only the Müeller-Hinton broth was found to be satisfactory; the reactions were either not clear-cut or not reproducible when the Minitek inoculum broth or the balanced salt solution was used.

The manufacturer recommends the use of the mineral oil overlay and it was used throughout the study of the 569 isolates. Fifty strains of *N. gonorrhoeae* were tested in duplicate, one set with the oil overlay and one without. We found that the mineral oil overlay was not essential for the reactions to occur.

At present, the dextrose disk as supplied by the manufacturer is a combination of substrates for dextrose fermentation and nitrate reduction determination. A dextrose disk without the NO_3 substrate is soon to be available; however, we found no problem using the dextrose-nitrate disk.

In summary, we found the paper disk method to be a simple, accurate, reproducible, and rapid method for determination of the fermentation reactions of the pathogenic *Neisseria* and the cost of the test is approximately one-half that of the CTA method.

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