

Technical methods

Identification of *Neisseria gonorrhoea* and *Neisseria meningitidis* by a carbohydrate disc technique

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Serum agar sugars are variable in performance when used to identify the pathogenic *Neisseria*. In these circumstances, it has been common practice to rely on the Gram stain and oxidase reaction to confirm the clinical diagnosis. However, with reports of isolations of *Neisseria gonorrhoea* from non-urogenital sites and isolations of *Neisseria meningitidis* from cases of neonatal ophthalmia (Barr and Danielsson, 1971; Cowan, 1969; Furmora, Wise, and Many, 1967; Hellgren, 1971; Iqbal, 1971; Rogers, 1972), it is of increasing importance that a reliable simple confirmatory test be used.

The method described uses a routine gonococcal isolation medium combined with easily prepared carbohydrate discs.

Materials

BBL GC agar base			
Polypeptone peptone	15 g
Corn starch	1 g
Dipotassium phosphate	4 g
Monopotassium phosphate	1 g
Sodium chloride	5 g
Agar	10 g
Distilled water (final pH 7.2)	1000 ml
BBL . . 2% haemoglobin solution			
BBL . . Isovitalex enrichment			
Colistin 8 µg/ml, vancomycin 3 units/ml, trimethoprim 3 µg/ml. The medium is prepared using the manufacturer's instructions.			

Cultures

Thirty known strains of *N. gonorrhoea* were obtained from the Department of Bacteriology, Birmingham General Hospital; six strains of *N. meningitidis* and

13 strains of saprophytic *Neisseria* isolated at Dudley Road Hospital were used.

Carbohydrate Discs

Whatman AA discs are heated to 160°C for 30 minutes (further heating causes charring and produces a less absorbent disc), and 30% carbohydrate solutions sterilized by Millipore filtration. The discs are saturated in the solution and dried at 37°C in the incubator. When dry, the discs keep for several months in the refrigerator. Each disc contains approximately 30 mg carbohydrate.

Method

Using a swab moistened with sterile water pick off several colonies from a purified culture. Spread the culture liberally over the surface of the prepared medium. Apply the glucose, maltose, and sucrose discs to the plate. Incubate overnight at 37°C in 5% CO₂. Examine the culture next day and, assuming a reasonably confluent growth, apply one drop of the phenol red to each disc. Within five minutes acid production will be indicated by a colour change from red to yellow.

Results

Twenty-eight strains of *N. gonorrhoea* fermented glucose after overnight incubation. The remaining two strains fermented glucose after 36 hours. Maltose, lactose, and sucrose were unchanged.

All six strains of *N. meningitidis* fermented glucose and maltose but not sucrose after overnight incubation.

The 13 strains of saprophytic *Neisseria* were tested in peptone water sugars and with the disc technique in parallel. Similar reactions were obtained by both methods. With the saprophytic *Neisseria*, the inhibitor was omitted from the medium.

An attempt was made to reproduce the results of the disc technique on horse blood 'chocolate' agar. Under these circumstances, all the strains of *N. gonorrhoea* appeared to ferment maltose. This is thought to be due to the presence of maltase in the horse blood.

Comment

A carbohydrate disc technique for the identification

of Enterobacteriaceae has been described (Sanders, Faber, and Cook, 1957), and the present method is a modification of that technique, designed to relieve the uncertainty of serum agar sugars. The technique is carried out on the conventional selective medium for *N. gonorrhoea*. The only special requirements, the carbohydrate discs, are available commercially, but are quite simple to prepare.

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The oxidase activity of *Chromobacterium*

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Various techniques have been used to determine the oxidase activity of bacteria, the most popular of which is that of Kovacs (1956) in which bacterial growth is smeared onto a filter paper impregnated with 1% aqueous solution of NNN'N' tetra methyl-p-phenylene diamine dihydrochloride. This method has been found to suffer from shortcomings when used for violet-pigmented bacteria. Recently, it became necessary to determine the oxidase reaction of purple-pigmented strains of *Chromobacterium violaceum* and *Chromobacterium lividum* so that appropriate entries could be made in an identification matrix for medically important Gram-negative bacteria (Lapage, Bascomb, Willcox, and Curtis, 1970). Many strains of *Chromobacterium* do not produce pigment except on delayed incubation and from others non-pigmented colonies can be used for the test. However, for those strains which are pigmented even after overnight incubation a modified technique was used to determine their oxidase activity.

Materials and Methods

Thirty-eight strains of assorted bacterial species, including six strains of *Chromobacterium violaceum* and 10 strains of *Chr. lividum*, were tested. Also included were three pigmented strains of *Serratia marcescens* and one strain of *Ps. aeruginosa* which produced a red diffusible pigment. All strains were grown on nutrient agar at 30°C for 48 hours except for the strains of *Chr. lividum* which were incubated at room temperature for 48 hours.

The oxidase activity of the strains was determined first by the conventional method of Kovacs (1956), and then by the following method. A single sheet of Whatman no. 1 filter paper was folded at three edges so that it could stand upright in a Petri dish. With the use of a platinum loop five smears were made about 1 cm from the base of the filter paper. These were placed in a horizontal line about 1 cm apart.

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