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.

We are grateful to the Bacteriology Department, Birmingham General Hospital, for providing the strains of *N. gonorrhoea*, and also to Dr J. D. Williams for advice.

References

- Barr, J., and Danielsson, D. (1971). Septic gonococcal dermatitis. *Brit. med. J.*, 1, 482-485.
- Cowan, L. (1969). Gonococcal ulceration of the tongue in the gonococcal dermatitis syndrome. *Brit. J. vener. Dis.*, 45, 228-231.
- Fiurmar, N. J., Wise, H. M., Jr., and Many, M. (1967). Gonorrheal pharyngitis. *New Engl. J. Med.*, 276, 1248-1251.
- Hellgren, L. (1971). Gonorrhoea and tonsillitis following genito-oral contact (Swedish). *Lakartidningen*, 68, 569-571.
- Iqbal, Y. (1971). Gonococcal tonsillitis. *Brit. J. vener. Dis.*, 47, 144-145.
- Rogers, K. B. (1972). In *Proceedings of Birmingham Antibiotic Conference*, p. 871. Churchill Livingstone, London.
- Sanders, A. C., Faber, J. E., Jr., Cook, T. M. (1957). A rapid method for the characterization of enteric pathogen using paper discs. *Appl. Microbiol.*, 5, 36-40.

The oxidase activity of *Chromobacterium*

S. K. DHAR¹ AND R. JOHNSON *From the Computer Trials Laboratory, Central Public Health Laboratory, Colindale, London*

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.

¹Present address: National Institute of Communicable Diseases, Delhi, India.

Technical methods

The filter paper was also appropriately marked so that each bacterial smear could be identified.

Approximately 2 ml of a freshly prepared 1% aqueous solution of NNN'N' tetramethyl-p-phenylene diamine dihydrochloride (Hopkin and Williams Ltd) was dropped along the base of the filter paper using a Pasteur pipette. As the reagent ascended the filter paper it came into contact with the smears and then passed higher up.

Shortly after coming into contact with the reagent, those non-pigmented, oxidase-positive strains turned blue and this was followed by a vertical blue streak appearing from the top of both pigmented and non-pigmented smears.

Those smears which turned blue and those which produced a vertical blue smear were regarded as oxidase positive. A distilled water control was also tested on all the cultures to check that the blue streaks from pigmented, oxidase-positive strains were not simply due to pigment diffusing outwards.

Results and Comment

Table I indicates the results obtained by the two methods. Two points arise from the table. First it was impossible using method 1 (smearing a colony on filter paper soaked in reagent) to determine the oxidase reaction of blue or purple pigmented strains. Secondly, the oxidase reaction obtained in method 2 was slower in making an appearance. The time varied from 20 to 300 sec as opposed to method 1 where the time varied from 10 to 20 sec. However, it is noticeable that apart from the *Chromobacterium* strains the two methods give identical results.

The distilled water control did not give rise to false positives. The method was reproducible and easy to perform.

Since there are very few purple-pigmented Gram-negative bacilli, determination of their oxidase reaction may not be very important as they can be adequately characterized by other tests. However,

Strain Designation	NCTC No.	Pigment	Oxidase Reaction and Time (sec)	
			Method 1	Method 2
<i>Pseudomonas aeruginosa</i>	1999	—	+ 10	+ 60
<i>Pseudomonas aeruginosa</i>	6749	Red, diffusible	+ 20	+ 30
<i>Proteus vulgaris</i>	4175	—	— 60	— 300
<i>Proteus vulgaris</i>	4636	—	— 60	— 300
<i>Proteus rettgeri</i>	10377	—	— 60	— 300
<i>Serratia marcescens</i>	10211	Red	+ 20	+ 90
<i>Serratia marcescens</i>	1377	Red	— 60	— 300
<i>Serratia marcescens</i>	9493	Red	— 60	— 300
<i>Citrobacter freundii</i>	9750	—	— 60	— 300
<i>Enterobacter cloacae</i>	9395	—	— 60	— 300
<i>Escherichia coli</i>	9014	—	— 60	— 300
<i>Escherichia coli</i>	9699	—	— 60	— 300
<i>Alcaligenes faecalis</i>	655	—	+ 10	+ 30
<i>Alcaligenes faecalis</i>	415	—	+ 10	+ 20
<i>Yersinia pseudotuberculosis</i>	10216	—	— 60	— 300
<i>Acinetobacter anitratus</i>	10304	—	— 60	— 300
<i>Acinetobacter anitratus</i>	7844	—	— 60	— 300
<i>Acinetobacter anitratus</i>	8698	—	— 60	— 300
<i>Acinetobacter wolffii</i>	10307	—	— 60	— 300
<i>Acinetobacter wolffii</i>	10308	—	— 60	— 300
<i>Pseudomonas maltophilia</i>	10259	Yellow	— 60	— 300
<i>Pseudomonas fluorescens</i>	9428	—	+ 10	+ 20
<i>Chromobacterium lividum</i>	8661	—	+ 10	+ 20
	9796	—	+ 10	+ 20
	9717	—	+ 10	+ 20
	7150	—	+ 10	+ 20
	Sneath D270-NA	Blue	?	+ 20
	Sneath D264	—	+ 10	+ 20
	Sneath D267	Blue	?	+ 20
	Sneath D273	—	+ 10	+ 20
	Sneath D281	—	+ 10	+ 20
	Sneath D286	—	+ 10	+ 20
<i>Chromobacterium violaceum</i>	9694	Blue	?	— 300
	9376	Blue	?	+ 90
	9696	Blue	?	+ 90
	9757	Blue	?	+ 300
	9371	Blue	?	+ 90
	9872	Blue	?	+ 90

Table Comparison of two methods of determining oxidase activity

for a probability matrix from which identification scores are derived by multiplication of the entries for each taxon, it is important that the entries should be complete. It was therefore necessary for our purposes to determine the reaction, and this paper serves to report our findings.

We would like to thank Professor P. H. A. Sneath of the MRC Microbial Systematics Unit for providing cultures of *Chromobacterium lividum*.

References

- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (Lond.)*, **178**, 703.
- Lapage, S. P., Bascomb, S., Willcox, W. R., and Curtis, M. A. (1970). Computer identification of bacteria. In *Automation, Mechanization and Data Handling in Microbiology*, edited by A. Baillie and R. J. Gilbert, pp. 1-22. Society for Applied Bacteriology Technical Series no. 4. Academic Press, London.

A screening test for IgG Deficiency

ANNE FERGUSON *From the Department of Bacteriology and Immunology (Western Infirmary), University of Glasgow*

Immunodeficiency syndromes with hypogammaglobulinaemia are rare (Medical Research Council, 1971). However, there are well defined groups of patients in whom the incidence of IgG deficiency is likely to be higher than in the population as a whole, for example, patients admitted to hospital with infections; children who fail to thrive; relatives of immunodeficient patients. This paper describes a method whereby these 'at risk' patients can be screened for IgG deficiency. The method fulfils several important provisos for a screening procedure. The diagnosis benefits the patient (early treatment) and his family (genetic counselling); the initial screening procedure is rapid and relatively inexpensive; and abnormal results can be confirmed or refuted by formal measurement of serum immunoglobulins.

Method

The screening technique, as described here, has been modelled on the Guthrie test for phenylketonuria screening (Guthrie and Susi, 1963; Newman and Starr, 1971).

COLLECTION AND TRANSPORT OF SPECIMENS

Wards and clinics are supplied with printed sample papers (see fig.), polythene bags, envelopes printed with the address of the Ig screening laboratory, and a printed instruction card. Schleicher and Schull

Fig. Printed filter paper which is used for collection and transport of dried blood spots

filter paper 903 is used for the sample papers; sheets of filter paper were printed by the University of Glasgow printing department. Blood is obtained by

Received for publication 12 December 1972.