J. Hyg., Camb. (1980), **84**, 429
With 2 plates
Printed in Great Britain

Technique for the cultural examination of urine using a single plate (the Macba plate)

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(*Received* 9 *October* 1979)

SUMMARY

This technique for the cultural examination of urine provides, within 24 hours, using a single Petri dish, the colonial appearance of any bacteria present on MacConkey agar and on blood agar, a rough colony count of the bacteria present, their sensitivity to four chemotherapeutic agents, isolated colonies from which further sensitivity tests can be done if necessary and the opportunity to scrutinize contaminating organisms that, even though present in only small numbers, may be of help in diagnostic problems.

INTRODUCTION

In recent years, interest in urinary tract infections has greatly increased, and the number of urine specimens that diagnostic laboratories are called on to examine has risen steeply. Numerous methods have been devised for the diagnosis of urinary-tract infections and for providing guidance in therapy (Leading article, 1968). The laboratory is torn between the desire to provide advice as rapidly as possible and the need to conserve labour and materials. Screening tests have been devised: these reduce the labour but delay the diagnosis and advice on treatment in the genuine urinary-tract infections. Detailed plate cultures and direct sensitivity testing provide advice rapidly but are extravagant in labour and materials.

This paper describes a technique which is something of a compromise, and was used most successfully for at least eight years at the Cambridge Laboratory of the P.H.L.S., often in combination with the use of the dip-slide (Naylor & Guttmann, 1967).

PRODUCTION OF PLATES CONTAINING TWO MEDIA – THE MACBA PLATE

The culture plates used consist of half blood agar and half MacConkey agar combined in one Petri dish.

There are two alternative methods of producing the plates.

(1) The Petri dishes are sloped on the pouring bench by resting them on a suitable raised surface such as a sloping board or a long piece of glass rod or tubing of about 7 mm diameter, which can be held on the bench with sellotape. One half of the sloping plates is filled with horse blood agar, about 9 ml being required for

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0022–1724/80/0092–1979 $01.00

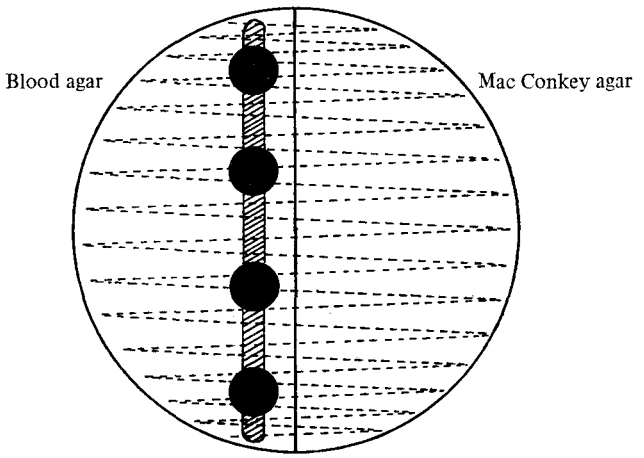

Fig. 1. Technique of sowing the Macba double plate. -----, Continuous zig-zag movement across both media with the standard loopful. , Heavily sown stripe across the diameter of the plate near the margin of the blood agar half. , Sensitivity discs.

each plate. When this has set, the plates are levelled and the remaining halves poured with MacConkey agar.

(2) In the second method the plates are kept flat throughout. The blood agar half is poured first using an automatic dispenser* set to deliver 6 ml into the flat plate. After a little practice in directing the flow of the 6 ml of blood agar, the viscosity and rate of setting of the blood agar is such that 6 ml flows evenly over half the dish. When this has set the remaining halves are formed with MacConkey agar as in the first method.

SOWING THE PLATES

The standard loop used to sow the plate is of 4 mm internal diameter of wire SWG 22.

The plate is sown in two ways. The loop is first dipped vertically into the urine and withdrawn vertically (standard loop) and then sown in a continuous zig-zag movement across both media (Fig. 1).

The loop is then charged by dipping horizontally into the urine, to obtain a larger inoculum, which is then spread in a single line across the diameter of the plate on the blood agar half, close to the junction of the blood agar with the MacConkey.

Four sensitivity disks selected to suit the individual laboratory can then be placed along this more heavily inoculated stripe. A carefully selected quartet of chemotherapeutic agents will cover most of the problems.

The urines can be sown on the plates when they first arrive at the laboratory to avoid the proliferation of bacteria in the urine that can occur if there is a delay

* Accuramatic: Jencons (Scientific) Ltd, Mark Road, Hemel Hempstead, Hertfordshire, England.

https://doi.org/10.1017/S0022172400026966 Published online by Cambridge University Press

until the urine has been examined microscopically. The sensitivity disks can then be applied later, if microscopy reveals any evidence of a urinary tract infection. This procedure avoids the unnecessary use of sensitivity disks.

EXAMINATION OF THE PLATE CULTURES

These Macba plates are simple to prepare, and provide on a single plate culture within 24 hours:

(1) Colonial appearance of any bacteria in the urine on two different media. This is of help in identification of an infecting organism and in differentiating the pure culture typical of infection from the mixed culture indicative of contamination (see Plate 2A–C).

(2) By using a standard loop a rough quantitative assessment of the number of colony forming units per ml of urine is obtained which is of help in distinguishing between growth on culture that reflects infection and that which is the result of contamination during collection (Kass, 1956; Cattell & Lefford, 1963; Guttmann & Stokes, 1963). The quantitive assessment is particularly discriminating over the range 10^4–10^5 colony forming units per ml, which is just the range that is most helpful in the diagnosis of urinary tract infections (see Plate 1A–D).

(3) Sensitivity to four antibiotics or chemotheraputic agents if required. The bile salts in the MacConkey agar lyse the horse red cells in the horse blood agar immediately adjacent to the MacConkey, where the heavily sown stripe is placed for sensitivity tests. The lysed horse cells neutralize sulphonamide inhibitors, which are sometimes present in nutrient media (Harper & Cawston, 1945). This increases the reliability of the sulphonamide sensitivity test.

(4) Scrutiny of contaminants of significance particularly on the heavily sown stripe on the blood agar, such as yeasts and haemolytic streptococci (see Plate 2 and legend).

(5) Isolated colonies for further sensitivity tests if needed. The two media are an advantage here: *Staphylococcus albus* grows well on blood agar, less well if at all on MacConkey, and proteus strains which swarm on blood agar do not swarm on MacConkey.

DISCUSSION

The technique thus provides the maximum amount of information for the minimum of effort in the shortest possible time. Diagnosis can be achieved rapidly and, in many instances, the patient can be started on treatment, based on laboratory criteria, within 24 h.

The technique has been well tried: it could be of particular use in laboratories examining up to 50 urines a day and may be of use in special circumstances such as the problem case or investigations.

I am extremely grateful to Dr A. E. Wright of the Public Health Laboratory at Gloucester for introducing me to the possibility of using two different media in the same Petri dish.

I am delighted to acknowledge with great gratitude the expert skill and experience of Mr V. C. T. Rolfe and the rest of the technical staff of the Cambridge

Public Health Laboratory, who did so much to develop, improve and refine this technique.

I also wish to thank Dr J. Boissard of the Public Health Laboratory at Cambridge for the result of the nose swab from the boy of five years with balanitis and for the type determination of the *Strept. pyogenes* isolated from his urine and from his nose swab (see Plate 2 D and legend).

Fig. 1 was drawn by Mr Colin Farrell and the photographs in Plates 1 & 2 were taken by the staff of the Department of Medical Photography and Illustration at Addenbrooke's Hospital, Cambridge, and I am very grateful to them all for their help.

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EXPLANATION OF PLATES

PLATE 1

Plate 1. The Macba plate: semi-quantitative cultures using a standard loop. Colony forming units (*Escherichia coli*) per ml: (A) 10^3, (B) 10^4, (C) 10^5, (D) 10^6. For many years, A, B, C, and D were mounted in line on a card propped in front of the person examining urine cultures.

PLATE 2

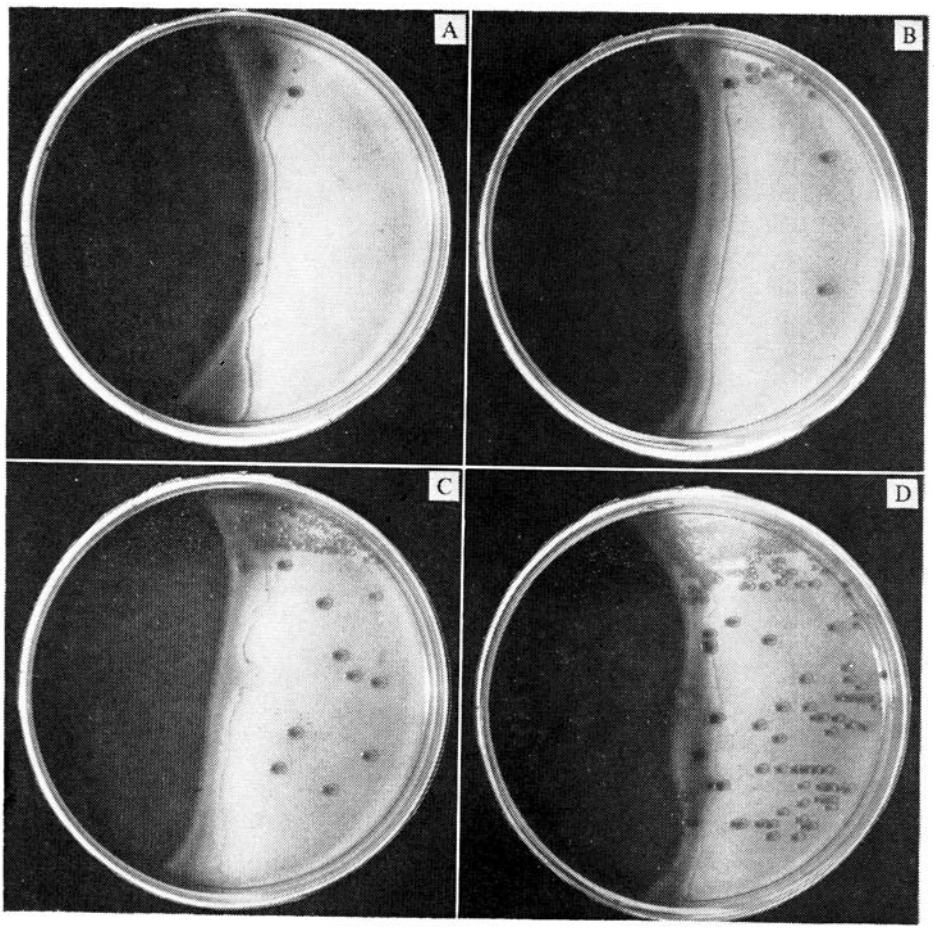
Macba plate cultures

(A) Typical urinary tract infection. There are more than 10^6 colony forming units of *E. coli* per ml and the causative organism is sensitive to nitrofurantoin (F), naladixic acid (NA), ampicillin (PN) and sulphonamides (G). Olds (1975) shows colour plates of typical urinary tract infections taken from a routine batch of Macba plates (see his figures 272 and 273, p. 161).

(B) Typical urinary tract infection: as A, but showing 'wedges' of growth when sensitivity discs are not placed directly over the line of growth across the diameter of the plate.

(C) Typical result with a urine contaminated with vulval material: there are about 10^5 colony forming units per ml but the growth is heavily mixed with a wide variety of colonial appearances.

(D) Illustrating the value of the more heavily sown stripe in detecting urinary contaminants of significance when not used for sensitivity tests. Growth of *Streptococcus pyogenes*, Group A, type 1: boy aged 5 years with balanitis. On investigation, his nose swab also grew *Strept. pyogenes* of the same type.



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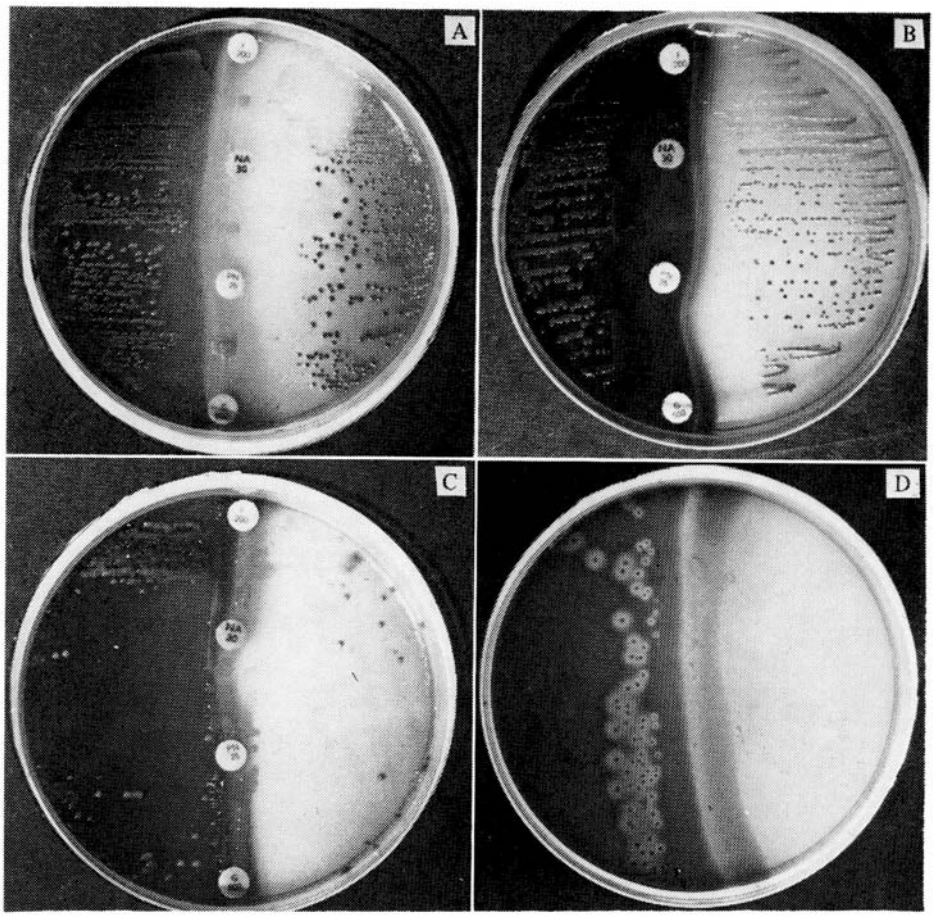
https://doi.org/10.1017/S0022172400026966 Published online by Cambridge University Press



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