

The localization of viable bacteria in tissue

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While simple qualitative cultures of tissues taken during surgery or necropsy have not presented a problem, their interpretation has been bedevilled by the difficulty of distinguishing between organisms actually present in tissue and contaminants, particularly any which might have been introduced during necropsy. Quantitative bacteriology in these circumstances is rarely attempted, and the precise anatomical correlation between viable bacteria and naked-eye or microscopically identifiable features has not been possible. The method described here is claimed to allow a distinction to be made between bacteria present in tissue and those which are surface contaminants, while simultaneously giving

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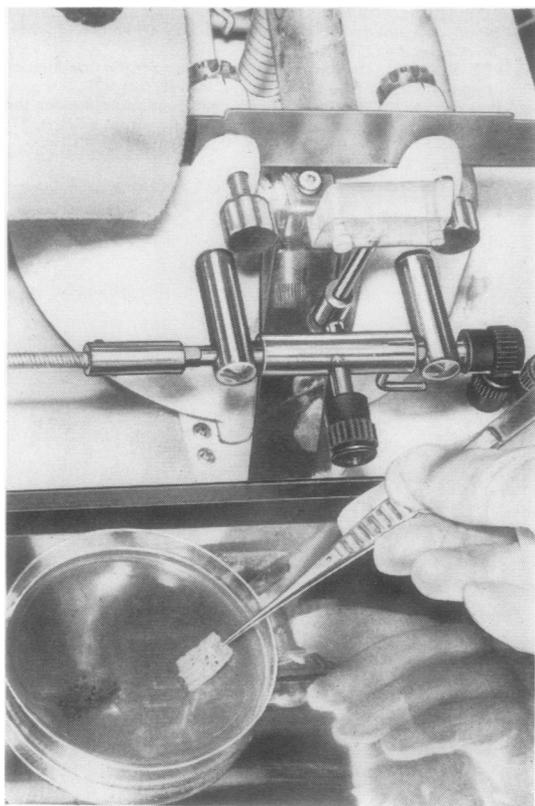


Fig. 1 *Mounting cryostat section on agar.*

excellent quantitative data and very precise anatomical localization of viable bacteria. The technique depends on the preparation of frozen sections which are placed onto nutrient media in petri dishes (Fig. 1). In this situation the tissue acts in the same way as a membrane filter, and any viable bacteria present develop into colonies on its surface. This development can be observed under varying conditions of incubation on plain or complex nutrient, selective, or where *Proteus* spp. are likely to be a problem, electrolyte-deficient media. The site of growth can be related to histological, histochemical, or immunofluorescent features by suitable examination of contiguous serial frozen sections of the tissue.

Method

Tissue for examination is removed with as little manipulation as possible and is placed in a sterile container. Depending on the structure being sampled the size of the specimen will vary, but a piece of lung 2.5 cm square \times 0.5 cm thick would be typical. The tissue is frozen as soon as possible, and it may be stored at -20°C until it is examined. If it is to be dealt with immediately it may be frozen conveniently with dichlorodifluoromethane (Polar Spray, Medical Aerosols Ltd, London). The frozen block is then trimmed to fit the chuck of the microtome, using sterile instruments. This step helps to remove surface contamination. The microtome is used to cut and discard the first millimetre or so of tissue and then sections for culture are cut at a thickness of between 25 and 30 microns. They are picked up with sterile forceps and laid on the surface of the chosen medium. The tips of the forceps used must be cold to prevent sections sticking to them. After a little practice no difficulty is experienced in applying the sections to the media. If they are small, several may be put into a single dish, and the size of the specimen to be examined is only governed by the capacity of the freezing microtome.

Results

When significant bacteria have been found in tissue by traditional means, we have never failed to isolate the same organism from frozen sections by the method described. On one occasion a slowly growing bacterium which was heavily overgrown by contaminants when examined by ordinary methods was obtained with the greatest of ease from a frozen section. On another occasion the method proved of value in the isolation of bacteria from the tissues of patients who died following the infusion of contaminated dextrose solution.



Fig. 2.

Fig. 2 Eighteen-hour culture on electrolyte-deficient medium of cryostat section of normal large intestine.

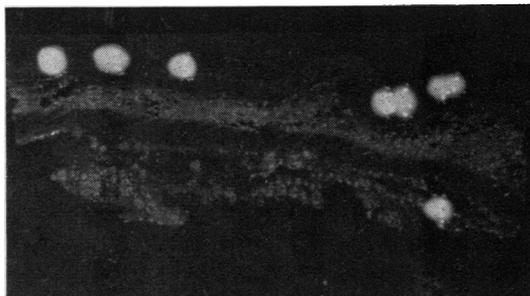


Fig. 3.

Fig. 3 Eighteen-hour culture on electrolyte-deficient medium of carcinoma of colon, showing organisms growing deep in the lesion.

The precision of bacterial localization possible is demonstrated in Figs. 2 and 3 where the growth obtained following culture of neighbouring specimens taken at operation for carcinoma of the colon is seen. Figure 2 is of normal colon showing scanty bacterial growth on its mucosal surface only, while in Fig. 3 bacteria have penetrated deeply into the malignant tissue.

An incidental lesson brought home by the success of this method is the potential risk of infection to operators of freezing microtomes and emphasizes the importance of disinfecting the apparatus.

We wish to thank Miss Sylvia Easton, Department of Medical Photography, Plymouth General Hospital, for the photographs.

Book reviews

The Thalassemia Syndromes 2nd edition By D. J. Weatherall and J. B. Clegg. (Pp. x + 374; illustrated. £7.00). Oxford, London, Edinburgh, and Melbourne: Blackwell Scientific Publications. 1972.

This excellent book, like the first edition, contains a detailed, up-to-date account of the thalassaemia syndromes. It does not suffer from the common 'second edition syndrome', namely, undue expansion in size, thus although the authors have added much new material, they have cut out what is now redundant. The text is strongest in the molecular and genetic aspects of the subject, many of the ideas expressed being supported by experimental results obtained in the authors' own laboratory. The clinical parts contain excellent descriptions of the various types of thalassaemia and their interaction with the abnormal haemoglobins; however, differential diagnosis and the interpretation of variations in foetal haemoglobin and Hb-A₂ level are less well covered. For example, I could not find the foetal haemoglobin levels expected in normal infants.

To sum up, here is an important book on an important subject. The general haematologist should read it, but its bias towards molecular pathology may not make it the ideal book to consult in clinical practice. The research worker or clinician in the field will certainly want to have it for the up-to-date account of our understanding of these diseases.

E. R. HUEHNS

Electronic Instruments in the Clinical Laboratory By Philip G. Ackermann. (Pp. x + 349; illustrated: £7.00). Boston: Little, Brown and Co. London and Edinburgh: Churchill Livingstone. 1972.

Starting with the general principles of electricity, the author proceeds to describe the theory of operation of the different instruments found in the modern clinical laboratory. The discussion includes not only the electronics involved, but also other physical or physio-chemical principles required for a complete understanding of the instruments.

The operation of vacuum tubes and semiconductors and their use in various types of circuits found in instruments is then described followed by a short introduction to the theory of computers and a chapter on computers in the laboratory. The final chapters deal with test instruments and methods of fault finding in the several types of instrument mentioned. A glossary of terms and suggestions for further reading conclude the text.

In 327 pages Dr Ackermann has packed an amazing amount of vital information and diagrams presented in a direct style which is sympathetic to the needs of the clinical laboratory worker. The book's American origin is evident in references to '115 volts mains supply at 60 Hz', but generally the text is universal.

An excellent book, strongly recommended.

J. M. RIDEOUT