Correspondence

Neomycin blood agar as a selective medium for vancomycin resistant *Enterococcus faecium*

We read with interest the article by Chadwick and Oppenheim¹ regarding the selective isolation of *Enterococcus faecium* and agree that further comparative studies of screening media are required for detection of vancomycin resistant enterococci (VRE) from clinical and environmental sources. Furthermore, we believe that an overall strategy for the isolation of these increasingly prevalent nosocomial pathogens should be developed.

Using cephalixin aztreonam arabinose agar (CAA),² a medium developed for the selective isolation of *E. faecium*, in association with a broth enrichment technique, we examined 92 swabs from 70 environmental sites and 22 rectal swabs during the investigation of a nosocomial outbreak on a renal unit. All swabs were plated directly onto CAA, and CAA containing 4 mg/l vancomycin (Eli Lilly). The swab was then placed into cephalixin aztreonam (CA) broth prepared by the addition of cephalixin and aztreonam to one litre of sterile brain heart infusion broth (Unipath). Plates were examined for *E. faecium*, following 24 and 48 hours' incubation at 37°C in air. CA broth was subcultured onto both of the above media following enrichment for 24 hours.

Thirty eight *E. faecium* strains were isolated from 92 environmental and patient samples. Of these, 28 (74%) were vancomycin sensitive and 10 (26%) were vancomycin resistant. When the isolation of *E. faecium* from direct culture and broth enrichment was compared, 16 strains (42%) were isolated on direct culture, and the remaining 22 strains (58%) were isolated from broth enrichment only. Of the 10 vancomycin resistant strains, only two (20%) were isolated on direct plating. It was interesting to note that vancomycin resistant strains often required 48 hours' incubation to produce typical colonies. This delayed growth was presumably because of the time required for the induction of the Van B resistance phenotype typical of our outbreak strains.

Our investigations show that the isolation rate of *E. faecium* during nosocomial outbreaks may be seriously underestimated if a broth enrichment procedure is not used, as only 16 (42%) of 38 *E. faecium* strains were isolated on direct culture. Moreover, only 20% of strains of VRE were isolated on direct culture. It is likely that the additional strains detected after broth enrichment were present in low numbers, which might easily have been missed if the broth enrichment step was not used, regardless of the type of selective media used. This effect might, however, be compounded if a selective medium inhibitory to VRE¹ was used without an enrichment stage.

In order to implement a successful infection control strategy it is essential that accurate information is available about the numbers of cases of clinical infection or colonisation, and the extent of any environmental contamination with VRE. Our study suggests that outbreak management based on results of screening exercises using only direct culture techniques may be inappropriate.

We agree with Chadwick and Oppenheim¹ that comparative studies of screening media

are warranted, but also recommend the use of a broth enrichment step in association with an appropriate selective medium such as CAA for the isolation of VRE during the investigation of nosocomial outbreaks.

M FORD
JD PERRY
FK GOULD
KE ORR

Microbiology Department,
Freeman Hospital,
Freeman Road,
High Heaton,
Newcastle upon Tyne NE7 7DN

- 1 Chadwick PR, Oppenheim BA. Neomycin blood agar as a selective medium for vancomycin resistant *Enterococcus faecium*. *J Clin Pathol* 1995;48:1068-70.
- 2 Ford M, Perry JD, Gould FK. Use of cephalixin aztreonam arabinose agar for the selective isolation of *Enterococcus faecium*. *J Clin Microbiol* 1994;32:2999-3001.

Coronary artery dissection

Bateman *et al*¹ describe an interesting spectrum of clinical presentation of spontaneous coronary artery dissection. Despite its rarity, the entity shows a striking constancy in the vessels involved and the presence of an inflammatory infiltrate rich in eosinophils. These features were also seen in a recent necropsy in our department. The patient, an obese 43 year old woman with no recent pregnancy, had a background of mild hypertension not requiring medical therapy. She complained of severe back pain one evening, and died the following morning. At necropsy, the heart weighed 400 g. The left anterior descending coronary artery was occluded by thrombus from its origin, and a dissection, clearly visible grossly, extended the length of the artery. There was no atheroma, and histologically, no abnormal accumulations of mucin and no evidence of systemic vasculitis. No intimal tear was identified. Like cases 2 and 3 reported by Bateman *et al*, in which there was an interval between onset of symptoms and death, there was an adventitial infiltrate with prominent eosinophils. The dissection in our case was mostly between the media and adventitia, internal to the external elastic lamina, with small foci in the outer media. We have seen dissection in this location in a previously reported case,² as have others.³ It seems likely that it overlaps with the dissection in the outer third of the tunica media, and does not justify the description of "unusual" as suggested.¹ Finally, increased awareness of this entity may mean that early presentation may result in salvage of some cases.

EE MOONEY
GSA McDONALD
Department of Histopathology,
St James's Hospital, Trinity College,
Dublin, Ireland

- 1 Bateman AC, Gallagher PJ, Vincenti AC. Sudden death from coronary artery dissection. *J Clin Pathol* 1995;48:781-4.
- 2 McDonald GSA. Spontaneous dissection of the coronary artery. *Ir J Med Sci* 1989;158:304-6.
- 3 Glasgow BJ, Tift JP, Alexander CB. Spontaneous primary dissecting coronary artery aneurysm. *Am J Forensic Med Pathol* 1984;5:155-9.

Drs Bateman and Gallagher comment:

We read Drs Mooney and McDonald's further report on spontaneous coronary artery dissection with interest. The intimal dissection in our first case remains unusual.¹

However, their comments regarding the plane of dissection in our second and third cases are entirely justified. We accept that coronary artery dissection has been observed both within the outer tunica media and between the media and adventitia.¹⁻⁴ Differences in the reported plane of dissection may result from sampling error. It is widely believed that spontaneous coronary artery dissection results from luminal blood entering the arterial wall via an intimal tear, although this feature is rarely identified at postmortem examination.³ Histological examination of coronary artery segments may therefore reveal dissection at varying levels within the tunica media as the blood flows towards the relatively impervious tunica adventitia.

Adventitial inflammatory cell infiltrates containing a high proportion of eosinophils remain a common feature of spontaneous coronary artery dissection, and their role has yet to be elucidated. Drs McDonald and Mooney suggested an intriguing association between autoimmune endocrine disease and coronary artery dissection in a previous report, although the composition of the inflammatory cell infiltrate differed between the involved coronary artery and the autoimmune thyroiditis.² The pathogenesis of spontaneous coronary artery dissection, particularly in patients in whom no associated factors are identified, remains an enigma.

- 1 Bateman AC, Gallagher PJ, Vincenti AC. Sudden death from coronary artery dissection. *J Clin Pathol* 1995;48:781-4.
- 2 McDonald GSA. Spontaneous dissection of the coronary artery. *Ir J Med Sci* 1989;158:304-6.
- 3 Glasgow BJ, Tift JP, Alexander CB. Spontaneous primary dissecting coronary artery aneurysm. *Am J Forensic Med Pathol* 1984;5:155-9.
- 4 Robinowitz M, Virmani R, McAllister HA. Spontaneous coronary artery dissection and eosinophilic inflammation: a cause and effect relationship? *Am J Med* 1982;72:923-8.

Effects of interleukins on the proliferation and survival of chronic lymphocytic leukaemia cells

Mainou-Fowler *et al*¹ report in their interesting study of the in vitro response of B chronic lymphocytic leukaemia (B-CLL) cells to interleukins that the effects of interleukin-4 (IL-4) are heterogeneous. They show that "IL-4 enhanced cell proliferation by... 235% (123-400%) in four of 12 B-CLL cases" and they propose that this variability in response is a result of variable B-CLL cell maturity and defective expression of receptors for growth factors.

We suggest that their observations may be a result of heterogeneous expression of the IL-4 receptor (IL-4R), as we have shown that B-CLL cells express IL-4R and have presented evidence of the expression of two species of high affinity receptor by these cells.² Briefly, the presence of high affinity IL-4R was determined by ¹²⁵I labelled IL-4 binding and Scatchard analysis using MLA-144 cells as a positive control.³ While a high affinity IL-4R was detected in all six samples examined, there was evidence in some cases of expression of a distinct, and previously unreported, high affinity IL-4R. Thus, four of six samples expressed the conventional⁴ high affinity IL-4R, K_D 17-95 pM, which was of similar affinity to the IL-4R expressed by MLA-144, K_D 22-188 pM, but two of six samples expressed a high affinity receptor, K_D 293-549 pM. The IL-4R was initially thought to be composed

of either a single chain or a homodimer capable of transducing a signal after binding to IL-4.⁵ However, it has been revealed recently^{6,7} that the γ subunit of IL-2R, γ_c , constitutes a functional component of IL-4R (K_D 79 pM) although IL-4 binding to the IL-4R can be detected in the absence of γ_c (K_D 266 pM). The variability we have observed in the K_D of IL-4R species in B-CLL may be the result of variable expression of γ_c and may explain the heterogeneity of B-CLL proliferative responses to IL-4. In addition, the inhibitory effects of IL-4 and IL-2 induced proliferation of B-CLL cells described by Mainou-Fowler *et al* may be the consequence of competition between IL-4 and IL-2 for their common receptor subunit, γ_c . In this context it would be helpful to display the range of the proliferative response following incubation of B-CLL cells with IL-4 and IL-2 and to correlate this with the expression of the γ_c receptor.

MH GILLEECE
Senior Registrar
in Haematology,
Manchester Royal Infirmary,
Oxford Road,
Manchester M13 9WL

CM HEYWORTH
NG TESTA
TM DEXTER
Department of
Experimental Haematology,
Paterson Institute,
Manchester

- 1 Mainou-Fowler T, Copplestone JA, Prentice AG. Effect of interleukins on the proliferation and survival of B cell chronic lymphocytic leukaemia cells. *J Clin Pathol* 1995;48:482-7.
- 2 Gilleece MH, Heyworth CM, Testa NG, Dexter TM. Interleukin-4 receptor expression in chronic lymphocytic leukaemia. *Exp Haematol* 1993;21:233.
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Book reviews

Tissue Culture Techniques. An Introduction. Martin BM. (Pp 250; £34.) Public Relations Birkhauser Verlag A.G. 1994. ISBN 3-7643-3643-9.

One noticeable trend in scientific publishing over the past few years has been the advent of the plastic comb-bound laboratory manual, full of detailed step by step recipes which can be followed at the bench. No doubt the popularity of these books is partly explained by the explosion in the number and complexity of techniques which have been de-

veloped in biomedical science, and because of the reluctance of some authors, despite the pleas of journal editors, to provide adequate details of techniques in refereed publications. There have been a large number of extremely useful publications which have found a permanent position in many laboratories, but I fear that this book is unlikely to join them on the shelves above the laboratory bench.

This book arose from a basic tissue culture course given by the author at the Boston University School of Medicine. It does contain some recipes, but the book is mainly an exposition of the basic principles of cell culture. It covers the usual aspects of basic technique, including aseptic technique and sterility, routine manipulation of cell cultures, preparation of primary cultures, and cell preservation as well as some more advanced aspects, including cloning, transformation and transfection. There are brief appendices about the cell cycle, formulation of media and salt solutions and a list of vendors aimed at the North American market. In a number of places in the text there are sets of problems and exercises and a set of model answers. These problems are generally of a conceptual, but of a rather self evident nature—for example, "What do you do if you drop a tray of culture dishes and some of the lids fall off?"; answer: "After reacting to this disaster in your own personal manner, calm down..." and then "... throw them out!". There are some factual questions along the lines of "how many 500 ml bottles will it take to contain 10 litres of medium?"

There are a number of technical matters described in the book with which I do not agree—for example, pouring from one bottle into another and I would not use a gas flame for routine cell culture purposes in a class II biological safety cabinet. I also have little faith in ultraviolet lamps in culture hoods as effective surface sterilisers. It is impossible to know the ultraviolet (as opposed to visible blue light) output of a lamp and how quickly the output is deteriorating. A single grain of dust can cast a shadow which can shade many millions of bacteria from the ultraviolet light. There are also a number of important topics which a student learning cell culture for the first time should beware of and which are not covered. A major omission is any discussion of cell culture cross-contamination. This is certainly more common than most people realise and the practical measures needed to prevent this and to detect that it has occurred should form part of any introductory book on cell culture.

There is no doubt that there is a need for laboratory manuals which describe particular techniques in detail, together with a realistic description of what can go wrong and how to troubleshoot, primarily aimed at the relatively experienced bench scientist who has mastered a number of techniques and can appreciate when he or she is going wrong, but who wishes to try something new. There is also a need for introductory books for the novice but this area is already well catered for with a number of better produced books available, with more and considerably better illustrations. I wonder to whom this particular book is likely to appeal? It is very doubtful that someone who has never done cell culture could pick up this book and start and for those who already know the basics there is very little in this book which would be useful.

J L DARLING

Notices

FNA Cytology using the Cytospin method

October 9 1996

Venue: Royal Preston Hospital, UK

This course, costing £60.00 to include coffee, lunch and tea, is aimed at Consultants and trainees, and MLSOs involved in Cytopathology.

For further information, please contact: Dr A J Howat, Department of Histopathology, Royal Preston Hospital, Preston PR2 4HG, UK. Tel: 01772 710141; fax: 01772 710181.

United States FDA Medical Device Update: Design Controls, GMP Requirements and Marketing Clearance

20-23 May 1996

Venue: Charles de Gaulle Hilton, Paris, France

An international meeting on FDA GMP and marketing regulations including the proposed new GMP requirements and how to comply with them. All three days will be presented by FDA's CDRH with a presentation by the European Commission. Sponsored by the French Government.

For further information, please contact: Sharon Goff, Advanstar Communications, Advanstar House, Sealand Road, Chester CH1 4RN (Tel: 01244 378 888; fax: 01244 370 011.)

Australian Institute of Medical Scientists

National Science Meeting

October 6-11 1996

On behalf of the Organising Committee, I extend a warm invitation to all Medical Scientists, Technical Officers and interested parties, to attend the above conference to be held in the Convention Centre in Adelaide, South Australia.

The Conference theme is Aboriginal Health and will cover this and many other related topics and scientific endeavours.

Persons interested in taking part in the scientific program by presenting a paper or poster should contact: Mr John Stirling, c/o Histopathology Dept, Finders Medical Centre, Bedford Park, SA 5042.

For further information, please contact: SAPMEA Conventions, 80 Broughams Place, North Adelaide, SA 5006. (Tel: +618 239 1515; fax: +61 8 239 1566.)

Brian Matthews, Chairperson Organising Committee.