It is interesting to note that one of the early reports of small intestinal disease in Waldenström's macroglobulinaemia[5] describes a patient who also had abnormal coagulation tests, probably false positive syphilis serology, and who sustained a cerebral artery thrombosis one year after diagnosis of Waldenström's macroglobulinaemia. Two other reports of intestinal Waldenström's macroglobulinaemia also noted abnormal coagulation and false positive syphilis serology,[5 9] and in the second of these phospholipid was found in association with the extracellular immunoglobulin in the small intestinal lymphatics. Unfortunately, most reports of lymphoma associated paraproteins with lupus anticoagulant activity or phospholipid specificity do not document extent or localisation of disease. Cooper *et al*, however,[8] did describe such a case in which gastrointestinal symptoms were prominent.

This case highlights the potential importance of the antigen specificity, usually unknown, of monoclonal paraproteins and also the ever present, though poorly understood, paradoxical risk of thrombosis associated with lupus anticoagulant. It leads us to suggest the possibility of a link between lymphoproliferative disorders secreting phospholipid specific immunoglobulin and a tendency to affect the lymphatics draining the small bowel. The mechanism behind this is unclear but we speculate that this might relate to an association between antigen specificity and recirculation patterns of the abnormal lymphoid clone. The high phospholipid antigenic load to which gut associated lymphoid tissue is exposed would increase the chances of a lymphoproliferative disorder arising here and having some degree of phospholipid specificity. Alternatively, lymphomas with phospholipid specificity arising elsewhere may localise in the lymphatic draining system of the small bowel due to a high content of the antigen in these areas.

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1 Conley CL, Hartmann RC. A haemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Clin Invest* 1952; **31**:621–2.
2 Gastineau DA, Kazmier FJ, Nichols WL, Bowie EJW. Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases. *Am J Hematol* 1985;**19**:265–75.
3 Bellotti V, Gamba G, Merlini G, *et al*. Study of three patients with monoclonal gammopathies and 'lupus-like' anticoagulants. *Br J Haematol* 1989;**73**:221–7.
4 Thiagarajan P, Shapiro SS, De-Marco L. Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specifity. *J Clin Invest* 1980;**66**:397–405.
5 Cabrera A, de la Pava S, Pickren JW. Intestinal localisation of Waldenström's disease. *Arch Intern Med* 1964; **114**:399–407.
6 Harris M, Burton IE, Scarffe JH. Macroglobulinaemia and intestinal lymphangiectasia: a rare association. *J Clin Pathol* 1983;**36**:30–6.
7 Bedine MS, Yardley JH, Elliott HL, Banwell JG, Hendrix TR. Intestinal involvement in Waldenström's macroglobulinemia. *Gastroenterology* 1973;**65**:308–15.
8 Cooper MR, Cohen JH, Huntley CC, Waite BM, Spees L, Spurr CL. A monoclonal IgM with antibodylike specifity for phospholipids in a patient with lymphoma. *Blood* 1974;**43**:493–504.
9 Pruzanski W, Warren RE, Goldie JH, Katz A. Malabsorption syndrome with infiltration of the intestinal wall by extracellular monoclonal macroglobulin. *Am J Med* 1973;**54**:811–18.

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Terminal subculture of blood cultures using a multipoint inoculator device

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Abstract

A multipoint inoculation method was used for the terminal subculture of blood cultures. This economical yet reliable technique successfully isolated important human pathogens such as *Haemophilus influenzae* from macroscopically negative blood cultures.

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Detection of bacteraemia is one of the most important functions of a clinical microbiology laboratory and accounts for a large part of laboratory workload. Traditional blood culture systems, involving enrichment broths and visual inspection of bottles, are usually supplemented with subcultures on to agar at various intervals. An early subculture (less than 24 hours) and a terminal subculture are usually recommended.[1] The time consuming nature of subculture, together with the low yield of clinically important organisms and the relatively large number of agar plates required, has led some workers to conclude that terminal subculture of blood cultures may not be necessary.[2 3] It has been shown, however, that in many blood culture systems important human pathogens such as *Haemophilus influenzae* and *Neisseria meningitidis* produce no macroscopic signs of growth.[4] In an effort to resolve these problems and to make the process of terminal subcultures more economical we used a multipoint inoculation method.

Methods

A total of 20 ml of blood was collected from each patient and 10 ml inoculated into both 100 ml fastidious anaerobe broth (Lab M) and 100 ml Hartley's digest broth (Oxoid) with 0·1% added glucose. Cultures were incubated at 37°C and observed twice daily for signs of growth. Subcultures were performed from the fastidious anaerobe broth after 24 hours using conventional techniques. Cultures were incubated for three days at which time they had terminal subcultures using a multipoint inoculator device. Any cultures with appropriate clinical details (? bacterial endocarditis, ? fungi, HIV positive, ? brucellosis) had terminal subcultures done after seven days or longer.

Terminal subcultures were performed using a hand operated multipoint inoculator (Don Whitley Ltd) fitted with a 36 pin head which delivered 0·001 ml of fluid. Sterile plastic cupules were placed into each well of a 36-well PTFE block, and using a sterile plastic pipette about 0·5 ml of agitated blood culture broth was placed into a cupule. The position of each cupule was noted on a template. Thirty five broths can be accommodated on the block, one of the places being taken by a marker pin. The multipoint pins were sterilised by immersion in alcohol and then flamed. The broths were inoculated onto the following agars:

1 Columbia heated blood agar (Oxoid) with 5% horse blood.
2 Columbia agar (Oxoid) supplemented with 5% horse blood.
3 Fastidious anaerobe agar (Lab M) supplemented with 5% horse blood and 10 mg/l nalidixic acid (Pro lab diagnostics).
4 Mycoplasma ureaplasma differential (MUD) agar.[5]
5 Sabouroud's agar (Oxoid) supplemented with 50 mg/l chloramphenicol (Parke Davies).
6 Listeria selective agar (Oxoid).
7 Deoxyribonuclease agar (Oxoid).
8 MacConkey agar (Oxoid).

All plates were incubated for 48 hours at 37°C in air plus 6% CO_2 except Columbia blood agar, fastidious anaerobe agar, and MUD agar which were incubated in anaerobic conditions and Sabouroud's agar which was incubated in air at 30°C.

Results

The microbiology department receives about 4000 blood cultures a year from a wide variety of inpatients, including many who are immunocompromised. The multipoint method described has now been used for four years, since then 16 052 blood cultures have been received from which there were 1504(9·4%) clinically important isolates. Of these isolates 51(3·4%) were detected only after terminal subculture and are listed below with the number of isolates in parentheses. *Acinetobacter* spp (2); *Bacteroides asaccharolyticus* (1); *Bacteroides distasonis* (1); *Bacteroides fragilis* (2); *Campylobacter jejuni* I (2); *Candida albicans* (5); *Candida glabrata* (2); *Cryptococcus neoformans* (1); *Enterobacter agglomerans* (1); *Escherichia coli* (3); *Enterococcus faecalis* (1); *Fusobacterium necrogenes* (1); *Haemophilus aphrophilus* (1); *Haemophilus influenzae* (4); *Klebsiella pneumoniae* (1); *Peptostreptococcus asaccharolyticus* (1); *Propionibacterium acnes* (3); *Pseudomonas aeruginosa* (3); *Pseudomonas vesicularis* (1); *Staphylococcus aureus* (6); *coagulase negative Staphylococcus* (7); *Streptococcus mitis* (1); *Streptococcus sanguis* (1).

Discussion

This method is considerably cheaper than conventional subculture methods for blood cultures, both in terms of media used and staff time. The range of media which can be economically used is much wider and therefore is more likely to recover a wider range of organisms, although this depends ultimately on the culture broths being able to support the growth of such organisms. With the exception of MUD agar, all plates have proved useful for the rapid recognition of organisms, although it is quite possible that fewer plates could achieve the same results. The method is very flexible as different media can easily be added as required. Mixed cultures present little problem as the range of both selective and differential agars makes them easy to detect. Spreading *Proteus* spp presented no problem as these organisms were always positive before terminal subculture.

1 Shanson DC. Blood culture technique: current controversies. *J Antimicrob Chemother* 1990;**25**:(Suppl C)17–29.
2 Campbell J, Washington JA. Evaluation of the necessity for routine terminal subcultures of previously negative blood cultures. *J Clin Microbiol* 1980;**12**:576–8.
3 Gill VJ. Lack of clinical relevance in routine terminal subculturing of blood cultures. *J Clin Microbiol* 1981;**14**: 116–8.
4 Szymczak EG, Barr JT, Durbin WA, Goldmann DA. Evaluation of blood culture procedures in a pediatric hospital. *J Clin Microbiol* 1979;**9**:88–92.
5 Davis S, Eggington R. Recovery of Mycoplasma hominis from blood culture media. *Med Lab Sci* 1991;**48**:110–3.