are likely to have been valid.[4] It is doubtful whether the same can be said for those who have cultured in air or for shorter periods. We recommend the routine use of an atmosphere of hydrogen plus carbon dioxide in this field and incubation for not less than 48 hours.

J MARKS
A PAULL
Department of Surgery and
**Department of Medical Microbiology,*
University of Wales College of Medicine,
Heath Park, Cardiff CF4 4XN.

References

1 Sherris JC. Problems in in vitro determination of antibiotic tolerance in clinical isolates. *Antimicrob Agents Chemother* 1986;**30**: 663–7.
2 Stratton CW. The role of the microbiology laboratory in the treatment of infective endocarditis. *J Antimicrob Chemother* 1987;**20**(Suppl A):41–9.
3 Eykyn SJ. The role of the laboratory in assisting treatment—a review of current UK practices. *J Antimicrob Chemother* 1987;**20**(Suppl A):51–64.
4 Powley L, Meeson J, Greenwood D. Tolerance to penicillin in streptococci of viridans group. *J Clin Pathol* 1989;**42**:77–80.

Limulus amoebocyte lysate (LAL) assay and rapid detection of Gram negative bacterial peritonitis in patients receiving CAPD

The limulus amoebocyte lysate (LAL) assay is a sensitive method for the detection of bacterial endotoxin.[1] Recently two studies reported that the LAL assay may be of value in the diagnosis of Gram negative peritonitis in patients receiving continuous ambulatory peritoneal diagnosis (CAPD).[2,3] In a preliminary study we used the simple gel clot LAL assay on all peritoneal fluids sent to our laboratory over four months. On receipt of the CAPD bags in the laboratory, a 25 ml aliquot was removed aseptically from each bag. After a cell count had been performed a 20 ml aliquot was centrifuged at 2000 × *g* for 10 minutes. A Gram film was made from the deposit and the deposit inoculated on to CLED agar and horse blood agar which was incubated aerobically and anaerobically at 37°C. If the white cell count was greater than 50 mm^3, a 3–5 ml volume was inoculated into Bactec bottles. A 5 ml sample from all CAPD bags was stored at —20°C in a pyrogen-free tube and subsequently tested in the LAL gel clot assay. Briefly, 0·1 ml of fluid was added to 0·1 ml of reconstituted LAL (Pyrogent, Whittaker MA Bioproducts, Walkerville, Maryland, USA) in a pyrogen-free tube. The tubes were incubated undisturbed for 60 minutes in a water bath at 37°C and then examined for gel formation—a gel clot denoting a positive result and the presence of endotoxin. The sensitivity of the lysate was determined with an endotoxin standard (*Escherichia coli* 0111:B4 Whittaker MA Bioproducts, Walkersville, Maryland, USA) which was serially diluted with distilled water, unused peritoneal dialysate, and freshly exchanged uninfected peritoneal dialysate. The sensitivity of the lysate was 0·13 EU/ml (0·012 endotoxin units/ml = 1 pg of endotoxin/ml) in water and 0·25 EU/ml in peritoneal dialysate. It has been shown that a single Gram negative bacterium contains 40 fg of endotoxin.[4] Theoretically, therefore, the LAL assay can detect 500 bacteria/ml in peritoneal fluid. In comparison, for bacteria to be seen by a Gram stain, at least 10^5/ml are necessary.

A total of 72 fluids from 21 patients with suspected peritonitis were tested. Culture data were correlated retrospectively with the results of LAL assays. All isolates were cultured from both the deposit and Bactec bottles except one isolate of a coagulase negative staphylococcus (*S sciuri*) which was isolated from Bactec bottles alone. There were 12 culture positive episodes of peritonitis (table). Three episodes were caused by Gram negative bacteria and nine episodes by Gram positive bacteria or yeasts. There were no false positive results: all LAL assays were negative on CAPD fluids from patients with confirmed Gram positive or yeast peritonitis. Bacteria were not detected in the Gram film in two of the three episodes of Gram negative peritonitis. In six episodes of peritonitis, despite a raised white cell count, no bacteria were cultured. In three of these episodes the patients had antibiotics added to the bag before presentation at the clinic. In a further three suspected episodes the white cell count was less than five and culture was negative.

In this centre, empirical treatment for peritonitis in CAPD consists of gentamicin and vancomycin administered intraperitoneally. To preserve residual renal function it would be advantageous to avoid unnecessary administration of two potentially nephrotoxic antibiotics. Gram's stain is insensitive for detecting Gram negative organisms in peritoneal fluid,[5] and standard microbiological culture techniques may take 24–48 hours to identify the causative organism. Gram negative bacteria may be responsible for 10–45% of cases of CAPD peritonitis and are associated with an increased morbidity. Rapid detection of such episodes is desirable. The LAL assay can confirm the presence of Gram negative infection within one hour. The cost, including controls, of an individual assay is about £4·50.

Table *Limulus amoebocyte lysate assay of peritoneal fluids in 21 episodes of peritonitis*

	LAL assay	
Culture	*Positive*	*Negative*
E coli	2	
Acinetobacter sp	1	
Coagulase negative staphylococci		6
S aureus		1
Streptococcus faecium		1
Candida albicans		1
No growth		9

Our preliminary results suggest that the LAL assay may be useful (i) to identify patients with Gram negative peritonitis at the time of presentation and (ii) to avoid use of aminoglycosides in patients with Gram positive peritonitis.

Our findings agree in general with those of the two recent studies[1,2] but we did not have any false positive results. We favour the rapid LAL gel clot assay as used by Clayman *et al*.[2] This method has the advantages of simplicity and ease of performance; the chromogenic modification of the assay used by Smalley *et al* is technically more complex and consequently less suitable as a rapid simple routine test.[2] A further prospective study on a larger number of patients is in progress.

A CHRISTINE MCCARTNEY
JN BRUNTON
GL WARWICK*
Department of Microbiology
*and the *Renal Unit,*
Glasgow Royal Infirmary,
Glasgow G4 0SF

References

1 Levin J. The reaction between bacterial endotoxin and amoebocyte lysate. In: Cohen E, Bang FB, Levin J, *et al*. eds. *Biomedical applications of the horseshoe crab (Limulidae)*. New York: Alan R Liss Inc, 1979:131–46.
2 Smalley DL, Baddour LM, Kraus AP. Rapid detection of gram-negative bacterial peritonitis by the Limulus amoebocyte lysate assay. *J Clin Microbiol* 1986;**24**:882–3.
3 Clayman MD, Raymond A, Cohen D, *et al*. The Limulus amoebocyte lysate assay: a rapid and sensitive method for diagnosing early Gram-negative peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. *Arch Intern Med* 1987;**147**:337–40.
4 Novitsky TJ. Pyroburden. In: Novitsky TJ, ed. *LAL update*. Woods Hole, Massachusetts: Associates of Cape Cod Inc. 1984:5.
5 Knight KR, Polak A, Crump J, *et al*. Laboratory diagnosis and oral treatment of CAPD peritonitis. *Lancet* 1982;**ii**:1301–4.