Pseudomonas sp by the API 20NE system. Most of those, identified to genus level only, belonged to the *Ps aeruginosa/fluorescens* group. With both systems 27% of isolates were identified to genus level only.

The population of *Ps aeruginosa* isolates examined was shown on the basis of the API 20NE seven digit biotype to be relatively homogeneous. Ninety five isolates yielded 13 different biotypes, the three major biotypes accounting for 84% of the isolates. Nevertheless, these isolates identified as *Ps putida* by the Sensititre method included isolates of a wide range of different API 20NE-derived *Ps aeruginosa* biotypes.

Fifty isolates identified as *Ps aeruginosa* by the API 20NE system and recorded as positive in the API 20NE nitrate reduction test were selected for further investigation.[5,6] Positive test results for growth at 42°C (95%) and pigment production on King's A medium (90%) were recorded.

Eighty isolates were examined in duplicate to assess the reproducibility of the Sensititre system. With 43 (54%) of the isolates, duplicates were identified to the same species, and with an additional 11 (14%) isolates, duplicate identification to genus level only was obtained. Only with 28 of these isolates did duplicates yield the same biocode.

Ps aeruginosa is a major pathogen and any acceptable system must be able to identify this organism rapidly and accurately. Reproducibility of the system even to species level is inadequate, and reproducibility of the system at biocode level would provide confidence in individual test performance and could potentially provide valid data for epidemiological studies. The identification of many isolates as *Ps putida* by the Sensititre system indicates that a modification of the database for identification is necessary. This may involve differential selective calibration of the system, the modification of existing tests, or more radically, the introduction of new tests.

JG BARR
AM EMMERSON
GM HOGG
ETM SMYTH
Department of Bacteriology, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BA, Northern Ireland

References

1 Lampe AS, van der Reijden TJK. Evaluation of commercial test systems for the identification of non-fermenters. *Eur J Clin Microbiol* 1984;3:301–5.
2 Von Graevenitz A. Clinical role of infrequently encountered non-fermenters. In: Gilardi GL, ed. *Glucose non-fermenting Gram-negative bacteria in clinical microbiology*. West Palm Beach, Florida: CRC Press Inc, 1978:119–53.
3 Von Graevenitz A, Zollington-Iten J. Evaluation of pertinent parameters of a new identification system for non-enteric Gram-negative rods. *Eur J Clin Microbiol* 1985;4:108–12.
4 Barr JG, Hogg GM, Smyth ETM, Emmerson AM. Comparison of identification of enterobacteriaceae by API 20E and Sensititre Autoidentification system. *J Clin Pathol* 1989;42:649–52.
5 Phillips I. Identification of *Pseudomonas aeruginosa* in the clinical laboratory. *J Med Microbiol* 1969;2:9–15.
6 King EO. Two simple media for the demonstration of pyocyanin and fluorescein. *J Lab Clin Med* 1954;44:301–7.

Effect of penicillin on endocarditis strains of viridans streptococci

Survival counts on viridans streptococci after exposure to penicillin are required for the determination of minimum bactericidal concentrations, serum bactericidal titre, killing curves, synergy tests and demonstration of tolerance and the Eagle effect. Measurement of bactericidal activity is well known to be prone to experimental error, and there have been three recent reviews of methodology in this field.[1–3] Surprisingly, not one of these considered the influence of atmosphere on survival counts or recommended a suitable incubation period. The third review noted that most series of laboratories questioned incubated counts for 24 hours or less. Examination of a series of 14 unselected endocarditis strains of viridans streptococci showed that an atmosphere of hydrogen plus carbon dioxide and incubation for 48 hours was generally desirable. The table compares the survival counts of organisms in the stationary phase after exposure to benzyl penicillin at 32 MIC when cultured for 48 hours in air, in air plus 5% carbon dioxide, and in hydrogen plus 5–10% carbon dioxide (BBL Gaspak method, Becton Dickinson). Similar counts were made after exposure to 2 MIC to show the Eagle effect, and the whole study was repeated on organisms in the logarithmic phase.

The table shows that the best results were obtained by culturing survivors in hydrogen plus carbon dioxide, as might be expected with microaerophilic organisms (preliminary experiments showed that lowering oxygen tension itself improved survival). In this atmosphere 10 strains seemed to be tolerant to penicillin (less than 99·9% kill at 32 MIC) and two borderline (within 50% of the critical survival count). In air only six strains seemed to be tolerant and one borderline. The Eagle effect (represented here by twice as many survivors or more at 32 MIC than at 2 MIC) was seen in hydrogen plus carbon dioxide with nine strains and one borderline (over 50% increase at 32 MIC) compared with five strains in air. Carbon dioxide was slightly adverse to one strain (14) but not to the extent of changing its classification as tolerant.

In agreement with the results of other workers the incidence of tolerance and the Eagle effect was somewhat lower with organisms in the logarithmic phase but the influence of atmosphere was much the same.

Some research workers have incubated their cultures in air plus carbon dioxide for 48 hours and in such cases their conclusions

Table *Survival counts of endocarditis strains of viridans steptococci made in different atmospheres after exposure to 32 MIC benzyl penicillin*

Strain and speciation	*MIC mg/l*	*No of cfu at risk*	*Survival counts culturing in*		
			air	*air + CO_2*	*H_2 + CO_2*
1 *S milleri* II	·03	21 000	0	40	290
2 *S mutans*	·015	24 000	0	53	108
3 *S milleri* II	·03	13 000	5	150	300
4 *S mutans*	·015	24 000	15	110	150
5 *S salivarius*	·03	50 000	0	9	40
6 *S sanguis* II	·015	9 000	90	170	590
7 *S bovis* II.1	·03	10 500	31	93	230
8 *S sanguis* I.1	·03	6 000	65	370	520
9 *S bovis*	·06	3 000	26	37	41
10 *S bovis* I	·06	6 500	120	120	140
11 *S suis* II	·03	15 000	15	11	19
12 *S agalactiae*	·06	12 000	1	0	1
13 *S sanguis/mitis*	·007	33 000	2	1	2
14 *S sanguis* II	·03	7 500	61	25	22

Arranged roughly in order of influence of atmosphere.
The counts cited derived from 20 µl samples from reaction volumes of 360 µl in Nunc well plates using digest broth with 0·1% glucose. A 99·9% kill for strain 1 would have required 21 survivors and so on.

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.