



Figure Pre-pyloric area of 58 year old man with acute myeloblastic leukaemia showing presence of bizarre megakaryocytes and erythroid nests (inset).

abnormality. He died after a brief septicaemic illness.

Post mortem examination showed hepatosplenomegaly and generalised lymphadenopathy. The entire pre-pyloric region of the stomach was diffusely thickened, indurated, and yellow, but the pyloric lumen was not grossly narrowed.

Histological examination of the pre-pyloric area showed a heavy and diffuse polymorphous infiltrate within the submucosa and muscularis externa. The infiltrate was composed predominantly of myeloid cells at all stages of maturation, the commonest single cell type being myelocytes. There were also occasional bizarre megakaryocytes (figure) and erythroid nests (inset). The appearances were therefore those of myeloid metaplasia. The affected part of the stomach contained no distinguishable submucosal or myenteric ganglion cells. As no gross pyloric narrowing was seen at barium meal or at necropsy it is presumed that infiltration by haemopoietic cells of the pre-pyloric and pyloric smooth muscle led to a functional gastric outlet obstruction. Similar foci of myeloid metaplasia were evident in the liver, spleen, renal parenchyma and lymph nodes. Histological examination of the bone marrow showed the typical changes of myelofibrosis, but with an excess of primitive cells (10–15%). Residual leukaemia was thus clearly present in the bone marrow, but the gastric infiltrate composed of haemopoietic cells showing

trilineage differentiation by definition represented myeloid metaplasia rather than a leukaemic infiltrate. The aggressive behaviour of the pre-pyloric myeloid metaplasia seen in this patient is in keeping with current views of the neoplastic nature of myeloproliferative disorders<sup>2,3</sup>; its apparent rarity may well be related to lack of recognition. Infiltrative myeloid metaplasia should therefore be considered as a possible cause of unexplained symptoms in patients with myeloproliferative disorders, particularly as the lesion may be radiosensitive.<sup>1,4</sup>

S M ISMAIL,\*

\*Department of Histopathology,  
University Hospital of Wales,  
Heath Park,  
Cardiff CF4 4XW,

K MYERS,  
Department of Haematology,  
West Wales General Hospital,  
Carmarthen, Dyfed

References

- Glew RH, Haese WH, McIntyre PA. Myeloid metaplasia with myelofibrosis: the clinical spectrum of extramedullary haematopoiesis and tumour formation. *Johns Hopkins Med J* 1973;132:253–65.
- Jacobson RJ, Salo A, Fialkow PJ. Agnogenic myeloid metaplasia: a clonal proliferation of hemopoietic stem cells with secondary myelofibrosis. *Blood* 1978;51:189–94.
- Fialkow PJ, Faguet GB, Jacobson RJ, Vaidya K, Murphy S. Evidence that essential thrombocythaemia is a clonal disorder with origin

in a multipotent stem cell. *Blood* 1981;58:916–9.

- Lieberman PH, Rosvold RV, Ley AB. Extramedullary myeloid tumours in primary myelofibrosis. *Cancer* 1965;18:727–36.

API-20NE and Sensititre Autoidentification systems for identifying *Pseudomonas* spp

*Pseudomonas* species are the most common non-enteric Gram negative rods associated with infection, and of these, *Pseudomonas aeruginosa* is most often implicated.<sup>1,2</sup> Many clinical laboratories often undertake the identification of these organisms as a prelude to epidemiological studies, and in previous comparative studies the API 20NE system has yielded the highest rate of correct identifications and fewer incorrect identifications than other comparable systems.<sup>3</sup>

The Sensititre Autoidentification system (Sensititre Ltd) is based on fluorospectrophotometry and provides a fully automated method for the identification of Enterobacteriaceae, oxidase positive fermentative rods, and non-fermentative Gram negative rods. The identification of Enterobacteriaceae by the API 20E and Sensititre systems has recently been compared.<sup>4</sup>

The table gives the results of the identification of 140 isolates by the two systems. Species identification was obtained with 102 (73%) isolates with both systems. The API 20NE system yielded an identification of *Ps aeruginosa* for 95 isolates: the Sensititre system identified 43 isolates as *Ps aeruginosa* and 56 isolates as *Ps putida*.

The identification of *Ps aeruginosa* by the Sensititre system was confirmed by a similar identification for most isolates by the API 20NE system. Those isolates identified as *Ps putida* by the Sensititre system were predominantly identified as *Ps aeruginosa* or

Table Identification of 140 isolates of *Pseudomonas* sp with API 20NE and Sensititre Autoidentification systems

Organism	Number (%) of isolates yielding acceptable identification	
	API-20NE	Sensititre
<i>Species identification</i>		
<i>Ps aeruginosa</i>	95 (68)	43 (32)
<i>Ps putida</i>	0	56 (40)
<i>Ps fluorescens</i>	3 (2)	0
<i>Ps stutzeri</i>	2	0
<i>Ps maltophilia</i>	2	2
<i>Ps alcaligenes</i>	0	1
Genus identification only:		
<i>Pseudomonas</i> sp	38 (27)	38 (27)

*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.<sup>5,6</sup> 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 EMERSON  
GM HOGG  
ETM SMYTH  
Department of Bacteriology,  
Royal Victoria Hospital,  
Grosvenor Road,  
Belfast BT12 6BA,  
Northern Ireland

#### References

- 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.

- 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.
- 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.
- 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.
- Phillips I. Identification of *Pseudomonas aeruginosa* in the clinical laboratory. *J Med Microbiol* 1969;2:9-15.
- 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.<sup>1-3</sup> 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 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 the extent of changing its classification to 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 streptococci 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 <sub>2</sub>	H <sub>2</sub> + CO <sub>2</sub>
1 <i>S milleri</i> II	·03	21 000	0	40	290
2 <i>S mutans</i>	·015	24 000	0	53	108
3 <i>S milleri</i> II	·03	13 000	5	150	300
4 <i>S mutans</i>	·015	24 000	15	110	150
5 <i>S salivarius</i>	·03	50 000	0	9	40
6 <i>S sanguis</i> II	·015	9 000	90	170	590
7 <i>S bovis</i> II.1	·03	10 500	31	93	230
8 <i>S sanguis</i> I.1	·03	6 000	65	370	520
9 <i>S bovis</i>	·06	3 000	26	37	41
10 <i>S bovis</i> I	·06	6 500	120	120	140
11 <i>S suis</i> II	·03	15 000	15	11	19
12 <i>S agalactiae</i>	·06	12 000	1	0	1
13 <i>S sanguis/mitis</i>	·007	33 000	2	1	2
14 <i>S sanguis</i> 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.