

## Results

In one year 112 blood culture sets had early subcultures. Sixteen (14%), from 14 patients, subsequently yielded clinically significant isolates. Of these, 10 (63%), from eight patients, were detected on solid media by the following morning. None was detected by initial visual examination. A further 16 (14%) isolates were regarded as contaminating organisms and only one of these grew on solid media as a result of the early subculture (Table). The contamination rate in the blood cultures taken between 4 pm and 9 am on weekdays and all those at weekends, which were processed by the routine method (subcultured on days 1, 3, and 7) during the same one year period, was 7%.

## Discussion

Blood culture results are important to the clinician in the management of individual patients and so early recognition of positive cultures is desirable. With this aim in mind, several rapid techniques, including automation, have been examined.<sup>7</sup> But for a number of reasons, including cost, the widespread adoption of automated equipment may be limited, and conventional techniques will still be necessary in some routine clinical laboratories.

Although the numbers are small during the period under review, the early detection—that is, within 24 h of receipt—of 10 (63%) significant isolates from eight bacteraemic patients made an appreciable contribution to the patients' management and compares well with the later detection of an additional four grown after subculture the day after receipt. In turn, this early detection resulted in the reporting of antimicrobial sensitivities 24 h earlier. Inevitably, the accompanying increased opening of the blood culture bottles produced a rise in contaminating organisms, thus increasing laboratory workload, although some of these would have been introduced at the time of collection of blood from patients. Silva and Washington<sup>5</sup> detected only two of

20 (10%) isolates after incubation for 1–6 h and recommended routine subculture after 6–17 h. If this was followed many blood cultures received during the working day would not be subcultured until the following morning in laboratories which perform routine work only between 9 am and 5 pm. Ganguli *et al*<sup>6</sup> avoided this by subculturing at 10 pm on the day of receipt. Although 119 of 237 bacteraemic patients were detected within 24 h by this practice, it may place an extra cost and burden on technical and medical staff outside the normal laboratory working day. Two other studies<sup>3,4</sup> found 85% and 40% of significant cultures after 4–14 h and 3–19.5 h incubation respectively, but the authors do not state the time of day that subcultures were performed.

We believe that by subculturing both in the morning and at 4 pm—that is, during normal laboratory hours—earlier detection of bacteraemia can be achieved. This regimen employs conventional microbiological techniques without the expense of new equipment, reagents, and out of hours work.

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## Letters to the Editor

### Annual and seasonal variation in the frequency of $\beta$ -haemolytic streptococcal infections

Dr Millar<sup>1</sup> reports that in Yorkshire over the past five years there has been a significant increase in the frequency with which streptococci of Lancefield group C have been found. In Shropshire, where the

population is principally rural or semi-rural, our experience has been different. There have been only modest fluctuations from year to year with no consistent pattern of change (Tables 1–4). We have, however, in common with others in the United Kingdom, noted a seasonal incidence in outbreaks of streptococcal skin sepsis among meat handlers<sup>2</sup>; this is an

occupational hazard directly related to the peak period in the autumn for animal slaughtering and processing, and is typically associated with group A streptococci.

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Table 1 Frequency of  $\beta$ -haemolytic streptococcal isolates from all sites

| Group | 1979 |    | 1980 |    | 1981 |    | 1982 |    | 1983 |    | 1984* |    |
|-------|------|----|------|----|------|----|------|----|------|----|-------|----|
|       | No   | %  | No   | %  | No   | %  | No   | %  | No   | %  | No    | %  |
| A     | 599  | 43 | 541  | 41 | 581  | 41 | 614  | 39 | 722  | 39 | 520   | 38 |
| B     | 242  | 17 | 302  | 23 | 365  | 25 | 424  | 27 | 557  | 30 | 417   | 30 |
| C     | 242  | 17 | 210  | 16 | 147  | 10 | 228  | 14 | 211  | 11 | 165   | 12 |
| G     | 287  | 20 | 244  | 18 | 301  | 21 | 290  | 18 | 356  | 19 | 263   | 19 |
| F     | 24   | 2  | 14   | 1  | 28   | 2  | 18   | 1  | 6    | 0  | 1     | 0  |
| Total | 1394 |    | 1311 |    | 1422 |    | 1574 |    | 1852 |    | 1366  |    |

A year = 1 January–31 December

Table 2 Frequency of  $\beta$ -haemolytic streptococcal isolates from respiratory swabs†

| Group | 1979 |    | 1980 |    | 1981 |    | 1982 |    | 1983 |    | 1984* |    |
|-------|------|----|------|----|------|----|------|----|------|----|-------|----|
|       | No   | %  | No   | %  | No   | %  | No   | %  | No   | %  | No    | %  |
| A     | 493  | 55 | 407  | 58 | 441  | 58 | 416  | 56 | 513  | 58 | 391   | 58 |
| B     | 82   | 9  | 71   | 10 | 82   | 11 | 92   | 12 | 102  | 11 | 83    | 12 |
| C     | 175  | 19 | 119  | 17 | 85   | 11 | 122  | 16 | 130  | 15 | 105   | 15 |
| G     | 130  | 14 | 101  | 14 | 132  | 17 | 109  | 14 | 135  | 15 | 98    | 14 |
| F     | 11   | 1  | 7    | 1  | 12   | 1  | 9    | 1  | 1    | 0  | 0     | 0  |
| Total | 891  |    | 705  |    | 752  |    | 748  |    | 881  |    | 677   |    |

†Throat, ear, and nose swabs and sputum

Table 3 Frequency of  $\beta$ -haemolytic streptococcal isolates from wound swabs

| Group | 1979 |    | 1980 |    | 1981 |    | 1982 |    | 1983 |    | 1984* |    |
|-------|------|----|------|----|------|----|------|----|------|----|-------|----|
|       | No   | %  | No   | %  | No   | %  | No   | %  | No   | %  | No    | %  |
| A     | 95   | 25 | 116  | 27 | 121  | 28 | 169  | 29 | 177  | 30 | 97    | 25 |
| B     | 73   | 20 | 107  | 25 | 107  | 25 | 157  | 27 | 143  | 25 | 100   | 26 |
| C     | 57   | 15 | 75   | 18 | 47   | 11 | 97   | 16 | 68   | 12 | 54    | 14 |
| G     | 136  | 37 | 124  | 29 | 141  | 33 | 154  | 26 | 187  | 32 | 134   | 35 |
| F     | 10   | 3  | 4    | 1  | 9    | 2  | 7    | 1  | 4    | 1  | 1     | 0  |
| Total | 371  |    | 426  |    | 425  |    | 584  |    | 579  |    | 386   |    |

Table 4 Frequency of  $\beta$ -haemolytic streptococcal isolates from high vaginal swabs

| Group | 1979 |    | 1980 |    | 1981 |    | 1982 |    | 1983 |    | 1984* |    |
|-------|------|----|------|----|------|----|------|----|------|----|-------|----|
|       | No   | %  | No   | %  | No   | %  | No   | %  | No   | %  | No    | %  |
| A     | 11   | 8  | 18   | 10 | 19   | 8  | 29   | 12 | 32   | 8  | 32    | 11 |
| B     | 87   | 66 | 124  | 69 | 176  | 72 | 175  | 72 | 312  | 79 | 234   | 77 |
| C     | 10   | 7  | 16   | 9  | 15   | 6  | 9    | 4  | 13   | 3  | 6     | 2  |
| G     | 21   | 16 | 19   | 10 | 28   | 11 | 27   | 11 | 34   | 9  | 31    | 10 |
| F     | 3    | 2  | 3    | 2  | 7    | 3  | 2    | 1  | 1    | 0  | 0     | 0  |
| Total | 132  |    | 180  |    | 245  |    | 242  |    | 392  |    | 303   |    |

\*Nine month period (January–September) only for 1984.

## References

<sup>1</sup> Millar M. High incidence of group C streptococci isolated from throat swabs. *J Clin Pathol* 1984;37:1314.

<sup>2</sup> Public Health Laboratory Service Working Group. The epidemiology and control of streptococcal sepsis in meat handlers. *Environmental Health* 1982;90:256–8.

## Separation of lymphocytes from peripheral blood: a revisitiation

Have we lost sight of the fact that, although separation of mononuclear cells from peripheral blood by centrifugation over density gradients such as Ficoll-Paque has been and no doubt will continue to be useful, at least half of the lymphocyte population of normal blood samples is lost during this procedure? Recently, we found that our mean recovery of lymphocytes from 72 normal subjects was only  $40.2 \pm 15.9\%$  (mean  $\pm$  SD) using 35 min centrifugation at room temperature (Markey *et al*; unpublished observations). The manufacturers of Ficoll-Paque, which we use, claim a recovery rate of lymphocytes from normal samples of blood of  $50 \pm 15\%$  (mean  $\pm$  SD).

We have performed a small study using blood samples from normal laboratory staff. Each sample was divided into four aliquots, (a), (b), (c), and (d), and these were separated as follows:

(a) Centrifugation (at 400 g) for 35 min at 4°C in a refrigerated centrifuge using Ficoll-Paque removed from a 4°C refrigerator immediately before use—that is, Ficoll-Paque at 4°C.

(b) Centrifugation (at 400 g) for 15 min at 19–21°C (room temperature); Ficoll-Paque at 4°C.

(c) Centrifugation (at 400 g) for 35 min at room temperature; Ficoll-Paque at 4°C.

(d) Centrifugation (at 400 g) for 35 min at room temperature; Ficoll-Paque equilibrated with room temperature before use.

The recovery of mononuclear cells and of lymphocytes is shown in the Table. Recovery of lymphocytes was greatest when Ficoll-Paque was allowed to equilibrate with room temperature before use ( $p = 0.001$  Student's *t* test). The recovery of cells was greatest when centrifugation time was shortened to 15 min, but this was because of the presence of a high proportion of monocytes and polymorphonuclear leucocytes. The proportion of non-lymphocytes present in the suspensions was significantly less ( $p = 0.01$ ) under conditions (d)—that is, centrifugation at room temperature for 35 min with Ficoll-Paque.

Attention to the detail of allowing sufficient time for equilibration of Ficoll-Paque with room temperature has improved our recovery rate. For the past 6 months our recovery of lymphocytes from normal blood has been  $56.7 \pm 13.5$ , which is significantly higher ( $p = 0.001$ , Mann Whitney U test) than previously. The manufacturers of Ficoll-Paque recommend that it be stored between 4°C and 25°C in the



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