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.[7] 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[5] 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*[6] 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[3,4] 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 β-haemolytic streptococcal infections

Dr Millar[1] 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[2]; 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 β-haemolytic streptococcal isolates from all sites*

Group	1979 No	1979 %	1980 No	1980 %	1981 No	1981 %	1982 No	1982 %	1983 No	1983 %	1984* No	1984* %
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 β-haemolytic streptococcal isolates from respiratory swabs*†

Group	1979 No	1979 %	1980 No	1980 %	1981 No	1981 %	1982 No	1982 %	1983 No	1983 %	1984* No	1984* %
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 β-haemolytic streptococcal isolates from wound swabs*

Group	1979 No	1979 %	1980 No	1980 %	1981 No	1981 %	1982 No	1982 %	1983 No	1983 %	1984* No	1984* %
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 β-haemolytic streptococcal isolates from high vaginal swabs*

Group	1979 No	1979 %	1980 No	1980 %	1981 No	1981 %	1982 No	1982 %	1983 No	1983 %	1984* No	1984* %
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.

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Separation of lymphocytes from peripheral blood: a revisitation

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 ± 15·9% (mean ± 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 ± 15% (mean ± 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 ± 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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