Letters to the Editor

Table 1 Frequency of β -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 β -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 β -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 β -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.

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subjects was only $40.2 \pm 15.9\%$ (mean $\pm$ SD) using 35 min centrifugation at room temperature (Markey *et al.*; unpublished observations).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 ± 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

References

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

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

Total cell and lymphocyte recovery from peripheral blood by differential centrifugation over Ficoll-Paque under different conditions of separation

	(a)	(b)	(c)	(d)
Cell recovery %				
Mean ($\pm$ SD)	52.5 ($\pm$ 15.7)	92 ($\pm$ 3.7)	69.2 ($\pm$ 11.5)	85.5 ($\pm$ 7.6)
Lymphocyte recovery %				
Mean ($\pm$ SD)	38.5 ($\pm$ 11.5)	52.8 ($\pm$ 6.7)	51 ($\pm$ 9.1)	65.8 ($\pm$ 8.2)

(a) = Ficoll-Paque 4°C; centrifugation 35 min at 4°C

(b) = Ficoll-Paque 4°C; centrifugation 15 min at room temperature

(c) = Ficoll-Paque 4°C; centrifugation 35 min at room temperature

(d) = Ficoll-Paque room temperature; centrifugation 35 min at room temperature.

dark. While we have been storing it unopened at room temperature, once opened we keep it in a 4°C domestic refrigerator to minimise the possibility of bacterial growth. We believe that many laboratories will be following this standard bacteriological practice and that it is worthwhile to draw their attention to the detail of making sure that equilibration of the Ficoll-Paque with room temperature is allowed to occur.

It is interesting to note that Coulter Clone recommend that separation of mononuclear cells be performed at 4°C over Ficoll-Paque,¹ and they state that the procedure was provided by Drs Schlossman, EL Reinhertz, and LM Nadler. In our hands, separation at this temperature was significantly inferior to each other modification. Likewise, we did not find shortening of centrifugation time to 15 min helpful as have others²; the recovery of lymphocytes was less and that of monocytes and polymorphonuclear leucocytes greater.

In conclusion, we have improved our lymphocyte recovery rate by this simple step. Nevertheless, there is still a deficit of 44%. It seems that major lymphocyte subset proportions are relatively accurately portrayed in the 50% or so of cells which is routinely recovered. There is no guarantee, however, that minor sub-sets and sub-sets in altered states of activation are not either selectively lost or, alternatively, selectively recovered. Therefore interpretation of profiles delivered by sophisticated technology such as fluorescent activated cell sorters may be inexact. Equally, to confine analysis of minor sub-sets only to those samples with a high recovery may not produce a representative picture. Perhaps it is time to direct further effort towards improving cell recovery before results obtained from an incomplete census of the lymphocyte population in normal subjects are indelibly printed not only in bulk on paper but also in our minds.

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- ¹ Coulter Clone. Monoclonal antibodies. *Coulter Immunology* 1983:1.
- ² Hicks MJ, Jones JF, Thies AC, Minnich LL. The effect of lymphocyte recovery on lymphocyte typing results. *Am J Clin Pathol* 1981;76:745-52.

When is it an osteoclast?

We read with interest Dr Kaye's recent paper¹ on the problems encountered in the identification of osteoclasts in tissue (bone) sections when conventional histological

and cytochemical techniques are used. Dr Kaye correctly emphasises that cellular multinuclearity, characteristic of osteoclasts, can often be proved only on serial sectioning. Moreover, conventional methods cannot differentiate between osteoclasts and cells of the mononuclear phagocyte series and are incapable of positively identifying mononuclear osteoclasts.

Our approach to such problems of cell identification, and hence enumeration, is the use of monoclonal antibody techniques. We have recently derived a series of hybridomas against human giant cell tumours of bone (osteoclastomas) which secrete antibodies specific for osteoclasts; further these antibodies fail to react with macrophages and thus effectively differentiate between these cell types (Horton *et al.* unpublished observations). A total of eight antibodies in six reactivity groups recognise osteoclast membrane antigens; a further two clones secrete antibodies to osteoclast restricted cytoplasmic determinants. The Figure illustrates typical specific membrane reactivity of monoclonal 23C6 (A4) on frozen section of giant cell tumour, visualised by the immunoperoxidase technique.

Although our antibodies fail to react in formalin fixed tissue, they do for the first time allow an approach to the specific detection of osteoclasts in bone and in other pathological material. Thus, any cell showing reactivity with osteoclast specific antibodies, whether mono- or multinucleate, can be categorised as an osteoclast. We predict that these and other monoclonal

Frozen section of osteoclastoma stained by immunoperoxidase technique using monoclonal antibody 23C6 (A4) showing membrane reactivity on multinucleate cells. $\times 390$.