Results and conclusion

A total of 5368 donors were screened. Fourteen of these (one in 383) had >10 IU/cm³, the minimum level required by the Scottish Blood Transfusion Service, and 11 (one in 488) had >15 IU/cm³, the level currently required by the Blood Products Laboratory for production of immunoglobulin. Our results were independently confirmed by the Blood Products Laboratory.

The method provides an easy, rapid, and economic way of screening large numbers of donors to find those with antibodies to HBsAg. These donors could then be further considered for possible plasmapheresis, with or without boosting of the antibody by hepatitis B vaccine. The method also provides a ready means, both of screening medical and other staff who may have been exposed to

hepatitis B virus before vaccination and of monitoring the progress of any vaccination undertaken.

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Letters to the Editor

Clostridium difficile and *Clostridium perfringens* in upper gut of infants with protracted diarrhoea

Although the role of *Clostridium difficile* in the pathogenesis of colitis is well established,¹ a possible role for *C. difficile* in the pathogenesis of chronic diarrhoea in children without colonic disease has been suggested.² On the other hand, the presence of *C. difficile* in the small bowel has only once been reported in an elderly person with chronic diarrhoea.³ We report here the presence of *C. difficile* in the upper gut of infants with chronic diarrhoea, which suggests that the organism has a potential pathogenic role in this part of the gastrointestinal tract.

We examined two groups of children to try to identify a role, if any, for clostridia in the upper gut of children: group 1 comprised 19 patients (mean age 19 months, range two to 48 months) presenting with protracted diarrhoea—that is, longer than two weeks—with no obvious cause at time of sampling; and group 2 comprised 10 control children (patients with coeliac disease in full clinical remission while taking a gluten free diet, mean age 39 months, range 18 to 59 months).

In all cases duodenal juice was collected under sterile conditions between the second and fourth part of the duodenum. Serial 10 fold dilutions of samples of duodenal juice were performed in an anaerobic cabinet and bacteriological cultures seeded on to both selective and non-selective media.

No clostridia were detected in any of the 10 controls, but they were found in six of the 19 patients with chronic diarrhoea: in four cases *Clostridium difficile*, in one case *Clostridium perfringens*, and in one case both, with a bacterial count ranging between 10^2 and 10^5 colony forming units/ml. Only one of the six patients had received treatment with antibiotics in the preceding two months.

Four patients had an eventual diagnosis of a definite gastrointestinal disease; interestingly, the remaining two were the only ones of the 19 patients who merely presented with the so called "postgastroenteritis diarrhoea."

To conclude, our findings, although needing further experimental evidence, suggest a potential pathogenic role for *C. difficile* and *C. perfringens* in the small bowel in children.

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Expression of interleukin 2 receptor on Hodgkin's and non-Hodgkin's lymphomas and macrophages

Interleukin 2, formerly termed T cell growth factor, is an important regulator of T cell activation. Until recently it was assumed that Interleukin 2 was produced by T lymphocytes and acted exclusively on cells of the same lineage. There are now numerous reports, however, describing the expression of the interleukin 2 receptor (Tac antigen) on leukaemic (hairy cell leukaemia)¹ and activated B lymphocytes.² Applying a sensitive immunoenzymatic staining procedure (alkaline phosphatase/antialkaline phosphatase staining) on frozen tissues and cytopreparations, our data fully support the relation of the Tac antigen to the B cell lineage. Moreover, almost all non-Hodgkin's lymphomas of low grade malignancy, including lymphocytic leukaemia of the B cell type, expressed the interleukin 2 receptor, whereas those high grade malignancy did not.

In most cases of Hodgkin's disease Hodgkin and Reed-Sternberg cells showed membrane staining with monoclonal antibodies against the interleukin 2 receptor. Surprisingly, positive reactivity with the anti-Tac antibody was also shown by most tissue macrophages and a variety of monocytoic cells. Starry sky macrophages in lymphoid tissue as well as the granulomas formed in patients with sarcoidosis of the lung showed Tac positivity. Most strikingly, two leukaemic cell lines (U 937 and HL-60) that are known to show macrophage features after having differentiated under the influence of a tumour promoter became positive for Tac after induction of TPA. In the skin the anti-Tac antibody reacted consistently with sweat gland epithelium. Thus the monoclonal antibodies against the interleukin-2 receptor react with at least three different cell lineages, also including non-lymphoid cell lineages. Clinical trials with purified or recombinant interleukin 2 should take into account the broader distribution of the Tac antigen.

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EDTA plasma rather than serum for B₁₂, folate, and ferritin estimations?

To establish the cause of macrocytic or microcytic red cells it is sometimes necessary to undertake vitamin B₁₂ and folate assays or an estimate of iron stores. Most laboratories use serum for the estimation of B₁₂, folate, and ferritin when a peripheral blood abnormality in ethylene diamine tetra-acetic acid (EDTA) anticoagulated blood has been found. A further sample of clotted blood is then requested as the abnormality was usually unsuspected at the time of the initial venepuncture. On most of these occasions, however, excess EDTA plasma is

available, unused, and unwanted in the original sample tube. Such plasma is, we suggest, entirely suitable for radioisotopic estimation of B₁₂, folate, or ferritin.

We compared plasma and serum B₁₂, folate, and ferritin assays in blood from 56 National Blood Transfusion Service donors. A 4 ml dipotassium EDTA anticoagulated bottle and a 10 ml plain glass bottle were filled after 450 ml venesection, and the samples were sent to arrive in the laboratory within six hours. The anticoagulated blood was processed in a Model S Plus blood count analyser (Coulter), and excess plasma and separated serum were then placed in plastic tubes, frozen, and stored at -20°C to be assayed within one week.

B₁₂, folate, and ferritin assays of plasma and serum were performed in duplicate using the B₁₂/folate Combostat II radioassay kit (Micromedical Systems, Hersham, England)¹ and the Micromedical ferritin radioassay kit. A close correlation between plasma and serum B₁₂, folate, and ferritin concentrations was found ($r = 0.98, 0.96, 0.97$, respectively) across the wide range of values represented in the donors studied (B₁₂ 95-900 ng/ml, folate 1.25-10.5 µg/l, and ferritin 100-500 µg/l). Furthermore, no consistent differences in values measured in plasma or serum could be detected using the paired *t* test. We were, therefore, unable to confirm the reported finding of consistently lower B₁₂ and folate measurements in plasma as opposed to serum with the Simultrac radioassay kit (Becton and Dickinson (UK), Wembley, Middlesex).² The accuracy and precision of measuring these three variables in plasma and serum were investigated by duplicate testing of paired plasma and serum samples from 10 healthy volunteers and replicate analysis of 11 measurements on a single plasma or serum sample, and no significant differences were apparent.

We believe our results should encourage others to exploit the advantages of measuring B₁₂, folate, and ferritin in the excess plasma remaining in the original "abnormal blood count" tube, which would obviate the need to request generation of a

further serum sample. Plasma assays relate directly to the sample in which the blood count abnormality was found, which can never be the case with serum samples. Much may happen clinically and therapeutically between showing an abnormal EDTA blood count and the subsequent arrival of a clotted blood sample in the laboratory. Indeed, failure to receive a serum sample is by no means uncommon. In a study of the importance of macrocytic red cells in patients attending a university hospital serum samples for B₁₂ and folate assays were obtained from only 48% of relevant patients despite specific requests by the laboratory for generation of samples for assays.³

In practice the ready availability of plasma B₁₂, folate, and ferritin estimations in our laboratory enables us as haematologists to comment more meaningfully on the importance of abnormal blood counts without the need to seek further samples. The potential hazards associated with delays are avoided, and we hope that the chances of belated recognition and thus treatment of B₁₂ deficiency in particular should be greatly reduced. We believe that plasma estimations greatly enhance the haematology laboratory's contribution to clinical care.

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Duplicate assay testing and replicate analysis of B₁₂, folate, and ferritin in paired plasma and serum samples

	Duplicate testing (SD)		Replicate testing (coefficient of variation)	
	Plasma	Serum	Plasma	Serum
B ₁₂ (ng/l)	15.1	15.4	3.4%	5.3%
Folate (µg/l)	0.08	0.05	5.6%	4.3%
Ferritin (µg/l)	3.24	1.27	6.5%	5.9%