

Table 1 Mean (SD) fluorescence obtained by two anti-D monoclonal antibodies with different red blood cells of known genotypes R^1R^1 , R^2R^2 , R^1r , R^2r

Antibody	Genotype			
	R^2R^2 (n 16)	R^2r (n 18)	R^1R^1 (n 14)	R^1r (n 19)
IgG ₁ 1λ anti-D H2D5D2 (25 μg/ml)	230 (50)	140 (20)	120 (30)	80 (10)
IgG ₁ K anti-D QA37C3 (5 μg/ml)	190 (40)	115 (20)	100 (20)	60 (10)

Table 2 Possible genotypes (control cells) compared with those of an RhD positive father

Genotype of control red blood cells	Mean fluorescence
R^0r^1	135
R^1R^0	200
R^1R^1	130
R^1r	85
rr	<5
Case 1	80

This man is a D + C + c + E - e + phenotype. Two genotypes are possible; R^1R^0 ; R^0r^1 . The cyto-fluorometric analysis data are compatible with heterozygous status. The child was rr^1 (D - C + c + E - e +).

mined conventionally was always consistent with the data from flow cytometry. Table 2 shows an example of a patient studied in our laboratory. For each case we used red blood cell controls corresponding to the possible genotypes.

Discussion

The technique outlined seems suitable for studying the zygosity of a given red cell character and makes it possible to determine if an Rh (D) positive subject is genetically $D//D$ or $D//d$ (or $D//-$). The process is simple and based on antibody binding. The same results were obtained with fresh and thawed red cells. All previous simple methods have depended on aggluti-

nation, where only the kinetics of the reaction are correlated with genotype.

The method described relies on two things. First, the use of monoclonal antibodies of high and homogenous affinity; and secondly, the use of flow cytometry, which allows direct quantitation of the amount of bound antibody.

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Simple reagent modification of Technicon H6010C that permits processing of uraemic samples

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The Technicon H6010C performs an automated differential leucocyte count. Leucocytes are classified

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according to their size and reaction with cytochemical dyes. This classification relies on prior lysis of erythrocytes. Blood samples from patients with a raised concentration of urea (> 15 mmol/l (90 mg/100 ml)) cannot be processed on the analyser because the erythrocytes will not lyse using standard Technicon reagents. Where lysis is incomplete, the instrument records a spuriously high leucocyte count and an increased number of cells classified as lymphocytes and "large unstained cells."

In hospitals with large renal units this may result in a considerable number of samples having to be processed on instruments that do not have this limitation. Alternative instruments, however, do not provide a full differential leucocyte count.

Technical methods

The H6010C reagent system was examined in detail to see if the chemistry could be modified to enable processing of uraemic samples without any special preparation. Preliminary investigation of these samples showed that substitution of plasma with saline overcame the problem. It was thought that if a substance capable of degrading urea in plasma was introduced into the reagent system this too could be successful in overcoming the problem.

Material and methods

Urease, with pH and temperature optima of 7.5 and 55°C, respectively, was an obvious choice, as similar physical conditions are used on the alkaline peroxidase manifold of the H6010C. Jack Bean urease was obtained from Sigma Chemicals. The enzyme was added in concentrations ranging from 3.2 to 160 micromolar units/ml to standard Technicon working alkaline peroxidase diluent and sample diluent and to several modified sample diluents designed to enhance its activity.

Results

The enzyme was found to be effective in overcoming the problem when added to either Technicon sample

diluent or working alkaline peroxidase diluent. It was quite unstable, however (50% loss of activity after 60 minutes at room temperature), in standard sample diluent with a pH of 4.5. Although this problem was overcome using various modified formulations of sample diluent, all of the modifications tested had an undesirable effect in either the red blood cell/platelet or Basophil manifolds. It was concluded, therefore, that addition of the enzyme to alkaline peroxidase diluent, which had no adverse effect on the instrument, was the best approach. A urease concentration of 16 micromolar units/ml was found to be adequate for samples with urea concentrations up to 60 mmol/l (360 mg/100 ml). Figs 1 and 2 give a comparison of the results obtained with a uraemic sample processed with the standard reagent system and added urease.

Discussion

The effectiveness of urease when added to working alkaline peroxidase diluent was surprising in view of the fact that shortly after (75 seconds) the sample is mixed with alkaline peroxidase diluent, a fixative reagent containing 8 g/l formaldehyde. Whether the urease is able to degrade urea in the sample within 75 seconds at ambient temperature or whether it remains active in the presence of formalin at 56°C is unclear.

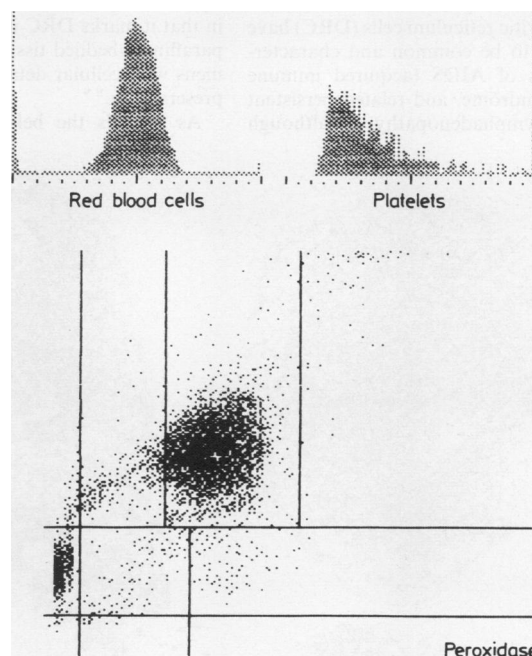
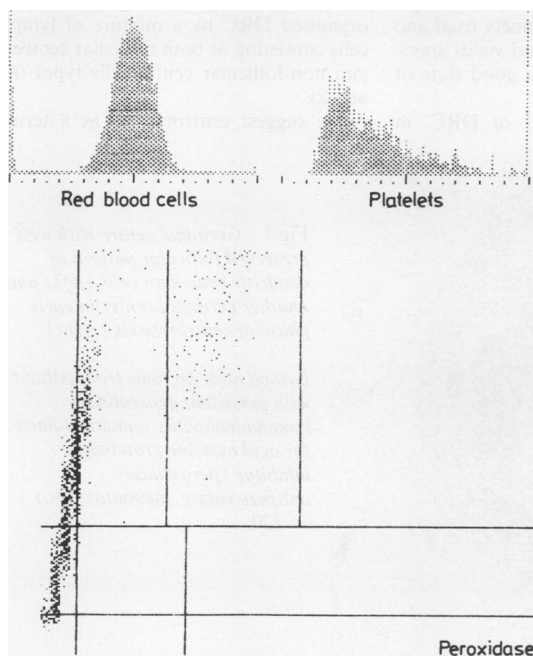


Fig 1 Standard reagent system. Note predominance of small and peroxidase negative cells to left and bottom of scattergram.

Fig 2 Reagent system with added urease. Note normal scattergram pattern.

We performed some preliminary experiments to show that urease at room temperature and the concentration used would not be capable of degrading the urea in a sample containing 15–50 mmol (90–360 mg/100 ml) urea. We were also able to show that urease can function in the presence of 3.6 g/l formaldehyde at 56°C, these being the conditions in the peroxidase fixation bath.

Working alkaline peroxidase diluent containing urease has been used in this laboratory for the past six months without any apparent adverse effects on the Technicon H6010C. Addition of the urease has doubled the cost of alkaline peroxidase diluent and added an additional 0.005 Australian dollars (0.5 cents) to the cost of processing each sample when the

semi-stat option is used.

It should be noted that although erroneous results obtained for grossly uraemic samples using the standard reagent system are easily recognised, invalid results for mildly uraemic samples (with only a slight spurious increase in leucocyte and lymphocyte counts) could easily be overlooked. Use of urease overcomes this problem and is recommended for use in the H6010C when its throughput includes blood samples for uraemic patients.

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Letters to the Editor

Centrorrhexis: a "new" reaction pattern of lymph nodes

Disruption and reorganisation of the network constituted by the follicular dendritic cells or dendritic reticulum cells (DRC) have been shown to be common and characteristic features of AIDS (acquired immune deficiency syndrome, and related persistent generalised lymphadenopathy,^{1–6} although

not specific.⁴ We immunostained lymph node tissues from 12 cases of persistent generalised lymphadenopathy (all came from the metropolitan area of Los Angeles and fulfilled the criteria for persistent generalised lymphadenopathy given by the Centers for Disease Control)⁷ by using antiserum raised against acid cysteine proteinase inhibitor (ACPI, isolated and purified from human squamous epithelia).⁴ The method is unique in that it marks DRC in routinely fixed and paraffin embedded tissues, and yields specimens with cellular detail in a good state of preservation.^{8,9}

As regards the behaviour of DRC, in

most cases of persistent generalised lymphadenopathy some or many germinal centres exhibit profound changes that can be summarised as follows: disruption and fragmentation of the normal follicular DRC pattern; haphazardly organised DRC, which tightly embrace groups of follicular centre cells; hypertrophy and intense ACPI immunoreactivity of occasional DRC; and infiltration between the groups of newly organised DRC by a mixture of lymphoid cells consisting of both follicular centre cells and non-follicular centre cells types (Figs 1 and 2).

We suggest centrorrhexis as a term for

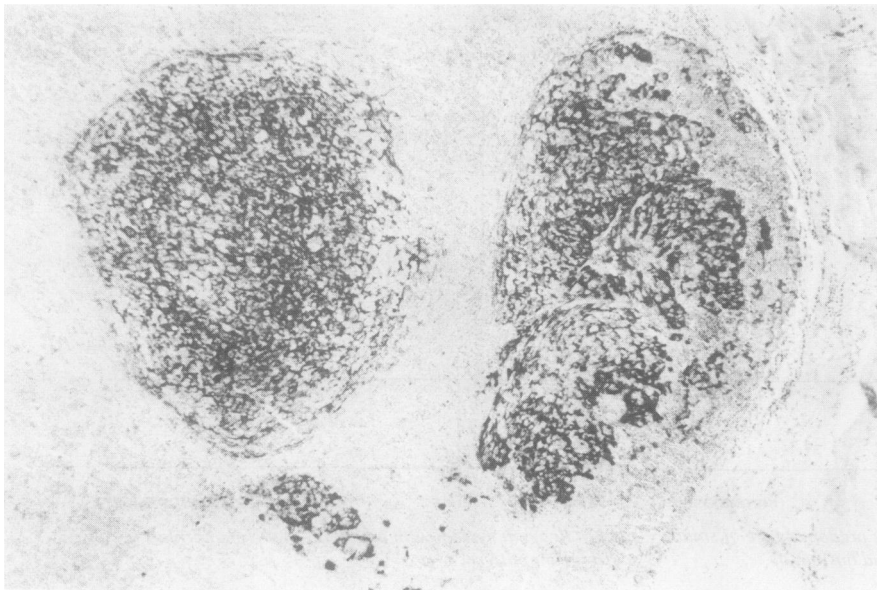


Fig 1 Germinal centre with well preserved follicular pattern of dendritic reticulum cells (left) and another germinal center in early phase of centrorrhexis (right).

Lymph node sections from patient with persistent generalised lymphadenopathy immunostained for acid cysteine proteinase inhibitor (peroxidase-antiperoxidase, haematoxylin.)
× 120.