Technical methods

Table 1 Recovery data ($n = 16$)

Step in procedure	Supernatant haemoglobin (g)
After thawing	0.45
First wash	1.6
Second wash	0.45
Third wash	0.12
Final product	0.023
After incubation	0.05

stability of the red cell preparation after incubation at 37°C for 30 minutes.

Recovery was calculated from the supernatant haemoglobin in relation to the initial total haemoglobin after thawing (table 2). Mean (SD) recovery was 70% (13.3), range 45–82% ($n = 16$). The mean (SD) volume of packed red cells available for use was 17 ml (6) ($n = 16$). All recoveries were sterile.

To date red cells from eight accredited donors have been stored in small aliquots for use in a five year boosting programme of 20 volunteers to maintain plasma anti-D concentrations of at least 50 IU/ml.

Over the past two years 35 doses have been given to 20 volunteers with no untoward effects. Small volumes (0.1–0.5 ml) were given intravenously. The frequency of administration depended on the initial anti-D value and the magnitude of the immune response to each booster injection.

Discussion

This method allows the storage and recovery of small

aliquots of fully phenotyped red cells from accredited donors and ensures that each volunteer is boosted repeatedly with red cells from the same donor over the whole five year period of the planned boosting and plasmapheresis programme. The volume of red cells recovered from any one aliquot was sufficient in all cases for boosting any number of volunteers. The technique is safe and conforms to international criteria of good manufacturing practice. Volunteers responded well to repeated small boosting doses with no adverse reactions.

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Table 2 Characteristic features of frozen recovered red cells ($n = 16$)

Characteristic features	After thawing	After washing (Mean (SD))	Final product
Supernatant haemoglobin (g)	0.45 (0.19)	2.2 (1)	0.02 (0.01)
Packed red cell mass (ml)	38 (6)	17 (6)	
Percentage recovery	94 (2.6)	70 (13.3)	

A rapid stain for *Pneumocystis*

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As the epidemic of acquired immune deficiency syndrome gathers pace laboratories will be faced with increasing numbers of specimens sent for diagnosis of *Pneumocystis carinii* pneumonia in bronchial biopsy specimens. Grocott's methenamine silver method is

generally used for this purpose,¹ but, although it stains the cysts of *Pneumocystis* satisfactorily, it has several disadvantages. It is a time consuming method requiring technical expertise. In understained preparations the cysts stain feebly or not at all, and in overstained preparations red cells are stained black and may be mistaken for parasites. The alternative technique described here, which is a modification of Gridley's method,¹ is both quicker and simpler.

Material and methods

Dewaxed paraffin sections are oxidised with chromic acid, stained with Schiff's reagent, and then counterstained.

Figure Purple staining of pneumocysts, low power (above) high power (below)

The following reagents are used:

- 1 5% chromic acid.
- 2 Schiff's reagent. Dissolve 10 g of pararosanilin base in 200 ml of N hydrochloric acid. Add 2 l of distilled water and 10 g of potassium metabisulphite. Store in a dark cupboard overnight to reach a straw colour the next morning. Decolorisation is unnecessary.
- 3 0.2% light green.

The staining procedure is as follows:

- 1 Take sections to distilled water.
- 2 Place in fresh chromic acid for one hour at room temperature or for three minutes at 60°C.
- 3 Wash in running tap water for five minutes.
- 4 Rinse in distilled water.
- 5 Place in Schiff's reagent for 15 minutes.
- 6 Rinse in distilled water.
- 7 Wash in running tap water for five minutes.
- 8 Counterstain with light green.
- 9 Dehydrate, clear, and mount as desired.

Discussion

In haematoxylin and eosin stained sections of lung infected with *Pneumocystis* a foamy eosinophilic exudate fills the alveoli, but the cysts are invisible. In routine periodic acid Schiff preparations the exudate stains a pale magenta, but the cysts are not seen. In preparations stained with Grocott's methenamine silver the exudate stains a weak brown and the cysts are stained black.

Both the periodic acid Schiff and Grocott methods stain polysaccharides after an initial oxidation step to produce aldehyde groups. Most organisms which contain polysaccharide encountered in tissue sections are equally well stained by either method. It is remarkable that pneumocysts are stained by the Grocott method but not by the periodic acid Schiff method. A possible explanation is that periodic acid oxidation is unsuitable for pneumocysts.

In the new stain the oxidation step is with chromic acid (the Bauer reaction,²) as in Grocott's method, but this is followed by staining with Schiff's reagent instead of a silver stain. This is similar to the Gridley method for fungi, but the subsequent fuchsin step in that method is not required. We use Schiff's reagent prepared in the laboratory, but commercial preparations are also suitable. The pneumocysts are stained a delicate purple (figure). There is no confusion with red cells since these are stained only by the counterstain.

The duration of exposure to chromic acid seems to be important. We have found that this step is more easily standardised at room temperature, but if a quicker result is required the oxidation may be carried out at 60°C. Five minutes' incubation at 60°C is too long and the method does not work. We have found three minutes to be a suitable time, but this may vary according to local conditions. It is important that the chromic acid is reasonably fresh.

The new technique uses simple reagents and is quicker and easier than the Grocott method currently used in most laboratories.

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