Table 2 Percentage sensitivity, specificity, and predictive values of positive and negative results on Staphaurex testing compared with reference methods

	Tube coagulase test	Slide coagulase test	DNase test	Staphaurex
Sensitivity	98.9	97.7	100	100
Specificity	100	99.3	99.3	98.3
Predictive value of positive result	100	98.9	98.9	97.3
Predictive value of negative result	99.3	98.6	100	100

Although false positive Staphaurex reactions have been described with non-*S aureus* staphylococci, previous workers have not attempted to investigate this further.^{2 8 9} The production of protein A by some isolates of *S saprophyticus* explains some of the false positive results. Three of the five organisms which were falsely positive with Staphaurex in our study were *S saprophyticus*. This limits its use in testing urinary isolates.

Staphaurex reagent may be expensive. The use of 20 µl for each test instead of the 50 µl dispensed by the droppers supplied in the kit should, however, substantially reduce the cost of this useful test.

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Storage and recovery of small aliquots of frozen red cells for anti-D boosting

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The boosting of volunteers with frozen recovered red cells from accredited donors was recommended by the World Health Organisation in 1981.¹ Frozen recovered red cells were successfully used in routine boosting of eight Rh D negative women previously immunised by pregnancy,² and for immunising and boosting Rh negative male volunteers.³ In view of the lack of specific markers for non-A non-B hepatitis and the possibility of transmitting human immunodeficiency virus to donors who volunteer to join the boosting programme, it is now even more important to implement all available safety measures. The safety

of immunisation and boosting programmes could be improved by storing frozen red cells from accredited donors in small aliquots. Each volunteer could then be boosted using the same donor cells during the whole programme so as to minimise antigenic exposure.

Red cell donations were selected from a panel of R2R2 longstanding regular donors and were phenotyped for the following antigens: ABO, rhesus, M, N, S, s, K, k, P, Le^a, Le^b, Fy^a, Fy^b, Lu^a, Jk^a, Jk^b. This allowed selection of suitable boosting cells and avoided the development of unwanted irregular antibodies.³ At each donation tests of liver function and for hepatitis B virus markers (hepatitis B surface antigen, hepatitis B surface antibody, and hepatitis B core antibody) were performed. Archive serum samples were used to test for antibody to human immunodeficiency virus to ensure that all available frozen cells were negative.

We describe a method using polyolefin freezing bags for storing and recovering Rh positive red cells in small aliquots for boosting. This method satisfies safety requirements and conforms with criteria of good manufacturing practice.⁴

Figure Red cell bags in cassette ready for freezing

Material and methods

Blood donors were chosen according to World Health Organisation recommendations.¹ The following guidelines for the accreditation of boosting cells were introduced by the Scottish National Blood Transfusion Service: donors are interviewed at monthly intervals for six months after donation and tested for liver function, antibody to human immunodeficiency virus, and hepatitis B markers.

Red cells were obtained from one unit of blood by removal of plasma and buffy coat after centrifugation at 4825 g for 10 minutes using a DPR centrifuge (Damon IEC). The red cells were mixed with an equal volume of the following solution: 420 g glycerol, 29 g sorbitol, and 6 g sodium chloride in 1000 ml of pyrogen free distilled water. Fifty millilitre volumes were transferred to each of eight individual freezing bags (Delmed 2030-2) using a standard AE8 plasma transfer set (Travenol Laboratories). The bags were sealed using a port tube sealer (Union Carbide) and the bottom and top of each bag was then folded to reduce its surface area and avoid brittle fractures. The bags were then placed in freezing cassettes (Union Carbide bag holder) and immersed in liquid nitrogen (figure). These bags were subsequently stored in the vapour phase in a liquid nitrogen refrigerator to permit access and retrieval. The frozen bags could have been stored in perforated envelopes (transit type code no 27/29

HMSO) in a smaller liquid nitrogen refrigerator.

Thawing was carried out by agitating individual bags in water at 42°C for five minutes. The red cell mixture was then transferred to a five tail wash pack (Tuta no 11-055). A volume of 200 ml of hypertonic saline (3.5 g/100 ml) was added with continuous mixing at room temperature to permit osmotic equilibration. The bag was then centrifuged at 4825 g for five minutes. The supernatant was replaced by 200 ml of physiological saline (0.9 g/100 ml). This procedure was repeated once and the red cells were finally resuspended in about 20 ml of the remaining supernatant saline to reach a final packed cell volume of 0.7 l/l. Samples were taken after thawing and at each wash for monitoring of haematological, biochemical, and serological variables and for retrospective sterility quality assurance.⁵ Boosting doses were dispensed in small dry plastic packs (Tuta 11-020) according to the number of volunteers and dispatched to be used within 12 hours of recovery. All open procedures were performed in laminar flow cabinets under aseptic conditions.

Results

Table 1 shows the supernatant haemoglobin values during the recovery procedure and establishes the

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.

We thank Mrs S Balmer for typing the manuscript.

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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.