

PREPARATION OF FROZEN FRESH PLASMA FOR FACTOR VIII CONCENTRATE
PRODUCTION AT THE BLOOD PRODUCTS LABORATORY ELSTREE (BPL).

INTRODUCTION

As Factor VIII is extremely labile, variations in processing, both during the preparation of frozen plasma and during its fractionation, may affect the yield of Factor VIII in the concentrate and its stability during storage. Increasing the scale of production will give an opportunity to investigate factors which may affect the yield. Because of the amount of work involved in making this product information leading to improved yield will be important both financially and from the point of view of organisation.

For these reasons:-

1. In each Regional Transfusion Centre fully defined and constant procedures should be used for the preparation, freezing, storage and transport of the plasma.
2. When the processing procedure is varied, resulting in more than one type of frozen plasma, the type should be segregated and pooled in separate bags (e.g. "8 hr" versus "24 hr" plasma; or ACD versus CPD plasma). By processing separate pools at the BPL it should be possible to accumulate evidence regarding the effect of variations in procedure on yield.

BASIC PROCEDURE

SELECTION OF DONORS

1. It will be assumed that donors will not have been selected for a high 'natural' Factor VIII level, unless the BPL is informed to the contrary.
2. To avoid any risk from blood group iso-agglutinins in the concentrate:
 - a) each 5L bag should contain plasma from donors in roughly the normal distribution of blood groups i.e. Each bag should contain at least one or two group B or AB units and approximately equal numbers of group A and group O units. In practice fairly large pools will be fractionated so that risks from this source should be small. However, bags containing only plasma from group A or from group O donors should be so marked.
 - b) plasma with a high titre of blood group antibodies (e.g. anti-A, or anti-D) should be excluded.
3. Plasma from the blood of 'malaria' donors is suitable.
4. If the plasma is from donors on regular plasmapheresis, it should be so marked (the BPL should be advised).

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TESTING FOR HB Ag and HB Ab

If '24 hr' plasma is being prepared it may be possible to screen before pooling into the 5L bags. In the absence of such screening, the use of blood from 'new' donors or previously unscreened donors would carry a risk of a 5L bag of plasma having to be discarded. 'New' donors should, therefore, be excluded when pooling precedes testing.

BLEEDING OF DONORS

1. Normal precautions appropriate for Factor VIII - rich plasma preparation: should be taken i.e.

- (1) Reject the donation for Factor VIII production
 - (a) if venepuncture has not been by clean first-time entry into the vein
 - (b) if bleeding has been slow (maximum 8 minutes) or intermittent.
- (2) Be sure that blood and anticoagulant are positively mixed during the bleeding
- (3) Avoid excessive frothing
- (4) In the case of blood bags, on completion of bleeding clip off the donor tube as close as possible to the bag and mix the residual blood in the tube adjacent to the bag with the blood in the bag containing anticoagulant. Clotting of uncitrated blood in the tube must be avoided.

2. Blood collected in heparin or EDTA is unsuitable.

3. Both ACD and CPD are suitable as anticoagulants, but if both are in use, the plasma should be segregated by type and the plasma despatch sheet marked ACD, CPD or ACD plus CPD as appropriate.

if possible

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COOLING AND STORAGE OF THE BLOOD

The blood should be cooled to 4° to 6°C and ^{kept} held at this temperature until processed.

Both frozen fresh "8 hr" plasma and frozen "24 hr" plasma are suitable for fractionation. It is probable that there will be some loss of activity with the latter, but further investigation is required. In the meantime "8 hr" plasma is to be regarded as the preferred starting material.

The term "8 hr" refers to plasma with a maximum period of 8 hours between bleeding the donor and freezing the centrifuged plasma. Likewise "24 hr" plasma refers to plasma from blood collected the previous day and processed within ~~anything from~~ 16 to 24 hours of blood withdrawal.

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65 min at 2000 G \equiv 13 min at 4500 G. or 5 min.
bag at 62 min = 7 1/2 min. It

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INSPECTION OF THE BLOOD

Blood which is seen on visual inspection to contain large or small clots, or clotted froth, or is unusually lipaemic is not suitable for Factor VIII preparation and should be rejected.

CENTRIFUGATION

Platelet - poor plasma is required. Centrifugation: for blood bags 4,500 x G, 5 mins, + 4°C; for bottles 2000 x G, + 4°C, 65 mins. At 2000 x G (2,500 rpm on a Mistral 6L centrifuge) blood bottles may require 'cushioning' with fluid external to the bottle, to avoid breakage.

Notes

- 1 When loading centrifuges with bags avoid 'kinks' on the bags which may entrap red cells and see that the top of the bag is not folded over. It is a general finding that unless great care is taken 'high-spun' plasma may contain many red cells, even if it is relatively free from platelets. A further important precaution is to avoid braking the centrifuge head by hand, as it slows down, especially during the last few turns. It must be allowed to come to rest spontaneously or the sedimented red cells will be disturbed.
- 2 The centrifuged blood should be handled carefully and should be inspected before the plasma is separated. Reject for ^{all present} presence of red cells or clots, or if the plasma is pink, very yellow, or unusually lipaemic. Siphoning of plasma should be done in such a way that platelets and red cells are, as far as possible, not disturbed.

Special mention about
platelets?

PLASMA RECOVERED FROM PREPARATION OF PLATELET CONCENTRATES

This is suitable for use as it will have been prepared by centrifugation in the manner described above.

SEPARATION OF THE PLASMA

1. A note, prepared at BPL, on the use of 5 litre plastic bags for pooling and freezing of plasma was distributed to the Regional Transfusion Centres (note dated May 1971; see also paper "Freezing Plasma in 5-litre Bags" L. Vallet, Vox Sang. 24 550-559 (1973).
- 2 Pooling equipment which has been used for time expired plasma should not be used for fresh plasma, prepared at the same session.
- 3 The outsides of the plastic bags and of the cardboard cartons should be kept as clean as possible. The cartons should be stored in clean, dry conditions. This will help to minimise any risk of contamination when the thawed plasma is pooled.
- 4 Separate plasma dispatch sheets should be used for frozen fresh plasma.

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LABELLING OF BAGS

INFORMATION IS TO FOLLOW

STORAGE OF PLASMA

Preferred storage temperature: below -30°C . If this is not available: -25°C . The Blood Products Laboratory should be informed of the actual storage temperature. Extended storage should be avoided; regular deliveries to BPL should thus be made.

TRANSPORT TO BPL

Precautions should be taken to ensure that the temperature does not rise above -25°C during transit. Exposure to fluctuating temperatures may be deleterious even if no visible thawing occurs.

It would be convenient for the BPL if the plasma dispatch sheets for frozen fresh plasma could be delivered (along with the plasma) in an envelope marked "CF LAB"*; and also if this plasma could be kept separate from time-expired plasma as it has to go to a different deep-freeze store on arrival.

* Abbreviation for 'Coagulation Factor Preparation Laboratory'

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Blood Products Laboratory

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