FACTS AND FIGURES

JUNE 2004 • NO 2

THE PREPARATION OF SINGLE DONOR CRYOPRECIPITATE

Revised Edition

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FEDERACIÓN MUNDIAL DE HEMOFILIA

Published by the World Federation of Hemophilia (WFH); 1997, revised 2004.

This publication is accessible from the World Federation of Hemophilia's web site at **www.wfh.org**. Additional copies are also available from the WFH at:

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The Preparation of Single Donor Cryoprecipitate

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Introduction

It is clear that for some of the developing nations the treatment of factor VIII deficient hemophilic patients with factor concentrates is not a possibility due to many constraints including financial ones. This article is designed to be used for the preparation of single donor cryoprecipitate, keeping in mind that there are minimum requirements and therefore minimum costs for equipment and consumables.

Many countries now have in place some form of blood donor recruitment and the general policies for recruiting, screening and retention of blood donors for cell products apply equally to those for cryoprecipitate production. The basic principles as stated in the WHO consensus statements WHO/LBS/93.2 and WHO/GPA/INF/93.1 are the same. Some simple guidelines are incorporated into the article as well as guidelines for the collection of donor blood so that maximum factor VIII can be extracted.

Although the production of cryoprecipitate is not technically difficult, training of responsible staff must be carried out.

Instructions have been kept to a minimum and where alternative techniques are possible and relevant these have been included.

The methodologies of achieving the highest possible yield of factor VIII have been the subject of many debates. However, a simple approach with a suitable yield for the treatment of factor VIII deficient patients can be used, and the resultant product can even be kept in short-term storage for home therapy for those patients that have access to a domestic deep freeze.

The method below results in three useful blood components: packed cells with a life span of up to 42 days (dependent upon the additive solution used, if any), a plasma component that can be used as a plasma expander, and a unit of cryoprecipitate.

All aspects of quality assurance apply to the production of cryoprecipitate. This includes development, implementation and use of standard operating procedures for each step of the process. Further to this, it is desirable to carry out factor VIII assays on a regular basis, to ensure that at least 75% of the product produced contains a minimum of 80 international units (IU) of factor VIII.

1. The Donor

1.1 Donors should be voluntary and non-remunerated.

1.2 Where a donor retention program exists, cryoprecipitate should be made from return donors in preference to new donors.

1.3 Risk factors for possible transfusion transmissible infections should be rigorously screened for.

1.4 To avoid adverse reactions in the donor, general donor health should be assessed during donor screening.

1.5 From the general donor pool, target those return donors who have given often for cryoprecipitate production.

1.6 Selecting known group A or AB donors can also be beneficial as these two ABO groups are known to yield higher levels of factor VIII.

2. Blood Safety

As no routine, simple method exists for viral inactivation of 'wet' cryoprecipitate, donor screening is of vital importance to prevent transmission of infection, particularly from those donors in the window period of infection.

2.1 All donors should be questioned carefully and preferably on a one to one basis. Lifestyle markers, such as association with commercial sex workers, and clinical manifestations (e.g. lymphadenopathy) should be rigorously investigated. Each region in question should establish its own risk behaviour pattern(s) and this should be used for designing a questionnaire.

2.2 All units of blood should be screened for:
- Antibodies to HIV 1/2.
- Hepatitis B surface antigen.
- Antibodies to hepatitis C.
- Syphilis (A useful lifestyle marker) - VDRL/RPR or TPHA.

Viral marker testing is not limited to the above, and is also dependent on the region's own testing policies or epidemiological data.

3. Quarantine and Release

3.1 It is possible, by using the regular donor pool, to release cryoprecipitate for use only when the donor returns to donate again and is found to be still negative for viral markers. Although labour intensive without a computer system, accurate records of donors' blood donation numbers can be kept and used for this procedure. Without viral inactivation, this is the best method for ensuring the lowest window period risk.

3.2 If the above is not possible, release the cryoprecipitate for use once viral marker testing has been done and found negative.

4. Collecting the Blood (Phlebotomy)

4.1 After screening the donor, prepare the donor for phlebotomy.

4.2 Use a closed system of blood packs (triple configuration).

4.3 Place the main pack that holds the anti-coagulant into the donor scales. It is preferable to place the pack upside down, so that blood entering the pack has immediate contact with the anti-coagulant. A simple electronic balance can be used instead of donor scales.

4.4 Mix the blood and anticoagulant as it is collected.
- Use an automated system that mixes and weighs the blood simultaneously.
  OR
- As soon as blood starts to flow into the pack, mix gently by inversion and repeat at least every minute during the donation time.

4.5 When the donor scale stops the flow of blood from the donor or when the desired weight of the main pack plus anticoagulant plus blood is reached, stop the flow of blood from the donor.
- It is essential that the blood to anti-coagulant ratio is not exceeded.
- If the donation time exceeds ten minutes, do not use the donation for cryoprecipitate production.

4.6 Label as per your donor collection procedures.

4.7 Allow the donated blood pack to cool to room temperature and deliver the pack to the responsible department within two hours for maximum factor VIII yield up to a maximum of six hours.

4.8 Superior factor VIII yields are obtained from blood that is not cooled to below 22° C before processing.

5. The Production

5.1 Using a simple electronic balance, ensure that the main blood pack is within the acceptable weight ranges. If not, do not use for cryoprecipitate preparation. (See Appendix, p. 4)

5.2 Centrifuge the main pack with its attached satellite bags in a refrigerated centrifuge for 20 minutes at 3000 rpm at 4°± 2° C.

5.3 Open the port connecting the main pack to the satellite bag and syphon off the plasma by gravity or using a plasma extractor.
- Detach the packed red cells. (This is done after adding the additive solution to the red cells, if applicable.) Leave the extracted plasma, and now empty the satellite bags attached to each other.

5.4 Prepare the freezing solution of alcohol (ethanol) and dry ice in the insulated bath.
- Pour the alcohol into the stainless steel sink. Add dry ice, broken into hand-sized bits, until the temperature reaches -60 ° C (Approximate ratio of 2 kg dry ice to 10 litres of ethanol).

5.5 Gently hang the bag containing the plasma into the freezing solution ensuring that the whole bag is immersed and that the attached satellite bag is placed on the side panels of the bath. Leave in the solution for ten minutes.

5.6 Remove the frozen plasma, and immediately commence thawing.
- Place frozen bag in a thermostatically controlled waterbath at 3° C until solution becomes "slushy" (approximately 20 - 30 minutes).
OR
- Place the bag in a refrigerator until solution becomes "slushy".

5.7 Immediately spin in a refrigerated centrifuge for 10 minutes at 2000 rpm at 2°-8° C. Optimum temperature is 3° C.

5.8 Remove the excess plasma into the empty satellite bag leaving approximately 10 ml with the deposited cryoprecipitate.
- Detach the excess plasma bag from the cryoprecipitate.

5.9 Label the products as per your protocol. Store the cryoprecipitate as follows:
- If storage is at -20° C, the expiry period is 6 months.
- If storage is at -40° C, the expiry period is 12 months.
- Store the protein poor fraction (excess plasma) below -20° C.

5.10 Immediately quarantine to await results for viral marker and other infectious disease testing, and formal release procedures.

6. Minimum Equipment Required

Unfortunately, cryoprecipitate cannot be produced without some capital expenditure on equipment and consumables. Some of the equipment can be made in-house and some guidelines for these are given below.

6.1 Equipment
- Refrigerated centrifuge.
- Simple electronic balance.
- Blood donor scales or electronic balance.
- Plasma extractor. Simple commercially produced ones can be obtained or home-made ones are quite simple.
- Insulated stainless steel bath for freezing solution. Home-made.
- Alcohol thermometer capable of reading to -80° C.
- Blood bank refrigerator. (This piece of equipment should already be in place and does not represent an extra outlay of capital).
- Deep freeze unit. (We have found that most chest deep freeze units can obtain a temperature of -20° C).
- Well-padded gloves for handling the dry ice and frozen units.
- Thermostatically controlled water bath (not essential; see section five above).

6.2 Consumables
- Donation blood packs.
- Dry ice.
  - Most countries can obtain dry ice commercially. Contract agreements can be sought with the suppliers to minimize costs.

  - Although dry ice can be made in-house, capital output will be required and in our experience, the dry ice obtained is rather friable and does not quite achieve the freezing temperature of - 60° C.
- Alcohol (ethanol).

7. Cost Effectiveness

Without imported freeze-dried factor VIII, most hemophilic patients would remain untreated or treated with fresh frozen plasma at best. By producing single donor cryoprecipitate, treatment with a much smaller volume, yet more concentrated product becomes a reality. Consumable costs for the production of cryoprecipitate can cost as little as US$.20 per I.U. of factor VIII. This production cost would also result in two other valuable blood products i.e. packed cells and protein poor plasma. By comparison, freeze-dried concentrate costs at least US$.24 per I.U. This may not appear to be a vast difference, but one must remember that the cost of cryoprecipitate production includes the cost of two other blood components.

Furthermore, if cryoprecipitate is used instead of fresh frozen plasma, the cost of production is increased by only 4%. The clinical benefits of treating a patient with one bag of cryoprecipitate as opposed to a minimum of four bags of fresh frozen plasma are of immense importance.

Appendix

Calculation for weight ranges for the primary donated pack

Formula:
Weight of primary pack with anticoagulant + Volume of blood to be donated x 1.032

The above formula gives the exact weight that the donated unit of blood should weigh. The acceptable range is +/- 8% of that weight.

Example:

1. Weight of primary bag with anticoagulant = 85 grams
2. Volume of blood to be donated = 450 ml
3. Multiply by 1.032 to get the weight of blood in grams
4. Weight of blood to be donated equals 450 x 1.032 = 464 grams
5. Therefore, desired weight of pack + donated blood = 85 + 464 grams = 549 grams
6. 8% of this weight (8% 549) = 44 grams
7. Desired weight range = 505–593 grams

Flow Chart for Preparation of Cryoprecipitate