Chapter 17

A Protocol for the Preparation of Cryoprecipitate and Cryodepleted Plasma

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Abstract

Cryoprecipitate is a concentrate of high-molecular-weight plasma proteins that precipitate when frozen plasma is slowly thawed at 1–6°C. The concentrate contains factor VIII (antihemophilic factor), von Willebrand factor (vWF), fibrinogen, factor XIII, fibronectin, and small amounts of other plasma proteins. Clinical-grade preparations of cryoprecipitate are mainly used to treat fibrinogen deficiency caused by acute bleeding or functional abnormalities of the fibrinogen protein. In the past, cryoprecipitate was used to treat von Willebrand disease and hemophilia A (factor VIII deficiency), but the availability of more highly purified coagulation factor concentrates or recombinant protein preparations has superseded the use of cryoprecipitate for these coagulopathies. Cryodepleted plasma ("cryosupernatant") is the plasma supernatant that remains following removal of the cryoprecipitate from frozen-thawed plasma. It contains all the other plasma proteins and clotting factors present in plasma that remain soluble during cold-temperature thawing of the plasma.

Key words: Plasma, Cryoprecipitate, Antihemophilic factor, Factor VIII, Cryodepleted, Fibrinogen, Proteomics, Cryosupernatant

1. Introduction

Cryoprecipitate (also known as cryoprecipitated antihemophilic factor) was first described in the mid-1960s as a method to concentrate factor VIII (antihemophilic factor) from plasma for use in patients with hemophilia, von Willebrand disease, or hypofibrinogenemia (1, 2). Cryoprecipitate is the insoluble concentrate of high-molecular-weight plasma proteins that precipitate when frozen plasma is slowly thawed at 1–6°C (2). Cryoprecipitate is enriched for plasma coagulation proteins, in particular factor VIII, fibrinogen, von Willebrand factor, factor XIII, and fibronectin. Small amounts of other plasma proteins, such as immunoglobulins, may also be present.

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Cryoprecipitate is a standard blood transfusion component manufactured by most blood transfusion services (3). Clinically, the main indications for use of cryoprecipitate is for the treatment of fibrinogen deficiency (hypofibrogenemia) caused by significant bleeding due to trauma, massive transfusion or disseminated intravascular coagulation, or dysfibrogenemia arising from functionally abnormal fibrinogen (3, 4). Previously, cryoprecipitate was used to treat hemophilia and von Willebrand disease, but with the advent of specific coagulation factor concentrates, it has been relegated to second-line therapy for these diseases. Over the years, attempts have been made to improve the yield in cryoprecipitate by the use of techniques such as thaw-siphon technique (5), the effect of temperature on freezing and thawing (6), or by the use of various additives, such as heparin (7) and sodium citrate (8, 9).

Cryodepleted plasma (cryosupernatant) is the plasma supernatant that remains following removal of the precipitated cold insoluble proteins (i.e., cryoprecipitate) from frozen-thawed plasma. It is, therefore, significantly depleted of fibrinogen, factor VIII, von Willebrand factor, factor XIII, and fibronectin, but contains all the other plasma proteins and clotting factors in similar concentrations as the original plasma. Clinically, cryodepleted plasma is used for plasma exchange in thrombotic thrombocytopenic purpura and in situations requiring rapid temporary reversal of warfarin anticoagulant therapy (10).

In the following protocol, the preparation of small research-scale cryoprecipitate and cryodepleted plasma is outlined and is based on the procedures used by blood transfusion services for the preparation of clinical-scale cryoprecipitate (10).

2. Materials

2.1. Blood Collection, Plasma Preparation, and Storage

1. Whole Blood Collection Tubes containing citrate anticoagulant (e.g., BD Vacutainer® with 3.2% sodium citrate anticoagulant, 4.5-mL tube BD Biosciences #366415; BD Vacutainer with acid–citrate–dextrose (ACD) Sol A anticoagulant (22.0 g/L trisodium citrate, 8.0 g/L citric acid, and 24.5 g/L dextrose), 8.5-mL tube, BD Biosciences #364606] (see Note 1).
2. Blood collection needles compatible with the blood collection tubes, e.g., BD Vacutainer® Safety-Lok™ Blood Collection Set #367283; 23 G butterfly needle with attached sterile tubing).
3. Alcohol and swabs for disinfection of the venipuncture site.
4. Gloves, gown, and eye protection.
5. Disposal container for biological hazards.
6. Polypropylene tubes (1.5 mL, 15 mL).

7. Labels for blood sample tubes.
8. Tube storage rack.
9. Centrifugation unit (with swing-bucket rotor, compatible with 1.5/15 mL tubes, programmable temperature setting; range 4–25°C).
10. Pipettes.
11. Freezer (−20°C or lower).

2.2. Cryoprecipitate/ Cryodepleted Plasma Preparation

1. Refrigerator or water bath set at 1–6°C.
2. Centrifuge, refrigerated (swing-bucket rotor, compatible with 1.5/15 mL tubes, programmable temperature setting).
3. 0.9% Saline solution.

3. Methods

3.1. Blood Collection/ Phlebotomy

1. Blood collection must only be performed by personnel trained in phlebotomy/venipuncture. Safety precautions for the collection and handling of blood must be employed at all times (see Note 2). Particular care must be taken with insertion of the needle into the vein to limit the possibility of activation of the coagulation factors in the blood, which could compromise the quality of the blood sample.
2. It is important to collect the volume of blood specified for the particular type of blood collection tube to ensure that the correct blood/anticoagulant ratio is achieved (see Note 3).
3. After blood collection, gently mix the blood by inverting the tube several times to ensure thorough mixing with the anticoagulant. For thorough mixing of blood collected into citrate tubes, it is recommended to invert the tube 3–4 times, while ACD tubes should be inverted eight times.
4. Blood samples should be maintained at temperate conditions (i.e., 20–24°C) and centrifuged within 4 h of blood collection. Superior factor VIII yields are obtained from blood that is maintained at 20–24°C before processing.
5. To separate the plasma, centrifuge the blood samples at 1,200 × *g* for 10 min at 22°C. If needed, RCF for a centrifuge can be calculated. For an online calculator tool, refer: http://www.currentprotocols.com/tools/g-forcerpm-conversion-tool.
6. After centrifugation, the plasma layer will be the upper layer of the separated blood. The plasma should be a clear, straw-yellow colored fluid. Mononuclear cells and platelets form a thin whitish mid-layer (buffy coat) that settles directly on top of the red blood cell layer.

7. Carefully, collect the plasma layer with an appropriate transfer pipette without disturbing the buffy coat layer. Do not attempt to collect all of the plasma. Do not allow the tip of the transfer pipette within 5 mm of the buffy coat layer and avoid touching the wall of the tube with the pipette. This helps to avoid inadvertent contamination of the plasma with cells that may only be softly sedimented in the buffy coat–plasma interface (see Note 4). If more than one tube is collected from the same donor, pool the plasma samples from both tubes into a 15-mL polypropylene tube. If necessary, aliquot plasma into smaller volumes. A practical minimum volume is 1–1.5 mL.
8. Close the tube tightly and place on ice or immediately freeze by placing in the freezer. This process should be completed within 30 min of centrifugation. Plasma should be frozen as quickly as possible to minimize loss of labile coagulation factors, such as factor VIII. Frozen plasma should be stored at below –20°C.

3.2. Cryoprecipitation

1. For the preparation of cryoprecipitate, remove tube(s) of frozen plasma from the freezer and immediately place in a thermostatically controlled water bath or refrigerator set at 1–6°C. Slowly, thaw the plasma until it becomes "slushy" (the required time will depend on the volume of plasma being thawed) (see Note 5). Optimum temperature for cryoprecipitate formation is 3°C.
2. Immediately, sediment precipitated proteins in a refrigerated centrifuge (1–6°C) at 5,000 × *g* for 15 min. A white precipitate should be evident at the bottom of the tube.
3. Carefully, remove the supernatant (Note: this is the cryodepleted plasma). If this cryodepleted plasma is required, aliquot into separate polypropylene tube(s). Leave a small amount of plasma above the deposited cryoprecipitate (5–10% v/v, 50–100 µL for 9–10 mL of blood collection volume). If the cryoprecipitate or cryodepleted plasma is not required immediately, freeze at –20°C (see Note 6).

3.3. Thawing of Cryoprecipitate/ Cryodepleted Plasma

1. Thaw cryoprecipitate or cryodepleted plasma in a water bath at 30–37°C. The cryoprecipitate should be evenly dissolved at warming temperature.
2. Cryoprecipitate can be suspended in diluent, such as 0.9% saline, at 20–24°C. Cryoprecipitate should be maintained at 20–24°C and used within 6 h of thawing. Progressive loss of labile proteins, including Factor VIII and fibrinogen, occurs following thawing (see Notes 7–10).
3. Thawed cryodepleted plasma can be maintained at 2–6°C.

4. Notes

1. *Anticoagulant.* For all physiological coagulation studies, sodium citrate or ACD is the anticoagulant of choice. Cryoprecipitate prepared by blood transfusion services for clinical use is prepared from whole blood collected into citrate–phosphate–dextrose (CPD) anticoagulant or from plasma collected into ACD anticoagulant by apheresis (11). Cryoprecipitate prepared from CPD anticoagulated plasma has been shown to give a higher yield of Factor VIII compared to ACD anticoagulated plasma (11). The preparation of cryoprecipitate from plasma anticoagulated with noncitrate anticoagulants (e.g., heparin) may yield a different profile of precipitated proteins (12).
2. *Safety.* All blood and biological specimens and materials coming in contact are considered biohazards. Use gloves, gowns, eye protection, other personal protective equipment, and controls to protect from blood splatter, blood leakage, and potential exposure to blood-borne pathogens. Use aseptic technique and sterile disposables (tubes, pipettes) throughout to prevent contamination of the blood. Risk factors for possible transfusion transmissible infections should be rigorously screened for prior to blood collection. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Refer to your institutional regulations regarding the screening of blood for specific infectious disease markers (i.e., HIV, Hepatitis B, Hepatitis C, etc.). Discard all blood collection materials in biohazard containers approved for their disposal.
3. *Evacuated blood collection tubes* (e.g., BD Vacutainers) are manufactured to draw the blood volume specified for the particular tube. Filling is complete when vacuum no longer continues to draw blood into the tube. Partially filled tubes will not have the correct blood/anticoagulant ratio and should not be used for the purpose of plasma sample preparation. If a citrated blood sample is the first sample to be collected from the donor, it is important to first collect a small volume of blood into a discard tube. This ensures that the "dead" volume in the needle/tubing set is filled with blood prior to the citrated tube being connected, thus ensuring that the correct blood volume is drawn into the tube.
4. *Quality control of plasma sample.* It is recommended that following the preparation of the plasma sample, the sample is monitored for hemolysis and cell contamination. This should be validated by your institution or testing laboratory. The plasma sample should also be observed with respect to its

color (normally straw-yellow) and clarity. The presence of high lipid content in a plasma sample can be observed by the opaqueness of the plasma. Pink or red coloration of the plasma is indicative of hemolysis of red blood cells. Plasma samples with high lipid content or hemolysis should be avoided for protein chemistry studies of normal plasma. Plasma that has been previously thawed and refrozen will give inferior yields if used for the preparation of cryoprecipitate.

5. *How to prevent cryoprecipitation when thawing plasma*. If plasma is to be used for coagulation studies, it is important to prevent cryoprecipitation from occurring. To avoid cryoprecipitation of the cold-insoluble proteins when thawing replete plasma, the plasma must be thawed quickly at 37°C. This can be achieved by placing the frozen plasma samples in a 37°C water bath or dry heating system set at 37°C. Such equipment must be maintained and kept clean to avoid inadvertent bacterial contamination of plasma samples.
6. *Cryoprecipitate and cryodepleted plasma storage*. The prepared cryoprecipitate and cryodepleted plasma have a shelf life of 12 months when stored at or below –18°C (10).
7. *Cryoprecipitate content and specifications*. A cryoprecipitate unit prepared from plasma obtained from a standard 450–500 mL CPD-anticoagulated whole blood donation and processed according to blood banking procedures should contain at least 150 mg of fibrinogen and a minimum of 80 international units (IU) of Factor VIII (10, 13). This equates to 30–70% of the factor VIII/vWF and fibrinogen content of the original plasma. Proportionally similar yields should be achieved when cryoprecipitate is prepared from smaller starting volumes of plasma, provided care is taken with the processing, freezing, and thawing of the plasma samples.
8. *Effect of ABO blood group*. Plasma/cryoprecipitate from blood group O individuals has lower levels of Factor VIII and vWF than A, B, or AB blood groups.
9. *Effect of pathogen reduction treatment on coagulation factor proteins*. Some blood transfusion services supply plasma units that have undergone an additional pathogen reduction process to further minimize the already very low risk of infectious disease transmission by blood transfusion. Various pathogen reduction technologies have been developed, including treatments with solvent/detergent, or photoactivating agents such as methylene blue, riboflavin, or psoralen (14). A trade-off for the increased safety rendered by pathogen reduction treatment is a reduced yield and activity of plasma coagulation factors, including fibrinogen and factor VIII (14–16). The nature of the biochemical changes that occur to the coagulation proteins following pathogen reduction treatment is yet to be fully determined.

10. *Platelet microparticle concentration*. The platelet membrane microparticle concentration of the cryoprecipitate has been shown to be 29-fold greater than that of the cryosupernatant plasma and 265-fold greater than that of the original plasma sample (17). There is a need to further clarify whether such microparticles have a role in hemostasis, vascular function, inflammation, or alloimmunoreactivity (17, 18). It is unknown whether the platelet membrane microparticles retain hemostatic function after processing and freezing of cryoprecipitate.

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