The primary goal of collection centers is to ensure that processes involved in the collection, preparation, storage, and shipment of blood and components result in a blood or blood component with optimal benefit for the recipient. Practices must prevent or delay physical and chemical changes detrimental to blood constituents, and minimize microbial contamination and proliferation.

As with other living systems, blood cells depend on a delicate biochemical balance of many materials for their integrity during storage, especially glucose, pH, and adenosine triphosphate (ATP). For red cells, balance is best preserved by storing at temperatures between 1-6 C, whereas platelets and granulocytes retain better function when stored at room temperature (20-24 C). Preservation of labile coagulation factors is satisfactory at temperatures of –18 C, and is probably better at –30 C or below. Refrigeration or freezing minimizes proliferation of bacteria that might have entered the unit during venipuncture or were present in the circulation of the donor. Anticoagulant-preservative solutions assist in metabolic stabilization and prevent clotting.

Anticoagulation and Preservation

CPD, CP2D and CPDA-1

CPD and CP2D are anticoagulant-preservatives approved by the Food and Drug Administration (FDA) for 21-day storage of Red Blood Cells (RBCs) maintained at 1-6 C. Blood collected in CPDA-1 may be stored for up to 35 days at 1-6 C. Even at 1-6 C, cells continue metabolic activity, consuming nutrients and depleting intracellular energy sources. For stored red cells, levels of ATP during storage correlate with posttransfusion viability in the recipient, although the low storage temperature slows glycolytic activity. Dextrose is present in all the anticoagulant-preservative and additive solutions in sufficient quantity to support continuing ATP generation by glycolytic pathways. (See Table 7-1.) The added adenine in CPDA-1 provides a substrate from which red cells can synthesize ATP, resulting in improved viability when compared to CPD without adenine. Citrate prevents coagulation by
chelating calcium, thereby inhibiting the several calcium-dependent steps of the coagulation cascade. Sodium biphosphate acts to prevent an excessive fall in pH during storage.

Commercially available blood collection bags contain 63 mL of anticoagulant-preservative solution, for an intended solution-to-blood ratio of 1:4 to 1:10. The standard collection volume is 450 mL ± 45 mL of blood (ie, 405-495 mL). If only 300-404 mL of blood is collected (300-404 mL), the red cells can be used for transfusion if the unit is labeled “Low Volume Unit __mL Red Blood Cells.” Other components should not be prepared from these units. If collection of less than 300 mL is planned, the volume of anticoagulant-preservative solution should be reduced proportionately. (See Table 4-1 for calculations.)

### Additive Systems

Presently approved additive systems consist of a primary collection bag containing anticoagulant-preservative, with at least two satellite bags attached, of which one is empty and one contains the additive solution. The additive solution contains sodium, dextrose, adenine, and other substances expected to enhance red cell survival and function. (See Table 7-2.) The use of additive solutions allows recovery of maximal amounts of plasma and preparation of RBC units with a final hematocrit of about 60%, a level that provides excellent flow rates and ease of administration. All the additive systems currently licensed by the FDA allow red cell storage for 42 days at 1-6 C.

If components are prepared from blood collected into CPDA-1 or an additive system, the plasma or platelet-rich plasma must be separated from the red cells within 8 hours of collection, during which interval storage at 1-6 C is not required. For platelet preparation, the blood should be transported and stored in a manner intended to reach 20-24 C. If platelets will not be prepared, the unit

### Table 7-1. Content of Anticoagulant-Preservative Solutions (g/L)²

<table>
<thead>
<tr>
<th></th>
<th>ACD-A</th>
<th>CPD</th>
<th>CP2D</th>
<th>CPDA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisodium citrate</td>
<td>22.00</td>
<td>26.30</td>
<td>26.30</td>
<td>26.30</td>
</tr>
<tr>
<td>Citric acid</td>
<td>8.00</td>
<td>3.27</td>
<td>3.27</td>
<td>3.27</td>
</tr>
<tr>
<td>Dextrose</td>
<td>24.50</td>
<td>25.50</td>
<td>51.10</td>
<td>31.90</td>
</tr>
<tr>
<td>Monobasic sodium phosphate</td>
<td>2.22</td>
<td>2.22</td>
<td>2.22</td>
<td>0.275</td>
</tr>
<tr>
<td>Adenine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 7-2. Content of Additive Solutions (mM)²

<table>
<thead>
<tr>
<th></th>
<th>AS-1 (Adsol®)</th>
<th>AS-3 (Nutricel®)</th>
<th>AS-5 (Optisol®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose</td>
<td>111.00</td>
<td>55.50</td>
<td>45.40</td>
</tr>
<tr>
<td>Adenine</td>
<td>2.00</td>
<td>2.22</td>
<td>2.22</td>
</tr>
<tr>
<td>Monobasic sodium phosphate</td>
<td>0</td>
<td>23.00</td>
<td>0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>41.20</td>
<td>0</td>
<td>45.40</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>154.00</td>
<td>70.00</td>
<td>150.00</td>
</tr>
</tbody>
</table>

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must be transported and stored in a manner intended to cool the blood continuously toward 1-6 °C until it arrives at the processing laboratory. The additive solution must be combined with the red cells within 72 hours after phlebotomy, in order to achieve maximal allowable dating.

Preserving Red Cell Function

The maximum allowable storage time for RBCs is described as “shelf life.” The accepted criterion for maintenance of viability and function during storage is that at least 75% of the original red cells be in the recipient’s circulation 24 hours after transfusion. Certain measurable biochemical changes occur when blood is stored at 1-6 °C; these changes, of which some are reversible, are known as “storage lesion.” The most striking biochemical changes that affect stored red cells are listed in Table 7-3, but these changes rarely have clinical significance, even in massively transfused recipients.

Oxygen Dissociation

Hemoglobin becomes fully saturated with oxygen in the lungs, but characteristically releases only some of its oxygen at the lower oxygen pressure (pO₂) of normal tissues. The relationship between pO₂ and oxygen saturation of hemoglobin is shown by the oxygen dissociation curve. (See Fig 7-1.) Release of oxygen from hemoglobin at a given pO₂ is affected by ambient pH, by intracellular levels of 2,3-diphosphoglycerate (2,3-DPG), and other variables. The oxygen dissociation curve remains constant in shape, but its position relative to the X axis is affected by these variables. The Pₕ₀ is the pO₂ level at which hemoglobin is 50% saturated. A high Pₕ₀ means the curve has shifted to the right; at a given tissue pO₂, more oxygen will be released. A left shift (lower Pₕ₀) means that less oxygen than normal is released at any given pO₂.

The concentration of 2,3-DPG in the red cells affect the release of oxygen to the tissues. High levels cause greater oxygen release at a given pO₂. Lower red cell levels of 2,3-DPG increase the affinity of hemoglobin for oxygen, causing less oxygen release at the same pO₂. In red cells stored in CPDA-1 or additive systems, 2,3-DPG levels fall at a linear rate to zero after 2 weeks of storage, and oxygen release is much less than in fresh cells. Upon entering the recipient’s circulation, stored red cells regenerate ATP and 2,3-DPG, resuming normal energy metabolism and hemoglobin function as they circulate in the recipient. It takes approximately 12 hours for severely depleted red cells to regenerate half their 2,3-DPG levels, and about 24 hours for complete restoration of 2,3-DPG and normal hemoglobin function.

Rejuvenation

It is possible to restore levels of 2,3-DPG and ATP in red cells stored in CPD or CPDA-1 solutions by adding a solution containing pyruvate, inosine, phosphate, and adenine. Only one such solution is currently licensed by the FDA. RBCs can be rejuvenated during storage or up to 3 days after expiration, and can then be glycerolized and frozen in the same manner as fresh red cells. If rejuvenated red cells are to be used within 24 hours, they can be stored at 1-6 °C; however, they must be washed before use to remove the inosine, which might be toxic to the recipient.

Blood Collection

To prevent activation of the coagulation system during collection, blood should be collected rapidly and with minimal trauma to tissues. There should be a single venipuncture and frequent, gentle mixing of the blood with the anticoagulant. Although the target collection interval is
Table 7-3. Biochemical Changes of Stored Red Blood Cells

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of Storage</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>35</td>
<td>35</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>% Viable cells</td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>79</td>
<td>71</td>
<td>76 (64-85)</td>
<td>83 ± 10</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>(24 hours posttransfusion)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (measured at 37°C)</td>
<td>7.20</td>
<td>6.84</td>
<td>7.55</td>
<td>7.60</td>
<td>6.98</td>
<td>6.71</td>
<td>6.6</td>
<td>6.5</td>
<td>6.5</td>
<td></td>
</tr>
<tr>
<td>ATP (% of initial value)</td>
<td>100</td>
<td>86</td>
<td>100</td>
<td>100</td>
<td>56 (± 16)</td>
<td>45 (± 12)</td>
<td>60</td>
<td>58</td>
<td>68.5</td>
<td></td>
</tr>
<tr>
<td>2,3-DPG (% of initial value)</td>
<td>100</td>
<td>44</td>
<td>100</td>
<td>100</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td>&lt;10</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>Plasma K+ (mmol/L)</td>
<td>3.9</td>
<td>21</td>
<td>5.10</td>
<td>4.20</td>
<td>27.30</td>
<td>78.50‡</td>
<td>50</td>
<td>N/A</td>
<td>45.6</td>
<td></td>
</tr>
<tr>
<td>Plasma hemoglobin (mg/L)</td>
<td>17</td>
<td>191</td>
<td>78</td>
<td>82</td>
<td>461</td>
<td>658.0‡</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>% Hemolysis</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>0.5</td>
<td>0.8</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

* Based on information supplied by the manufacturer.
† From Simon, et al.
‡ Values for plasma hemoglobin and potassium concentrations may appear somewhat high in 35-day stored RBC units; the total plasma in these units is only about 70 mL.
usually 4-10 minutes, one study of collection time and component quality showed Platelets (PLTs) and Fresh Frozen Plasma (FFP) to be satisfactory after collection times of up to 15 minutes. Blood destined for component preparation should be drawn into a primary bag with integrally attached satellite bags so that the contents are not exposed to air or outside elements during preparation and separation of components (ie, a “closed” system). If the airtight system is entered, it becomes an “open” system; aseptic techniques and pyrogen-free equipment must be used, and allowable storage times change. Components stored at 1-6°C must be used within 24 hours, and within 4 hours if stored at 20-24°C. If the open-system pool or component will be stored frozen, they must be placed in the freezer within 6 hours after the seal is broken. When these components are thawed, they must be transfused within the timeframes for other open system components. Sterile connection (docking) devices allow the system to be entered, and bags and tubing to be introduced, without breaking the integrity of the system. The shelf life of components thus prepared is the same as those prepared in a closed system except for Platelets, which expire 4 hours after pooling.

When the final container is not the container used for collection, the source of the contents (ie, identification of the original unit) must be written on the label of the final container. Records must indicate that the transfer has been made. A secondary container must be labeled while still attached to the primary container, either with the original unit number or by some other means to relate it to the original unit number.

### Preparation of Components

Blood from individual donors is collected into systems of tubing and satel-

![Figure 7-1. Oxygen dissociation curves under different conditions.](image-url)
lite containers that can be used to process the blood into one or more of the following components: RBCs, PLTs, FFP and Cryoprecipitated AHF (CRYO). Large pools of plasma harvested from whole blood or obtained by apheresis can be processed to yield derivatives such as albumin, plasma protein fraction, Factor VIII concentrate, immune serum globulin preparations, and concentrates of Factor IX and Factor IX complex (II, VII, IX, and X). Preparation of blood components and their appropriate therapeutic use expands the number of patients who benefit from a limited resource—human blood. Centrifugation is the primary step in separation of plasma from red cells and in preparation of single-donor components.

**Centrifugation**

Rotor size, speed, and duration of spin are the critical variables in preparing components by centrifugation. Method 10.5 describes the use of the centrifuge for platelet preparation, but each centrifuge must be calibrated for optimal speeds and times of spin for each component prepared. Times listed include only the time of acceleration and “at speed,” not the deceleration time. Currently used automatic electronic braking devices allow prompt deceleration with minimal resuspension of centrifuged elements. Rapid manual braking often causes unacceptable resuspension of cells. Good safety practice requires use of interlocking mechanisms that keep the centrifuge cover from opening until the rotor has come to a complete stop.

Large centrifuges rotate at high speeds, exerting gravitational forces of thousands of pounds. Blood bags sometimes have imperfections through which centrifuge pressures may force blood bags to rupture or leak. Occasionally bags rupture or the seals between tubing segments leak during centrifugation. If leakage and rupture are a concern, overwrapping the units with plastic bags will contain any spill. Bags should be positioned so that a broad side faces the outside wall of the centrifuge to reduce centrifugal force on the sealed margins.

Contents in opposing cups must be equal in weight; uneven weight distribution impairs efficiency of centrifugation and may damage the rotor. Dry balancing material is preferable to liquids. Weighted rubber discs and large rubber bands are excellent, and are available in several thicknesses to provide flexibility in balancing without the need to cut discs. Swinging cups provide better separation between cells and plasma than fixed-angle cups. The most practical way to evaluate centrifugation is to scrutinize quality control data on components prepared in each centrifuge. For example, if platelet concentrate yields are inconsistent, the variables to be monitored include calibration of the centrifuge (see Method 10.5); the initial platelet count in the donors; storage time and conditions between blood collection and platelet preparation; and sampling technique and counting method. Records of component preparation should identify each individual performing a significant step in the preparation of components.

**Whole Blood**

The clinical indications for the use of stored Whole Blood are extremely limited. After 24-hour storage, platelet function is lost and concentration of labile coagulation factors decreases; the unit becomes a suspension of red cells in the protein solution that is liquid plasma. Transfusion of Whole Blood can be appropriate when both red cell mass and total blood volume must be restored, such as for massive hemorrhage.
Red Blood Cells

RBCs are usually separated shortly after collection of Whole Blood. The cells may be allowed to sediment during refrigerated storage of the Whole Blood, or centrifugation can be used to separate cells and plasma at any time during the shelf life of the Whole Blood. A procedure for preparation of RBCs is given in Method 9.4.

Red Blood Cells separated in a closed system and stored without additive solutions at 1-6 C have the same expiration date as the Whole Blood from which they were separated, provided the hematocrit does not exceed 80%. At hematocrits above 80%, insufficient preservative solution remains to support prolonged storage. It is important to verify consistent preparation of RBCs at the appropriate hematocrit; this can be done by testing outdated units. If additive solutions are used, the appropriate expiration date and the volume and nature of the additive must be noted on the label.

If red cells have been separated in a system that was open for any reason, the resulting unit must be transfused within 24 hours, and the new date and time of expiration must be noted on the label and in the records.

Leukocyte-Reduced Red Blood Cells

Clinical Considerations

White blood cells have long been implicated in certain adverse consequences of transfusion. The relationship between febrile nonhemolytic (FNH) reactions and number of white cells in the transfused component has long been known. Explanations include alloimmunization of the recipient to transfused HLA or granulocyte antigens, adverse effects of transfused microaggregates on the microcirculation, and fragmentation of stored donor granulocytes with release of enzymes and other bioactive compounds. Present thinking attributes most FNH reactions to the actions of cytokines, predominantly those produced by leukocytes in the transfused unit, but sometimes produced in the recipient after alloimmune interaction with the donor’s leukocytes.

Cytokines. Leukocyte reduction has been used with considerable success to prevent FNH reactions, but in many cases even near-complete removal of leukocytes, at the time stored units were transfused (poststorage leukoreduction), is ineffective. It is now known that cytokines are generated during storage, even at 1-6 C but to a much greater extent at 20-24 C. Levels of interleukin 1 alpha and beta (IL-1α and IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) rise sharply in units stored with resident leukocytes, compared with similar units stored after leukocyte reduction (prestorage leukoreduction). These studies were performed on the cells and supernatant plasma of platelet concentrates, but the results can be extrapolated to red cell components as well. Cytokine levels rise in direct proportion to the number of leukocytes.

Other Effects. Several other adverse effects have been attributed to the presence of leukocytes in components. The simultaneous presence, in the transfused unit, of antigen-presenting cells and cells that express foreign HLA antigens is thought to increase the likelihood of HLA alloimmunization in the recipient. When present in the circulating blood, cytomegalovirus (CMV) resides entirely within the cytoplasm of white cells. Removing leukocytes from cellular components, therefore, very effectively reduces the danger of transfusion-transmitted CMV infection or reinfection.

Preparation

The designation “Leukocyte-Reduced Red Blood Cells” applies to RBCs pre-
pared by a method known to retain at least 80% of the red cells originally present. When intended for prevention of febrile reactions, a unit of RBCs should contain no more than $5 \times 10^8$ leukocytes; for other purposes such as preventing transmission of CMV or alloimmunization to HLA antigens, the number of residual leukocytes should be no more than $5 \times 10^6$. The most effective way to remove leukocytes is the use of specially designed filters, although washing cells has some degree of utility.

**Filtration Methods.** High efficiency (99%) of leukocyte removal can be achieved using commercially available adsorption filters before or after storage. Multiple-bag systems with additive solution and in-line filters to remove leukocytes before red cell storage allow preparation of multiple components, including leukocyte-reduced RBCs that can be stored for up to 42 days. Another approach is to use a sterile connection device to add a filter to a unit after testing has been completed. The timing of leukocyte removal may be significant.

Leukocyte removal increases as the time between collection and depletion is shortened. During storage, many leukocytes degranulate, fragment, or die, releasing substances that may promote febrile and allergic transfusion reactions. Early removal of leukocytes does not enhance multiplication of bacteria present at the time of collection, and may reduce the likelihood of significant bacterial contamination.

Bedside filtration may also be effective in preventing reactions in multitransfused patients. However, cytokines that accumulate during storage (particularly in platelet components) may account for some failures of bedside filtration to prevent febrile reactions.

**Washing Red Blood Cells.** Washing is not the best way to prepare leukocyte-reduced RBCs because the resulting unit is expensive and time-consuming to prepare, has a short shelf life, contains up to 20% fewer red cells than the original unit, and is less leukoreduced than a filtered component. However, washing RBCs does remove most of the plasma. Transfusion of washed RBCs reduces the incidence of febrile, urticarial, and, possibly, anaphylactic reactions. IgA-deficient patients with clinically significant anti-IgA may require blood from IgA-deficient donors, but administration of RBCs washed with high volumes of saline (3000 mL or more) or of deglycerolized RBCs may be satisfactory.

Automated cell washers are more efficient than manual washing by centrifugation but are more expensive. The wash process removes most of the plasma proteins, microaggregates, and cytokines. Varying amounts of platelets and leukocytes are removed by overriding the photocell on automated machines or, in manual washing, taking care to remove the buffy coat. Storage interval before washing does not affect posttransfusion survival, but washed RBCs must be used within 24 hours because preparation is in an open system.

**Plasma Components**

Along with water and electrolytes, plasma contains albumin, globulins, coagulation factors and other proteins. Its primary use is to replace coagulation factors in patients with demonstrated deficiencies, and as a replacement fluid in therapeutic plasma exchange. Most of the coagulation factors are stable at refrigerator temperature, except Factor VIII and, to a lesser extent, Factor V. To maintain adequate levels of Factors V and VIII, freshly collected plasma must be stored frozen. Plasma should be frozen in a manner that makes it easy to detect whether there has been thawing and subsequent refreezing. Some methods to accomplish this are:
1. Press a tube into the bag during freezing to leave an indentation that disappears if the unit thaws.

2. Freeze plasma flat in a horizontal position, but store it upright. During freezing, an air bubble forms at the uppermost point; this will be on the side of the bag after horizontal freezing, but will move to the top of the bag if there has been thawing and refreezing in the vertical position.

3. Place a rubber band to constrict the bag of liquid plasma before freezing, and remove it after the unit is frozen, leaving an indentation that disappears with thawing.

The intended manner of thawing may influence the freezing protocol since the configuration of the frozen unit may be critical for use of some thawing equipment. For example, the thickness of the frozen unit restricts its fit into some microwave thawing devices.

**Fresh Frozen Plasma**

To be labeled “Fresh Frozen Plasma,” plasma must be separated from the red cells and placed at –18 °C or below within 8 hours after collection into CPD, CPDA-1, or CP2D. FFP can be prepared from Whole Blood or from plasma collected by apheresis. Stored at –18 °C or below, FFP has a dating period of 12 months after donation of the original unit of blood. If not used within 12 months, it can be redesignated as “Plasma” and, suitably relabeled, can be stored for an additional 4 years. Rapid freezing can be achieved by placing units: 1) in a dry ice-ethanol or dry ice-antifreeze bath; 2) in layers between blocks of dry ice; 3) in a blast freezer; or 4) in a mechanical freezer maintained at –65 °C or lower. If plasma is frozen in a liquid freezing bath, the container must be overwrapped for protection from chemical alteration. When a mechanical freezer is used and many units are being prepared, care must be taken to avoid undue slowing of the freezing process by introducing an excessive number of units at one time. A procedure for preparation of FFP appears in Method 9.8.

**Plasma, Liquid Plasma, Recovered Plasma, and Source Plasma**

Plasma from a single donor may be separated from the unit of Whole Blood at any time before, or up to 5 days after, the expiration date of the Whole Blood. When stored frozen at –18 °C or lower, this component is known as Plasma and may be kept up to 5 years. If not frozen, it is called Liquid Plasma; this is stored at 1-6 °C and has a shelf life no longer than 5 days after expiration of the whole blood from which it was prepared. Separation techniques are the same as for FFP (see Method 9.8).

Plasma prepared from outdated whole blood differs from plasma originally prepared as FFP, the major changes being high levels of potassium and ammonia in plasma prepared after long contact with red cells. See Table 7-3 for storage changes in refrigerated blood. If cryoprecipitate has been removed, this must be stated on the label of the Plasma unit and the residual plasma may not be designated FFP because it has been depleted of Factor VIII, fibrinogen, and other coagulation factors.

Liquid Plasma may be useful in treating stable clotting factor deficiencies for which no concentrates are available. Very few other clinical indications exist for the use of Liquid Plasma or Plasma, although controversy exists over CRYO-reduced plasma in treating thrombotic thrombocytopenic purpura (TTP). Blood centers often convert Liquid Plasma and Plasma to an unlicensed component, Recovered Plasma, which is usually shipped to a fractionator for processing into such derivatives as albu-
min and/or immune globulins. In order to ship Recovered Plasma, the collection agency must have a “short supply agreement” with the manufacturer. Since Recovered Plasma has no expiration date, records for this component must be maintained indefinitely. Plasma intended for further manufacturing use that has been collected from donors by plasmapheresis is designated Source Plasma.

**Cryoprecipitated AHF**

Cryoprecipitated AHF (CRYO) is the cold-insoluble portion of plasma that precipitates after FFP has been thawed between 1-6 C. The precipitated material contains approximately 50% of the Factor VIII (AHF), 20-40% of the fibrinogen, and some of the Factor XIII originally present in the fresh plasma. CRYO contains both the procoagulant activity (Factor VIII:C) and the von Willebrand factor of the Factor VIII molecule.

Plasma may be obtained by apheresis or from Whole Blood; CPDA-1, CPD, and ACD are suitable anticoagulants. A procedure for preparation of CRYO appears in Method 9.9.

**Quality Control of Cryoprecipitated AHF**

**Standards** requires that all tested individual units of CRYO contain a minimum of 80 IU of Factor VIII and 150 mg of fibrinogen. Samples from at least four donor units should be tested, either individually or in a pool, every month. The amount of fibrinogen in CRYO is quite variable, depending, in part, on the volume of plasma processed.

**Cryoprecipitated AHF Pooled**

Units of CRYO can be pooled prior to labeling, freezing, or storage. They should be pooled promptly after preparation, using an aseptic technique, and then refrozen immediately. The resulting component is labeled “Cryoprecipitated AHF Pooled” with the number of units pooled stated on the label. The volume of saline, if added to facilitate pooling, must appear on the label. The instruction “Use Within 4 Hours After Thawing” must be included on the label unless uniform labeling is used. In this case, the statement should appear in the Circular of Information rather than on the container label. The facility preparing the pool must maintain records of each individual donor traceable to the unique identifier used for the pooled component. For pooled CRYO units, there should be quality control evaluation of at least two containers each month. Each pool must have a Factor VIII content of at least 80 units times the number of donor units in the pool; for fibrinogen, the content should be 150 mg times the number of donor units.

**Platelets**

A platelet concentrate prepared from a single unit of Whole Blood should contain at least $5.5 \times 10^{10}$ platelets and, under optimal conditions, is expected to elevate the platelet count by approximately 5000-10,000/µL in a recipient weighing 75 kg. Platelet transfusions are used to prevent spontaneous bleeding or stop established bleeding in patients with a deficiency in platelet number or function.

Whole Blood intended for processing into Platelets should not be cooled below 20-24 C before the platelet-rich plasma is removed, and separation must occur within 8 hours after phlebotomy. The platelets may then be concentrated by additional centrifugation and removal of most of the supernatant plasma. A procedure for preparation of Platelets from single units of Whole Blood appears in Method 9.11. Calibration of a centrifuge for optimal platelet yield is described in Method 10.5.
The use of hemapheresis to prepare Platelets, Pheresis is discussed in Chapter 6.

**Drugs That Affect Platelet Function**

Several pharmacologic agents impair aspects of platelet function but the altered platelets can, in the presence of normal platelets, provide significant hemostatic effect. Damage from drug intake becomes a consideration only when the individual donor is the only source of transfused platelets for a patient. Adult patients usually receive either multiple platelet concentrates or a single preparation collected by apheresis. However, for infants and small children, the entire dose may be one platelet concentrate. Units of PLTs prepared from donors who have ingested aspirin or aspirin-containing medications within 3 days of blood donation should be labeled so that the transfusing facility will not use the unit as a patient’s sole source of platelets. Prospective apheresis donors should be deferred until the drug effects are over.

**Platelet Storage Lesion**

Collection and storage affect many aspects of platelet morphology and function. Metabolic activity continues during storage, and there is release of granule contents and discharge of cytosolic contents. Morphologic changes occur in the cytoskeleton, surface membrane, and integrity of antigens and ligands. Factors that affect the viability and function of stored platelets include: 1) anticoagulant-preservative solution, which influences pH, metabolism of glucose, lactate, and HCO₃⁻; 2) storage temperature, which influences pH, glucose consumption, and lactate production; 3) composition, size and surface area of the plastic container, which influence oxygenation and metabolism; 4) type of agitation, which affects the release reaction; and 5) volume of plasma, which influences metabolism, pH, and lactate generation.

**Leukocyte Content**

Many patients receiving repeated platelet transfusions develop a refractory state and platelet counts do not rise as expected. The platelet-refractory state may result from many factors, including splenomegaly, fever, sepsis, platelet consumption in disseminated intravascular coagulopathy (DIC), or development of antibodies against HLA or platelet-specific antigens. The use of HLA-matched donors or serologically crossmatched platelets may improve the response of patients refractory to randomly selected platelet units. In some patients, administration of leukoreduced platelets may delay or prevent alloimmunization as a cause of refractoriness. Release of cytokines from leukocytes in stored platelet preparations may be responsible for the febrile reactions that many patients experience during a platelet transfusion.

Several methods reduce the leukocyte content of platelet concentrates or hemapheresis preparations. These include centrifugation or filtration, during collection or pooling or at the time of infusion. Careful preparation of PLTs is important in minimizing the number of leukocytes remaining. The standards for residual leukocyte numbers in platelet components are the same as those for red cells.

**Quality Control of Platelet Units**

There must be at least $5.5 \times 10^{10}$ platelets per bag in at least 75% of the PLTs tested, and at least four units must be tested each month. The pH must be 6.0 or higher at the end of the allowable storage period. Leukoreduction to reduce
febrile reactions must achieve a level of $5 \times 10^8$ or fewer leukocytes per unit; to prevent alloimmunization or CMV transmission, leukocyte content should be $5 \times 10^6$ or less. Platelets should be inspected and units with grossly visible platelet aggregates after storage should not be used for transfusion.

**Granulocytes**

Granulocytes are usually prepared by leukapheresis, which is discussed in Chapter 6. Granulocytes may be stored at 20-24°C for up to 24 hours, but it is best to transfuse them as soon as possible after collection.

**Frozen Cellular Components**

When unprotected cells are frozen, damage may result from cellular dehydration and from mechanical trauma caused by intracellular ice crystals. At rates of freezing slower than 10°C/minute, extracellular water freezes before intracellular water, producing an osmotic gradient that causes water to diffuse from inside the cell to outside the cell. This leads to intracellular dehydration. Moderate to severe dehydration and resulting hypertonicity cause significant cell injury. When the temperature drops at a rapid rate, the osmotic gradient does not develop and dehydration and volume reduction are minimal. The problem with rapid freezing, however, is spontaneous formation of intracellular ice crystals and accompanying cell damage.

Prevention of freeze-thaw injury requires finding a cooling rate for each tissue or cell suspension optimal for minimizing dehydration and ice crystal formation. At the ideal cooling rate, enough water leaves the cell to produce mild intracellular hypertonicity and retard intracellular ice formation, but not so much that there is significant dehydration. Controlling the freezing rate, however, is not sufficient by itself to prevent cellular damage, so cryoprotective agents must be used.

**Cryoprotective Agents**

Cryoprotective agents are classified as penetrating and nonpenetrating. Penetrating agents such as glycerol and dimethyl sulfoxide (DMSO) are small molecules that freely cross the cell membrane into the cytoplasm. The intracellular cryoprotectant provides an osmotic force that prevents water from migrating outward as extracellular ice is formed. A high concentration prevents formation of ice crystals and consequent membrane damage. Glycerol, a trihydric alcohol, is a colorless, sweet-tasting, syrup-like fluid that is miscible with water. Pharmacologically, glycerol is relatively inert. If incompletely deglycerolized cells are infused, systemic effects are negligible except for shifts in intracellular fluid volume. DMSO is a colorless liquid with a sulfur-like smell. It is highly polar and dissolves many water- and lipid-soluble substances. Because DMSO releases heat upon mixture with water, it should be added to the cryoprotectant solution and the mixing should be cooled before it is added to a cell suspension. DMSO, given intravenously, may cause nausea, vomiting, local vasospasm, and an objectionable garlic-like odor and taste.

Nonpenetrating cryoprotective agents (eg, hydroxyethyl starch) are large macromolecules that do not enter the cell. These molecules protect the cells by a process called “vitrification” because they form a noncrystalline “glassy” shell around the cell. This prevents loss of water and dehydration injury, and alters the temperature at which the solution undergoes transition from
liquid to solid. Hydroxyethylstarch is a polymeric cryoprotectant initially used for red cells but it has also been used, in conjunction with DMSO, for the cryopreservation of hematopoietic progenitor cells.

### Freezing Red Blood Cells

Frozen preservation of red cells is primarily used for storing units with rare blood types and autologous units. Frozen cells can be effectively stockpiled for military mobilization or civilian disasters, but their high cost and the 24-hour shelf life after deglycerolization make them less useful for routine inventory management. A plastic bag system has been described that allows removal of glycerol in a closed system and storage of the deglycerolized cells for 35 days.

Table 7-4. Comparison of Two Methods of Red Blood Cell Cryopreservation

<table>
<thead>
<tr>
<th>Consideration</th>
<th>High-Concentration Glycerol</th>
<th>Low-Concentration Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final glycerol concentration (w/v)</td>
<td>Approx. 40%</td>
<td>Approx. 20%</td>
</tr>
<tr>
<td>Initial freezing temperature</td>
<td>−80°C</td>
<td>−196°C</td>
</tr>
<tr>
<td>Freezing rate</td>
<td>Slow</td>
<td>Rapid</td>
</tr>
<tr>
<td>Freezing rate control</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Type of freezer</td>
<td>Mechanical</td>
<td>Liquid nitrogen</td>
</tr>
<tr>
<td>Storage temperature (maximum)</td>
<td>−65°C</td>
<td>−120°C</td>
</tr>
<tr>
<td>Change in storage temperature</td>
<td>Can be thawed and refrozen</td>
<td>Critical</td>
</tr>
<tr>
<td>Type of storage</td>
<td>Polyvinyl chloride; polyolefin</td>
<td>Polyolefin</td>
</tr>
<tr>
<td>Shipping</td>
<td>Dry ice</td>
<td>Liquid nitrogen</td>
</tr>
<tr>
<td>Special deglycerolyzing equipment required</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Deglycerolyzing time</td>
<td>20-40 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>55-70%</td>
<td>50-70%</td>
</tr>
<tr>
<td>WBC removed</td>
<td>94-99%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Two concentrations of glycerol have been used to cryopreserve red cells, as shown in Table 7-4. This chapter, and Methods 9.6 and 9.7, discuss in detail only the high-concentration glycerol technique used by most blood banks that freeze red cells. Modifications have been developed for glycerolizing, freezing, storing, thawing, and deglycerolizing red cells. Several instruments are available that partially automate glycerolization and deglycerolization of red cells. The manufacturer of each instrument provides detailed instructions for its use. Blood intended for freezing can be collected into CPD or CPDA-1 and stored as Whole Blood or RBCs (including RBCs in AS-1 or AS-3). Ordinarily, red cells are glycerolized and frozen within 6 days of whole blood collection, but RBCs preserved in AS-1 and AS-3 have been frozen after up to 42 days of...
liquid storage, and rejuvenated red cells can be frozen for prolonged storage. Some glycerolization procedures require removal of most of the plasma or additive from the RBCs, while others do not. The concentration of glycerol used for freezing is hypertonic to blood. Its rapid introduction can cause osmotic damage to red cells, which becomes manifest as hemolysis only after thawing. Glycerol should be introduced slowly enough to allow equilibration with the red cells.

The US Department of Defense has adopted a method for high-concentration glycerolization that uses an 800 mL primary collection container suitable for freezing (see Method 9.7). Since the original container is used for both the addition of cryoprotective agent and for freezing, there is less chance of contamination and/or identification error. In addition, the amount of extracellular glycerol is smaller and it is more efficient to store and ship units prepared by this method.

Storage Containers

Composition of the storage bag affects the hemolysis associated with the freezing process; less hemolysis seems to occur in polyolefin than in polyvinyl chloride (PVC) bags. Contact between red cells and the PVC bag surface may cause an injury that slightly increases hemolysis upon deglycerolization. In addition, polyolefin bags are less brittle at –80 C and less likely to break during shipment and handling than PVC bags.

Freezing and Storage

Red cells with a final glycerol concentration of 40% (w/v) should be stored at temperatures of –65 C or lower. Metal canisters are usually used to protect the plastic bag during freezing, storage, and thawing. Although up to 18 hours at room temperature can elapse between glycerolizing and freezing without increased postthaw hemolysis, an interval no longer than 4 hours is recommended. With the currently used high-concentration glycerol methods, control of the freezing rate is unnecessary and freezing can be accomplished simply by placing the RBC container into a –80 C freezer.

The FDA licenses Red Blood Cells, Frozen for storage up to 10 years, and AABB Standards allows up to 10 years of frozen storage of blood for routine transfusion. Units stored for up to 21 years have been transfused successfully, and for blood of rare phenotypes, a facility’s medical director may wish to extend the storage period. The distinctive nature of such units and the reason for retaining them past the 10-year storage period should be documented. As units are put into long-term storage, it is prudent to freeze samples of serum that can be subjected to any new donor screening tests that are introduced in the future. Frozen rare RBCs that have not been tested for new markers should be transfused only after weighing the risks and benefits to the patient and after consideration of available alternatives. The label should indicate that the unit has not been tested.

Thawing and Deglycerolizing

The protective canister and enclosed frozen cells may be placed directly in a 37 C dry warmer or can be overwrapped and immersed in a 37 C waterbath. Units frozen in the primary collection bag system should be thawed at 42 C. The thawing process takes at least 20-25 minutes and should not exceed 40 minutes. Gentle agitation may be used to speed thawing, prolonged immersion is not recommended. The thawed cells will contain a high concentration of glycerol, which must be gradually reduced to
avoid in-vivo or in-vitro hemolysis. Deglycerolization is achieved by washing the red cells in a series of solutions of decreasing osmolarity. In one commonly used procedure, a wash with 150 mL of 12% saline is followed by 1 L of 1.6% saline, and then by 1 L of 0.2% dextrose in 0.9% saline. The progressive decrease in osmolarity of the washing solutions causes osmotic swelling of the cells, so each solution must be added slowly with adequate time allowed for mixing and osmotic equilibration. Any of the commercially available instruments for batch or continuous-flow washing can be used to deglycerolize red cells frozen in a high concentration of glycerol. Since there are so many small but potentially important variations in deglycerolization protocols for each instrument, personnel in each facility should not only follow the manufacturer’s instructions, but should also validate their process locally.

When deglycerolization is complete, the integrally connected tubing should be filled with an aliquot of red cells and sealed in such a manner that it can be detached for subsequent compatibility testing. The label must identify both the collecting facility and the facility that prepares the deglycerolized unit. When glycerolized frozen red cells from persons with sickle trait are thawed and deglycerolized, they form a jelly-like mass and hemolyze. In some cryopreservation programs, donations are screened for the presence of hemoglobin S before freezing is undertaken.

Storing Deglycerolized Red Cells. When glycerolizing or deglycerolizing involves entering the container, the system is considered “open” and the resulting suspension of deglycerolized cells can be stored for only 24 hours at 1-6 C. A longer postthaw shelf life would make frozen RBCs more useful for inventory management. Sterile connection devices or plastic containers of suitable configuration can be used for preparation and subsequent deglycerolization in a closed system. When deglycerolized cells are stored at 1-6 C for periods up to 14 days, the major observed change is increased concentration of potassium and hemoglobin in the supernatant fluid. Deglycerolized cells have been stored in AS-1, AS-3, or experimental additive solutions, and have had posttransfusion survival of well over 70% after 24 hours. Red cells that have undergone gamma irradiation and subsequent storage at 1-6 C tolerate freezing with no more detectable damage than unirradiated cells.

Refreezing Deglycerolized Red Cells. It may occasionally be desirable to refreeze thawed RBC units that have not been used as expected or have been unintentionally thawed. Units that were deglycerolized, stored 20 hours at refrigerator temperature, and then reglycerolized and refrozen showed no loss of ATP, 2,3-DPG, or in-vivo survival, and red cells subjected three times to glycerolizing, freezing, and thawing exhibited 27% loss of total hemoglobin. AABB Standards does not address refreezing thawed units, since this should not be considered a routine practice. If thawed units are refrozen, the records should document the valuable nature of such units and the reasons for refreezing them.

Transportation

Red cells cryopreserved with high-concentration of glycerol tolerate fluctuations in temperature between –85 C and –20 C without significant effects on in-vitro recovery or 24-hour posttransfusion survival. Frozen RBCs can be transported on dry ice or in a container that maintains the temperature at –65 C or lower. AABB Standards requires that components ordinarily stored frozen should be transported in a manner designed to maintain them frozen.
Clinical Considerations

Deglycerolized red cells are nearly comparable in volume, hematocrit, and efficacy to standard, liquid-stored red cells. Virtually all the plasma and anticoagulant and most of the leukocytes and platelets have been removed. Consequently, they are generally safe for IgA-deficient patients with clinically significant anti-IgA antibodies or for patients with severe immune reactions to transfused plasma proteins. Freshly deglycerolized cells have reduced levels of potassium in the supernatant fluid, but potassium levels rise as the cells are stored after deglycerolization.

In-vivo survival and function are comparable to freshly drawn, liquid-stored red cells because ATP levels, 2,3-DPG content, and oxygen dissociation curves are unchanged from pre-freeze values. The process of cryopreservation results in some hemolysis, so total red cell mass of a deglycerolized unit is never as large as the original RBC unit. With less than 1% of the original leukocyte and platelet content, deglycerolized red cells have been used to prevent some alloimmune complications. Recurrent febrile nonhemolytic reactions, transmission of CMV, and HLA alloimmunization are reduced when deglycerolized red cells are transfused, but high-performance leukoreduction filters are both less expensive and more effective at leukoreduction than freezing and deglycerolization. Some viable lymphocytes remain after freezing and deglycerolization, and deglycerolization cannot substitute for gamma irradiation to prevent graft-vs-host disease.

Cryopreservation of Platelets

Perhaps because of their greater complexity, platelets appear to sustain greater injury during cryopreservation than red cells, although several protocols have successfully used DMSO as a cryoprotectant. Because postthaw platelet recovery and function are significantly reduced as compared with liquid-stored platelets, the clinical use of cryopreserved platelets is not widespread. The major application is autologous platelet support of patients undergoing chemotherapy or bone marrow transplantation.

Cryopreservation of Hematopoietic Progenitor Cells

See Chapter 23.

Labels

The following information is required in clear, readable letters on a label firmly attached to the container of all blood and component units:

1. The proper name of the component, in a prominent position.
2. A unique numeric or alphanumeric identification that relates the original unit to the donor and each component to the original unit.
3. The amount of blood collected, which shall be accurate within ±10%, and the kind and quantity of anticoagulant (not required for CRYO or for frozen deglycerolized, rejuvenated, or washed RBCs).
4. For Platelets, Low Volume Red Blood Cells, Fresh Frozen Plasma, pooled components, and components prepared by apheresis, the volume of component in container, which shall be accurate within ±10%, except that the quantity of cryoprecipitate need not be stated on the label.
5. The expiration date, including the date and year; if the shelf life is 72 hours or less, the hour of expiration must be stated.
6. Recommended storage temperature.
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7. ABO and Rh type (Rh type not required for CRYO) and interpretation of unexpected RBC antibody tests when positive (not required for frozen deglycerolized, rejuvenated, or washed RBCs).

8. Results of unusual tests or procedures performed when necessary for safe and effective use. Routine tests done to ensure the safety of the unit need not be on the label if they are listed in the Circular of Information.

9. Reference to the Circular of Information, which must be available for distribution and contains information about dosage, directions for use, route of administration, and contraindications.

10. Essential instructions or precautions for use, including the warning that the component may transmit infectious agents, and the two statements: “Caution: Federal law prohibits dispensing without a prescription” and “Properly Identify Intended Recipient.”

11. The appropriate donor classification statement, “autologous donor,” “paid donor,” or “volunteer donor” in type no less prominent than that used for the proper name of the component.

12. If intended for autologous use, the statement “For Autologous Use Only.”

13. Any additives, sedimenting agents, or cryoprotective agents that might still be present in the component.

14. For pooled components, the name and final volume of the component and a unique identifier for the pool. The number of units in the pool, and their ABO and Rh type (Rh type not required for CRYO) must be on the label or an attached tie tag. Identification numbers of the individual units in the pool should not be on the label but must be in the records of the facility preparing the pool.

15. For licensed components, the name, address, and FDA license number of the facility that collected the blood and/or prepared the component. For components, the label must include the name and location of all facilities performing any part of component preparation, but there should not be more than two alphanumeric identifiers on the unit.

Storage and Shipment

Freezers and Refrigerators

The same refrigerator may be used to store blood and blood components, blood derivatives, blood samples from patients and donors, and reagents for blood bank tests. Separate, well-demarcated sections must be used if anything other than transfusable components are stored. In a refrigerator, the temperature in all areas must be between 1 and 6 C; either a fan or the capacity and design of the unit must ensure that the designated temperature is maintained throughout. The interior should be clean and adequately lighted, and storage areas should be clearly organized and designated for: 1) unprocessed blood, 2) labeled blood suitable for allogeneic transfusion, 3) rejected, outdated, or quarantined blood, and 4) autologous blood.

If blood is kept in sites outside the blood bank, such as surgical or obstetric suites, it must be stored in refrigerators that meet the same standards. Temperature records are required for such refrigerators at all times when blood is present. Blood must never be stored in unmonitored refrigerators. It is usually more practical for blood bank personnel to be responsible for monitoring these refrigerators.
Monitoring Temperature

Recording thermometers and audible alarms are required for all blood storage equipment. Because warmer air rises, the sensors in a refrigerator should be on a high shelf and must be immersed in a volume of liquid no greater than the volume of the smallest component stored. RBC units are usually 250-350 mL, but if split units or pediatric units are stored, the sensors should be kept in a unit with smaller volume. Either a glass container or a plastic blood bag may be used.

The alarm signal must be activated at a temperature that allows personnel to take proper action before the stored blood reaches undesirable temperatures. An acceptable range is 1-6 °C. The electrical source for the alarm system must be separate from that of the refrigerator; either a continuously rechargeable battery or an independent electrical circuit served by an emergency generator is suitable.

In large refrigerators or freezers it is advisable to have at least two independent thermometers, one immersed with the continuous sensor and the other in a similar container on the lowest shelf on which blood is stored. The temperatures in both areas must be between 1 and 6 °C at all times. For large walk-in refrigerators, enough thermometers should be placed to detect all areas of temperature fluctuation. Thermometers should be checked periodically. If the thermometer immersed with the recorder sensor does not agree within 1 °C with that shown on the automatic recorder, both should be checked against a National Institute of Standards and Technology certified thermometer, and suitable corrective action taken. It is desirable to record the temperatures from the two independent thermometers on the recorder chart when the chart paper is changed regularly. When temperature charts from recording devices are changed, they should be dated inclusively and labeled to identify the facility, the specific refrigerator or freezer, and the person changing the charts. Any departure from normal temperature should be explained in writing on the chart beside the tracing. A chart that habitually traces a perfect circle suggests that the recorder is not functioning properly or is not sensitive enough to record the slight variations in temperature that occur in any actively used refrigerator.

Some refrigerators have, in addition to automatic alarms, automated temperature monitor and digital readout systems, with continuous surveillance at preset areas within the unit. Another approach is a central monitor and alarm system capable of monitoring numerous refrigerators simultaneously. When temperatures are monitored continuously, there must be a hard-copy record of the temperature at least every 4 hours. Temperature records must be retained as part of the blood bank records for at least 5 years.

Quality Control of Controlled-Temperature Equipment

Thermometers and alarms should be checked periodically for proper functioning. Method 10.1 details a way to check temperatures of activation for refrigerator alarms.

Freezers must be equipped with a continuously recording thermometer and an audible alarm. Ideally, the alarm sensor should be accessible and located near the door of the freezer, but some older units have the sensor between the inner and outer freezer walls where it is neither apparent nor accessible. In these cases, the location of the sensor can be obtained from the manufacturer and a permanent mark placed on the wall at that location. The approximate temperature of activation can be determined by
placing a flexible container (ie, water bottle) filled with cold tap water against the inner freezer wall where the sensor is located. When the alarm goes off, usually in a short time, the recording chart should be checked immediately to determine the temperature of activation. More details about checking freezer alarms are in Method 10.2. A thermocouple that responds at the desired temperature range is useful for freezers held at –65 C or below. A digital recording device measures the difference in potential generated by the thermocouple, and this difference can then be converted to temperature.

If there is no source of emergency power independent of standard house circuits, an alternative plan for storing blood and components must be included in the procedures manual for quick reference and use. Alarms must have a continuous power source, which should be tested periodically to ensure proper function. There must be written instructions for personnel to follow when the alarm sounds. These instructions should include steps to determine the immediate cause of the temperature change and ways to handle temporary malfunctions, as well as steps to take in the event of prolonged power failure. It is important to list the names of key people to be notified and what steps should be taken to ensure that proper storage temperature is maintained for all blood, components and reagents.

**Blood and Component Inspection**

All stored blood and components should be inspected immediately before issue for transfusion or shipment to other facilities. Preissue inspections must be documented; records should include the date, donor number, description of any abnormal units, the action taken, and the identity of personnel involved. Whole Blood or RBC units abnormal in color or other appearance should not be transfused. Contamination should be suspected if the color of the segments is much lighter than that of the bag, if the red cell mass looks purple; if a zone of hemolysis is observed just above the cell mass; if clots are visible; or if the plasma or supernatant fluid is murky. Purple, brown, or red plasma suggests unsuitability for transfusion. Although a green hue from light-induced changes in bilirubin pigments need not cause the unit to be rejected, units with grossly lipemic plasma, identified by its milky appearance, are usually considered unsuitable for transfusion. The presence of blood or plasma in the ports or at sealing sites in the tubing suggests inadequate sealing or closure, and the unit should, at the very least, be quarantined. Visual inspection cannot always detect contamination or other deleterious conditions.

**Platelet Storage**

Platelets from single units or collected by hemapheresis are stored at 20-24 C with continuous gentle agitation. The collection system and the type of plastic used determine the expiration date of the component. Elliptical, circular, and flat-bed agitators are available. Elliptical rotators are not recommended for use with storage bags made of polyolefin without plasticizer (PL-732 or PL-2209). If the seal of any bag is broken, the Platelets should be used as soon as possible, within 4 hours if stored at 20-24 C. The temperature in the immediate vicinity of the platelet storage area must be monitored and recorded, to ensure continuous maintenance of appropriate storage conditions.
the plastic bag should be inspected for cracks. Unusual turbidity of the thawed component may be cause for discard.

**Bacteriologic Examination**

Bacterial contamination of transfusion components is rare, due to the use of aseptic technique, closed systems for collection and preparation, and careful monitoring of storage conditions. A rising number of reports of bacterial sepsis has paralleled the rise in the number of platelet transfusions. Various studies of bacterial contamination give positive culture results in 0-10% of platelet concentrates, 0-4.9% of apheresis platelets, and 0-0.2% of deglycerolized RBC units.

Routine sterility testing of blood or components is not required by the AABB, but is required by the FDA in the licensing of certain blood products. If a transfusion component has an abnormal appearance, or if an adverse clinical reaction appears to be related to contaminated donor blood, culturing may be desirable, and a Gram’s-stained smear of supernatant plasma should be examined. Making separate cultures from a sealed segment and from the contents of the bag can give useful diagnostic information. Positive cultures should arouse suspicion of inadequate donor arm preparation, problems with disposables or storage conditions, or improper pooling technique. The donor’s health should be reviewed, and other components prepared from that collection should be evaluated.

**Shipping**

**From Mobile Collection Facilities**

Blood should be transported from the collection site to the component preparation laboratory as soon as possible. Unless platelet preparation is planned, units should be cooled promptly. For unrefrigerated units, the time between collection and the separation of components must not exceed 8 hours.

**Whole Blood or Red Blood Cells**

Blood should not unnecessarily remain at room temperature. It is desirable to observe routine operations to ensure that personnel do not allow blood to stay out of the refrigerator too long. This can be monitored by including a fluid-filled container with a thermometer in the same batch as units of blood removed from refrigeration for testing or labeling. If the temperature in this container rises to near 6 C, the blood should be returned to the refrigerator without delay.

Blood issued for transfusion should not be allowed to remain unnecessarily long at room temperature. Delays in delivery to the patient, in assembly of equipment or personnel to begin transfusion, or infusion are all undesirable. Mishandling of donor units can be reduced by transfusion therapy teams trained in handling and infusing blood and components.

**Platelets and Granulocytes**

Every reasonable effort must be made to ensure that Platelets and Granulocytes are maintained at 20-24 C during shipment. A well-insulated container without added ice, or with a specific coolant designed to keep the temperature at 20 to 24 C, is recommended. Containers with a mobile power source can accurately maintain temperatures between 20 and 24 C.

**Frozen Components**

Frozen components must be maintained during transport at or below the required storage temperature. This can be achieved with a suitable quantity of dry ice in well-insulated containers or in
standard shipping cartons lined with insulating material such as plastic air bubble packaging or dry packaging fragments. The dry ice, obtained as sheets, should be layered at the bottom of the container, between each layer of frozen components, and on top.

Shipping facilities should determine optimal conditions for shipping frozen components, depending on the temperature requirements of the component, the distance to be shipped, the shipping container used, and the ambient temperature encountered. All procedures and shipping containers should be validated and periodically monitored. The receiving facility should always observe the shipment temperature, and report unacceptable findings to the shipping facility.

**Thawing Fresh Frozen Plasma for Transfusion**

FFP must be thawed either at temperatures between 30 and 37 C or in an FDA-approved microwave device. Plasma thawed in a waterbath must be protected from water contamination of the entry port. This can be accomplished by wrapping the container in a plastic overwrap, or by positioning the container upright with entry ports above the water level. Microwave devices should be shown not to exceed temperature limits and not to damage the plasma proteins, and there should be a warning device to indicate if the temperature rises unacceptably. As for any device, there should be a procedure for quality control of the indicated functions.

**Thawing Cryoprecipitated AHF**

CRYO must be thawed at temperatures between 30 and 37 C, but should remain at that temperature for no more than 15 minutes. Thawed CRYO is stored at room temperature and should be transfused as soon as possible, within 6 hours if it is used as a source of Factor VIII. If entered for pooling or any other reason, the component can be kept only 4 hours at 20-24 C.

**Disposition**

Exposure to temperatures above 10 C does not necessarily render blood unsuitable for transfusion, but if the units reach temperatures outside the 1 to 10 C range, they should be discarded. Exceptions may be made under unusual circumstances, such as for autologous units or cells of rare phenotype, but the records should document the reasons for preserving the unit, the evaluation of its continued suitability for transfusion, and the identity of the person responsible for the decision. Factors to consider include the length of time in shipment, mode of transportation, magnitude of variance over 10 C, presence of residual ice in the shipping box, and the appearance of the unit(s). The age of the unit and its probable subsequent storage before transfusion should also be considered. The shipping facility should be notified when a receiving facility observes unacceptably high temperatures.

Units that are questionable for any reason should be quarantined until a responsible person decides their disposition. Evaluation of a questionable unit should include inverting it gently a few times to mix the cells with the supernatant fluid because a great deal of undetected hemolysis, clotting, or other alterations may have occurred in the undisturbed red cell mass. If, after resuspension and resettling, the blood no longer appears abnormal, it may be returned to inventory. Appropriate records should be maintained.

When blood cannot be released for transfusion, it should be returned to the provider or the nature of the problem should be investigated and the results reported to the blood supplier. Findings may indicate the need for improvement in do-
nor techniques, in screening of donors or in handling blood units during processing. Disposal procedures must conform to the local public health codes for biohazardous waste. Autoclaving and/or incineration is recommended. If disposal is carried out off-site, a contract with the waste disposal firm must be available and should specify that appropriate Environmental Protection Agency, state and local regulations are followed.

Reissuing Red Blood Cells or Whole Blood
Units that have left the blood bank and been returned to the blood bank must not be reissued for transfusion unless the following conditions have been met:

1. The container closure has not been penetrated or entered in any manner.
2. The blood has been maintained continuously between 1 and 10 C, preferably 1-6 C. Blood centers and transfusion services should not reissue red cells that have remained out of a monitored refrigerator for longer than 30 minutes because, past that time, the temperature of the component will rise above 10 C.
3. At least one sealed segment of integral donor tubing remains attached to the container, if the blood has left the premises of the issuing facility.
4. Records indicate that the blood has been reissued and has been inspected prior to reissue.

Records
Records must be made concurrently with each step of component preparation, must be legible and indelible, must identify the person immediately responsible, must include dates of various steps and must be as detailed as necessary for clear understanding. For specific records to be kept for component preparation, storage, and shipment, see Chapter 1.

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37. Myhre BA, Nakasako YUY, Schott R. Studies on 4 C stored frozen reconstituted red blood cells. III. Changes occurring in units which have been repeatedly frozen and thawed. Transfusion 1978;18:199-203.