Preparation and Storage of Whole Blood Components

Revised: 6/26/15

Anticoagulants and Preservatives

- Whole blood is collected into a bag that contains an approved anticoagulant-preservative solution
  - Prevents clotting
  - Maintains cell viability and function

Anticoagulant-Preservative Solns.

	CPD	CP2D	CPDA-1
Ratio(mL soln to blood)	1.4:10	1.4:10	1.4:10
Shelf Life (days)	21	21	35
Content			
Sodium citrate	1660	1660	1660
Citric acid	188	188	188
Dextrose	1610	3220	2010
Monobasic sodium phosphate	140	140	140
Adenine	0	0	17.3

Effects

- RBCs continue metabolic activity during storage, despite low temps
  - Dextrose – ATP generation via glycolytic pathway
  - Adenine – substrate for ATP synthesis
    - Results in improved RBC viability
  - Sodium biphosphate – buffer to control ↓ in pH
    - From production of lactic acid
  - Citrate – prevents coagulation by chelating Ca^{++}

Additive Solutions (AS)

- Contain sodium chloride, dextrose, adenine, others to support RBC survival
- Outdate is 42 days
- Final product is RBC unit with hct of about 60%
  - Easy administration

Whole Blood Processing

- Whole blood is collected into primary bags to simplify the separation or fractionation into component parts
- Centrifugation
  - Specific gravities
    - RBCs – 1.08-1.09
    - Platelets – 1.03-1.04
    - Plasma – 1.023

Production

- Each centrifuge must be QC'd for optimal speed and time (check periodically)
- Records of all events involving the donor unit and its components must be kept for at least 5 years

Purpose of Separate Components

- Mission is to provide for each patient's specific needs while maximizing donor resources
- Benefit for several reasons:
  - Separation allows optimal survival of each component;
  - Patient only needs a specific portion
  - Able to avoid unnecessary transfusions that may cause problems (hypervolemia)

Whole Blood (WB)

- Contains all blood elements
- Most often used as source for component production
- After 24 hours, essentially becomes red cells suspended in a protein solution equivalent to liquid plasma
  - Minimum hct = 33%

Red Blood Cells (RBCs)

- Units of WB with most of plasma removed
- CPD, CP2D, or CPDA-1
  - Hct must be ≤80% in CPDA-1 units
- AS-RBCs have less plasma
  - Hct 55-65% because of added AS
  - AS must be added within first 72 hrs of storage
- Stored at 1-6C

Pre-Storage RBCs Leukocytes Reduced (LR)

- In-line filtration of WB
  - Filtered blood can be manufactured into RBCs and plasma
  - Plts remain in filter
- Sterile-docked filter – WB or RBCs
  - Should occur as soon as possible after collection
- In-line filtration of RBCs
  - Plasma is removed first
  - Additive transferred to RBCs
  - RBCs run through filter

QC of RBCs LR

- Should contain $<5x10^6$ residual wbcs
- Retain 85% of original red cells
- 95% of units sampled should meet this specification

AABB TM (17th ed.)

Plasma Components

- FFP
  - Prepared from WB or apheresis collection
  - Frozen at –18C or colder
  - Contain maximum levels of labile and nonlabile clotting factors
    - About 1 IU/mL
  - Shelf life of 12 mos from date of collection
  - Frozen and maintained at –65C or colder – 7 years

Thawed FFP

- Thawed at temps between 30-37C OR
- In an FDA-approved microwave
- Outdate is 24 hours from time of thaw
- Stored at 1-6C if not tx'ed immediately
- Thawed in waterbath
  - Protect ports from water contamination

Thawed Plasma

- FFP that has been thawed but not tx'ed within 24 hours
- Stored at 1-6C for 5 days from time of thaw
- Similar to FFP except for reduction in Factor V and VIII, particularly VIII

Plasma Frozen Within 24 Hours

- Separated and frozen at –18C or colder between 8 and 24 hours of collection
- Contains all stable proteins found in FFP
- Once thawed, tx immediately or store at 1-6C for up to 5 days

Cryoprecipitated AHF

- Cold-insoluble portion of plasma that precipitates when FFP is thawed between 1-6C
- Also called CRYO
  - Prepared from single WB unit
  - Suspended in <15mL plasma
- Contains
  - ≥80IU Factor VIII (AHF)
  - ≥150 mg of fibrinogen
  - Most of Factor XIII originally present in fresh plasma
- CRYO is refrozen w/in 1hr of production, stored at –18C or colder from 1 yr after date of phleb

Thawing and Pooling CRYO

- Thawed at 30-37C
  - Do not allow to remain at that temp after thaw to minimize degradation of Factor VIII
  - Protect ports from water
- Single unit
  - Stored at RT for 6 hours
- Pooled CRYO
  - Stored at RT for 4 hours

Platelets (Plts)

- Prepared from WB that has not cooled below 20C
  - Platelet-rich plasma (PRP) is separated w/in 4 hours of collection
  - Plts are concentrated by centrifugation and most of supernatant plasma is removed
  - Final component is 45-65mL
    - Must have enough plasma to maintain pH and normal levels of stable coag factors
  - Not made from collections that take >10 min
- Storage is 20-24C with gentle continuous agitation for 5 days after phlebotomy

Drugs That Affect Plt Function

- Many drugs impair plt function, but impaired plts can still help when in presence of normal plts
- Imparied plts must not be sole source of plts for a pt
  - Newborns
- Aspirin
  - Must be labeled as such

Other Plt Information

- QC
  - 90% of plts tested must have:
    - $\geq 5.5 \times 10^{10}$ plts
    - Plasma pH of ≥ 6.2 at end of storage
- Modifications
  - Pooled (4 hours outdate [OD] from time of pool)
  - Irradiated
  - Leukocyte-reduced
  - Volume-reduced

Granulocytes

- Collected by apheresis
  - Contains leukocytes, plts, and rbcs
  - $1.0x10^{10}$ – granulocytes
- Can also be harvested from buffy coats of fresh WB units
  - $1.25x10^{9}$ granulocytes
  - For newborns in urgent situations
- Effectiveness is controversial
- 20-24C without agitation for up to 24hrs

Special Components

- CMV-Negative
  - From seronegative donors
- Washed
  - RBCs
    - Removes 99% of plasma proteins, electrolytes, and abs
    - Can lose up to 20% of unit
    - OD is 24 hours
  - Plts

Irradiation

- Components that contain viable lymphocytes
  - RBCs, plts, granulocytes, non-frozen plasma
- Prevent proliferation of tx'ed T lymphs
- Prevents GVHD
- Some damage is done to RBCs and viability is affected
  - Outdate is original expiration or 28 days, whichever comes first

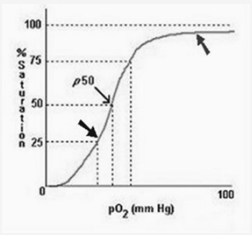
Effects of Storage on Blood Components

- "Storage lesion"
  - Biochemical changes that occur during RBC storage – some changes are reversible
    - ↓ in % viable cells
    - ↓ pH
    - ↓ATP
    - ↓ 2,3-DPG
    - ↑ plasma K^+
    - ↑ plasma hgb

Oxygen Dissociation

- Release of oxygen from hgb at a given pO_2 is affected by:
  - Ambient pH
  - Intracellular levels of 2,3-DPG
  - Other variables

Oxygen Dissociation Curve



2,3-DPG

- Increased amounts cause greater oxygen release
- Decreased amounts increase hgb affinity for oxygen
- In stored cells, 2,3-DPG levels fall
  - Oxygen release is much less than in fresh cells
  - When stored cells enter recipient circulation, stored RBCs regenerate ATP and 2,3-DPG
    - Takes about 24 hours to return to normal levels