RBC Depletion

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Why RBC Deplete?

WHY

- Recipient safety
  - To prevent hemolytic reactions due to antibodies in the recipient (major ABO mismatch)
- Volume reduction of umbilical cord blood units
  - Long-term storage issues
Why RBC Deplete?

WHAT

- RBC depletion is performed on high RBC content
- Bone marrow
- Apheresis products
- Cord bloods

Caveat: it’s better to split an apheresis product than to RBC deplete due to relatively few mls of RBC and ACD-A interference.
Why RBC Deplete?

WHO

- ABO mismatched recipient
- Antibody sensitized recipient
- Umbilical cord blood
RBC patient limits

Clinical Guidelines

Adult (Hillman Cancer Center, University of Pittsburgh Cancer Institute)
- 20ml/recipient

Pediatric (Children’s Hospital of Pittsburgh)
- smaller volume of 20ml/recipient or 0.3ml/kg actual body weight

MCW
- 20ml/recipient or 0.3ml/kg actual body weight
Methods of RBC Depletion

- Hetastarch
- Hetastarch with centrifugation
- Cell Washer
- Cell Washer with Density Gradient
- Ficoll-Hypaque
- Others
  - Sepax
  - Optipress
  - Filters
Hetastarch

Advantages
• Errors other than leakage are recoverable
• Not extremely operator dependent
• Closed system
• Non-toxic

Disadvantages
• Long sedimentation period
• Citrate-containing anticoagulants interfere with rouleaux formation
Hetastarch

Materials
• Hetastarch
• 1L Transfer pack(s)
• Plasma extractor
• Analytical balance
• Hemostats
• 1L or 2L Transfer pack for collection
Hetastarch

Method

• Split product into equal parts that will fit into a 1L or smaller bag
• Add 20% Hetastarch
• Mix well
• Hang from bar in hood or, lacking that, an IV pole
• The RBCs will rouleaux with the hetastarch, becoming heavier than the remaining cells
• Allow to gravity sediment for 45 minutes
• Using the plasma expressor, squeeze out the clear layer, and clamp off the line with a hemostat prior to any introduction of the RBC layer into the collection bag
• Determine recovery of WBC and/or CD34s. This procedure can be repeated on the “waste” product by addition of saline, homogenization and re-rouleaux of the RBCs. This can increase recovery to the expected outcome.
Hetastarch

Expected outcomes

- **Process:**
  - HCT should be ≤ 6%
  - WBC recovery should be ≥ 80%
- **Product:**
  - Packed erythrocyte volume ≤ 20 mL

Time

- **Single Process:**
  - 45 minute gravity sedimentation
  - ~65 minutes total
- **Double Process**
  - 90 minute gravity sedimentation
  - ~120 minutes total
Hetastarch with Centrifugation

Advantages
• Same advantages as hetastarch alone
• Rapid processing for time critical procedures (such as intra-operative)
• Great for small volumes of bone marrow

Disadvantages
• Increased risk of bag breakage due to centrifugation
• Cumbersome for product volumes over 250ml
• Citrate-containing anticoagulants interfere with rouleaux formation
Hetastarch with Centrifugation

Materials
- Hetastarch
- 300ml Transfer pack(s)
- Plasma extractor
- Analytical balance
- Floor model centrifuge capable of spinning bags
- Hemostats
- Transfer pack for collection
Hetastarch with Centrifugation

Method

• 20% by volume HES added to product
• Centrifuge at 50g for 7 minutes
• Using plasma expressor, squeeze or syringe out stem cell rich fraction
• Test for WBC recovery, CD34 recovery
Hetastarch with Centrifugation

Expected outcomes

• Process:
  - HCT should be ≤ 6%
  - WBC recovery should be ≥ 80%
• Product:
  - Packed erythrocyte volume ≤ 20 mL

Time

• Single Process:
  - 7 minute centrifugation
  - ~20 minutes total
• Double Process
  - 14 minute centrifugation
  - ~35 minutes total
Cell Washer

Advantages
• Closed system
• Errors other than leakages are recoverable

Disadvantages
• Time
• Older technology
• Requires significant investment in equipment
Cell Washer

Materials
• COBE 2991
• “Donut” bag and tubing
• Hemostats
• Transfer pack
• Saline
Cell Washer

Method

• Priming of lines
• Dilution of product with saline
• Gradual addition of product to donut bag with periodic removal of plasma
• Centrifugation of donut bag
• Once buffy coat formed, slow removal of buffy coat through tubing to transfer pack as centrifugation continues
Cell Washer

Outcomes
- 110ml
- Goal is $>2 \times 10^8$ nucleated cells/kg for transplant
- Minimum acceptable $1 \times 10^8$

Time
- ~2 hours

Expected Recovery
- WBC >40%
- CD34s >50%

Expected RBC content
- RBC < 20ml
Density Gradient

Advantages
• Familiar process, common in research labs
• GMP grade now available
• Common equipment
• Open system

Disadvantages
• Usually reserved for research use or processing of products under an IND, not routine clinical use
• Tube method rarely used for large volume products
• Operator dependent
• Known toxicity to cells
Density Gradient

Materials

• Conical tubes
• Density gradient medium (Ficoll-Hypaque)
• Pipettes and pipettors
• Centrifuge, floor model or counter top
Density Gradient

Method

- Under or overlayer the ficoll, depending on the operator’s preference and skill level or the lab’s SOP
- Centrifuge for validated time
- “No brake” set on the centrifuge
- Harvest buffy coat using pipettes
Density Gradient

Cells to be separated

Density cushion

Plasma/Medium/Platelets

Mononuclear Cells

Red Cells/Granulocytes

BEFORE  AFTER

*Photo courtesy of Adrian Gee, M.I.Biol., Ph.D.,
CAGT, Baylor College of Medicine
Density Gradient

Expected outcomes

Recovery of WBC/CD34s
- A cellular (TNC) recovery of at least 10%
- CD34 recovery of at least 25%

Time
- ~90 minutes

Reduction in RBC content
- at least 90%
Cell Washer with Density Gradient

Advantages
• Closed system
• Large volume
• Errors other than leakages are recoverable

Disadvantages
• Time
• Older technology
• Requires significant investment in equipment
Cell Washer with Density Gradient

Materials

- **COBE 2991**
- Triple set “donut” bag and tubing
- Hemostats
- Transfer pack
- Saline
- Density gradient medium
Cell Washer with Density Gradient

Method

• Buffy coat prep to reduce volume (bag 1)
  – Collect deeper into RBC layer to reduce cell loss
  – Suspend to ≤300 mL in saline

• Add density gradient medium to donut bag, turn on centrifuge. (bag 2)

• Gradually introduce diluted buffy coat using peristaltic pump

• Collect supernatant to waste & interface to collection bag

• Wash interface (bag 3)
Cell Washer with Density Gradient

Expected outcomes
- Volume <100 mL
- Extensive RBC depletion

Time
- ~2.5 hours

Expected Recovery
- WBC ~25%
- CD34s ~65%

Expected RBC content
- RBC < 5 ml
Summary

Methods

- Hetastarch
- Hetastarch with centrifugation
- Cell Washer
- Density Gradient
- Cell Washer with Density Gradient
QUESTIONS?