# Hematopoietic Progenitor Cell Product Characterization

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# Product Testing and Characterization Goals

- Required to ensure product:
  - Safety
  - Purity
  - Potency
- Questions to be addressed this talk:
  - What to test
  - When to test
  - How to test

# What Testing (1)

- Function of product type and processing
- All Product Types and Processing
  - TNC
  - Viability
  - Sterility
- HPC Products {HPC(A), HPC(M), HPC(CB)}
  - Hematopoietic stem cell content (CD34)
- TC Products {TC-T, TC-CTL, etc}
  - TC content (e.g. T cells)

# What Testing (2)

- Based on Processing
  - RBC removal
    - RBC content
  - Mononuclear Cell Enrichment
    - MNC content
  - Subset enrichment or depletion
    - Enriched or depleted target cells

# When To Test

- At receipt
  - TNC
  - Viability
  - Sterility-Not required but may be useful
- Post Processing known to affect content
  - Relevant subset (s)

Example: Density gradient separation to remove plasma and red blood cells. Also expect loss of CD34+ cells, therefore should repeat testing for CD34+ cells in addition to assessment of rbc content.

# When To Test

- Prior to Infusion or/Cryopreservation
  - TNC
  - Viability
  - HPC content if relevant
  - Sterility-Required
- Testing requirement for product release must be defined

# How To Test

Total Nucleated Cell Counts
- Surrogate measure of graft quality
- Does not measure potency

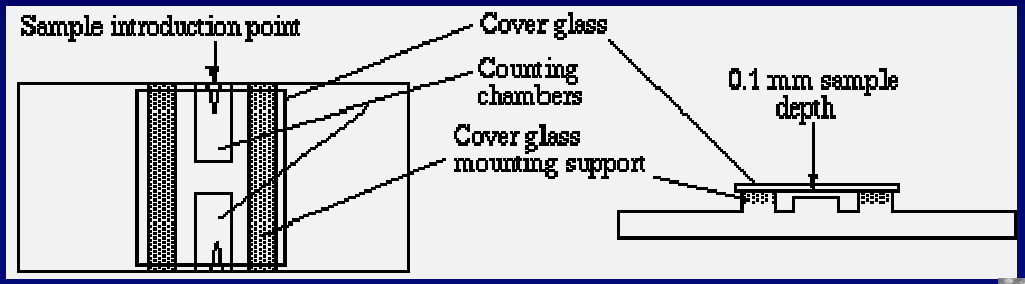
| Expected % CD34+ HPC by HPC Product | | |
|---|---|---|
| Allo-HPC(M) | HPC(CB) | Allo-HPC(A) |
| 1.0%±0.33% | 0.93%±0.44% | 0.85%±0.46% |

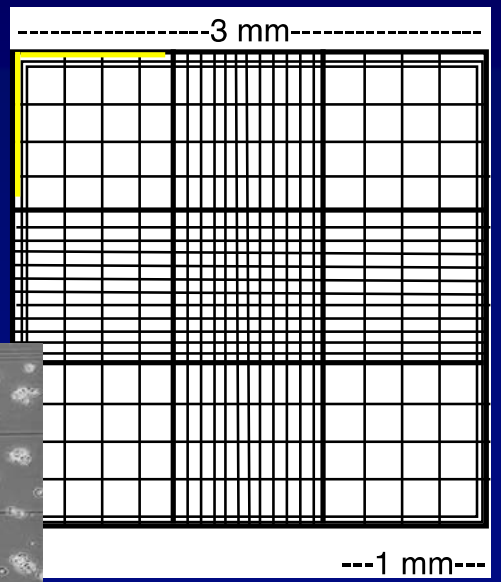
Manual Methods

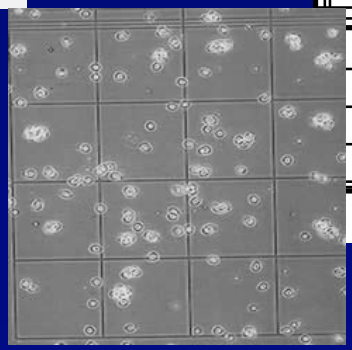
Electronic Methods

# Manual Counting Method

1. Take representative sample- Ensure product is well mixed
2. Prepare dilution- Typically 1:10 for apheresis products. Target ≤100 cells per quadrant.
3. Load Counting Chamber, both sides, Do not overfill



1. Count WBC in 4 outer Quadrants, determine average

Average cells in 4 outer quadrants
x $10^4$ x dilution= cells per mL

# Electronic Counting Methods

- Method
  - Take representative sample- Ensure product is well mixed
  - Prepare dilution- Typically 1:10 for apheresis products
  - Apply sample to hematology analyzer
  - Results report from analyzer

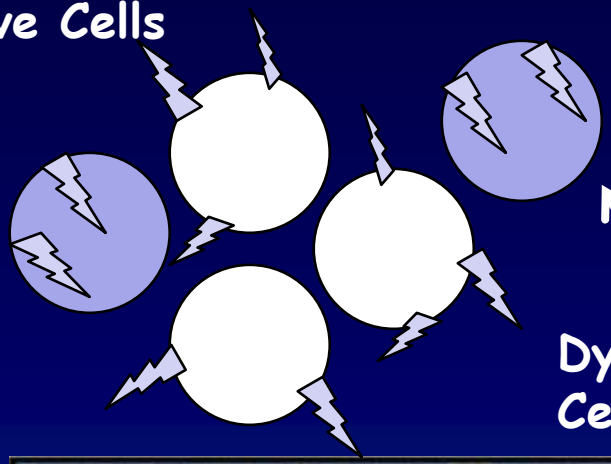
| Aspect | Manual Method | Electronic Method |
|---|---|---|
| Red Blood Cells | Manual Lyse, or Distinguish | Lysed automatically |
| Accuracy | Fewer events but better for marrow | More events but may ct marrow fat |
| Precision | Less (more manual steps) | More |
| Cost | Less | More (could share) |
| Subjectivity | More | Less |

# Viability Methods

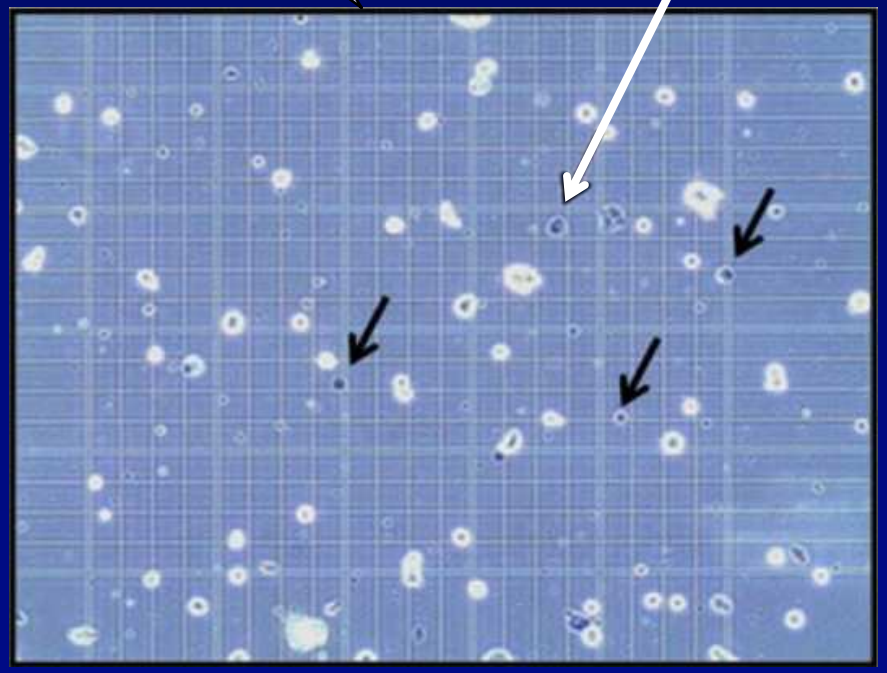
- Dye Exclusion Assays- Taken up by dead cells, excluded by cells with intact membranes
- Light or phase contrast Microscope
  - Trypan Blue-Most common in HPC laboratories
  - Erythrosin B
- Fluorescent Microscope
  - Acridine Orange with Propidium Iodide- Detects living and dead cells with two dyes
- Flow Cytometry Based Assays
  - 7-amino-actinomycin D (7-AAD)- Most common
  - Propidium Iodide (PI)

# Trypan Blue Viability

Dye excluded= Live Cells



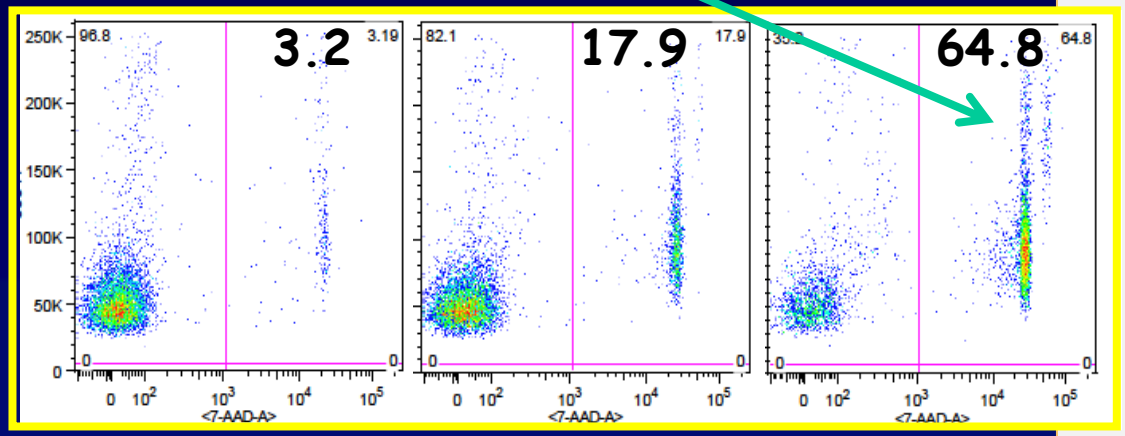
Dye Uptake=Dead Cells




% Viability= (# Living ÷ (#Living + # Dead)) X 100

# 7-AAD Viability

Dead cells take up 7-AAD




Titration with 100% Dead Cells


Advantages compared to Trypan

- Less subjective
- Can be used in conjunction with CD34 assessment
- Most accurate with lyse/no wash methods for assessing thawed products (e.g. cord blood)
- Documentation of results

# Sterility

- To assess Aerobic and Anaerobic Bacteria and Fungus
- Culture Based Methods
  - Bactec/ BacTAlert- Most Common in US Labs
  - Requires validation by each laboratory for the products and reagents used. Should include:
    - Innoculum size (most 0.5 to 1 mL product)
    - Detection of appropriate range of bacteria and fungus for your situation
    - Guidance available in US
  - Typically product samples innoculated in processing laboratory, cultures performed in institution Microbiology Laboratory-Must have notification system
- Rapid Methods- May be needed for extensively manipulated products (e.g. cultured cells)
  - Gram stain
  - Endotoxin testing
  - Mycoplasma testing

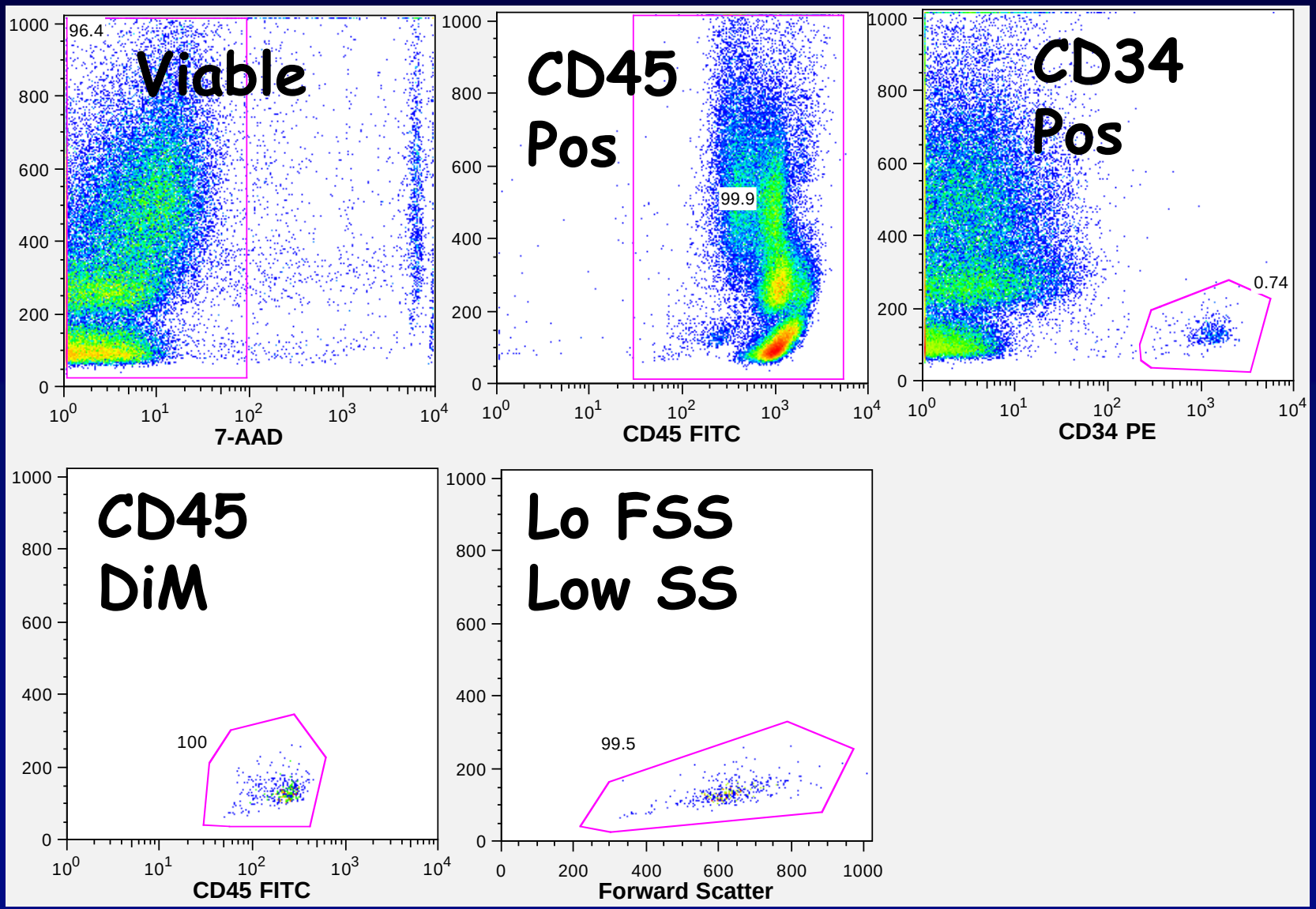
# Hematopoietic Stem Cell Assessment (Potency Assays)

- Assays to detect HSC found to be associated with engraftment
  - Flow Cytometry Based
    - CD34+ Cell content- Most common
    - CD133+ Cell content
    - Aldehydehyrogenase bright (ALDH) cells
  - Hematopoietic Colony Forming Cell Assays (CFU)- Required for Cord Blood Banks, not typically performed for other HPC products

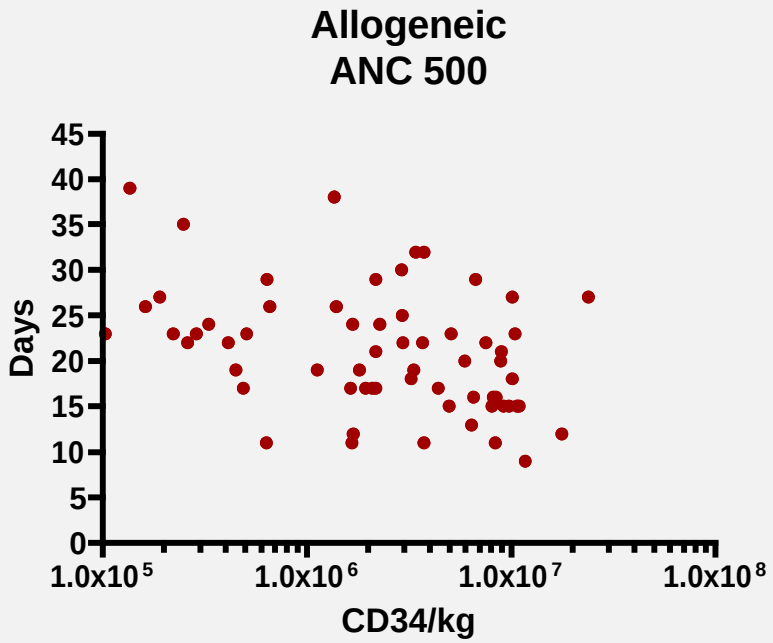
# CD34+ Cell Assessment

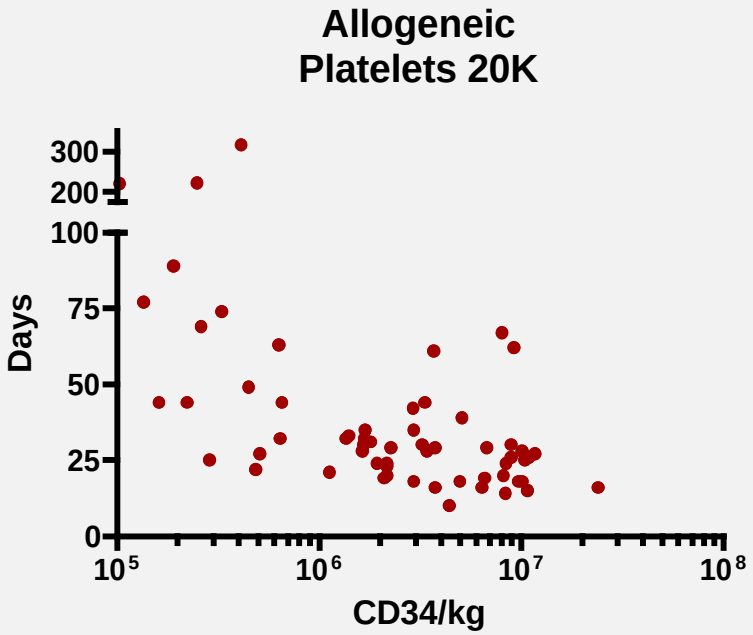
- Assay Requirements
  - Class III CD34 antibody conjugated to bright fluorochrome (PE or APC recommended)
  - CD45 in every tube
  - Viability dye in every tube-7-AAD recommended
  - Use multiple parameters to ensure accurate detection to include:
    - Moderate to bright expression of CD34
    - Moderate to dim expression of CD45
    - Low forward and low side scatter
  - Acquire sufficient cells to collect minimally 100 events in final CD34+ gate (if possible)

# ISHAGE Gating Method-CD34



# CD34 Dose



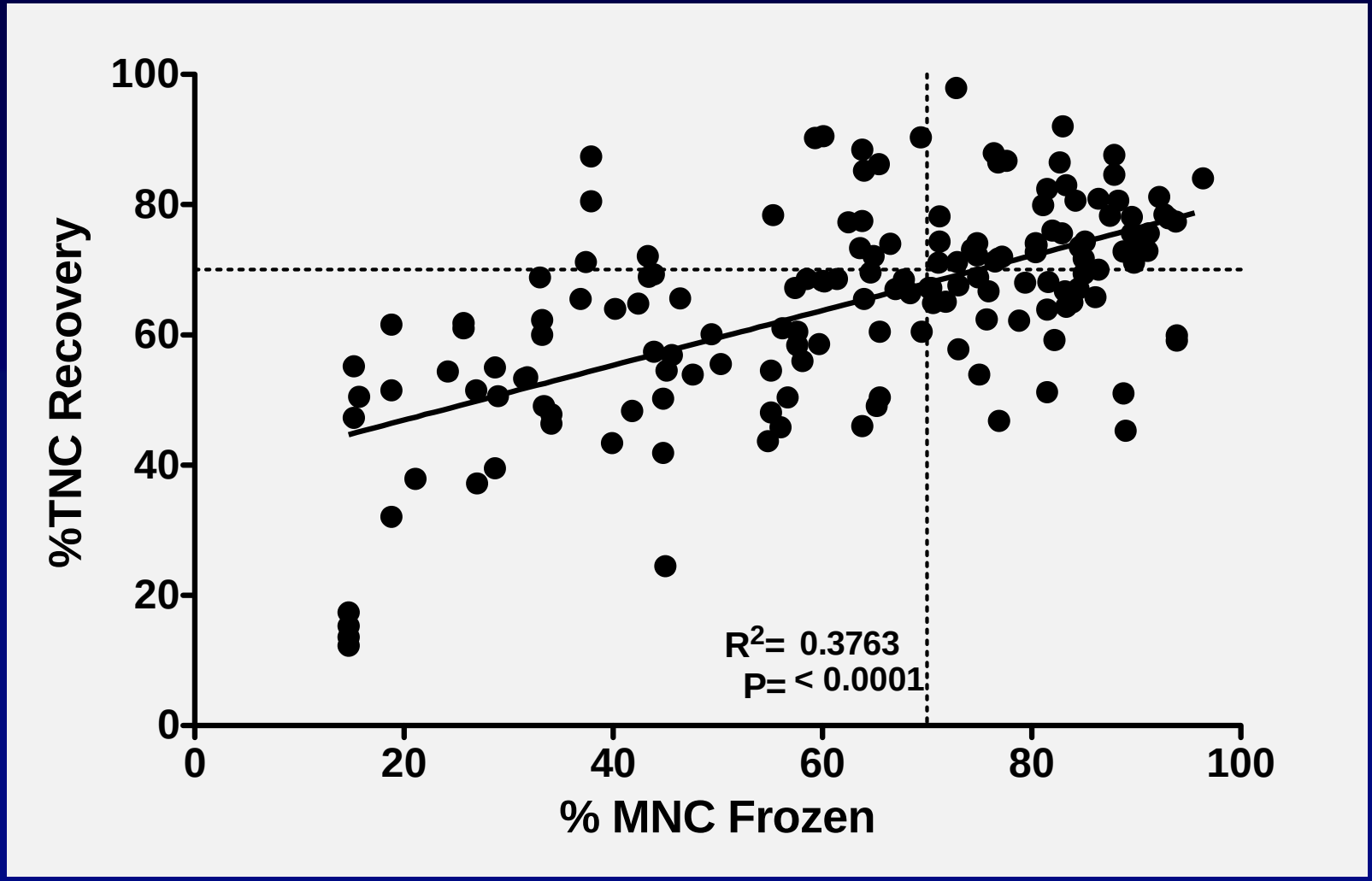




Day of engraftment versus CD34 dose per kg. Allogeneic patients.

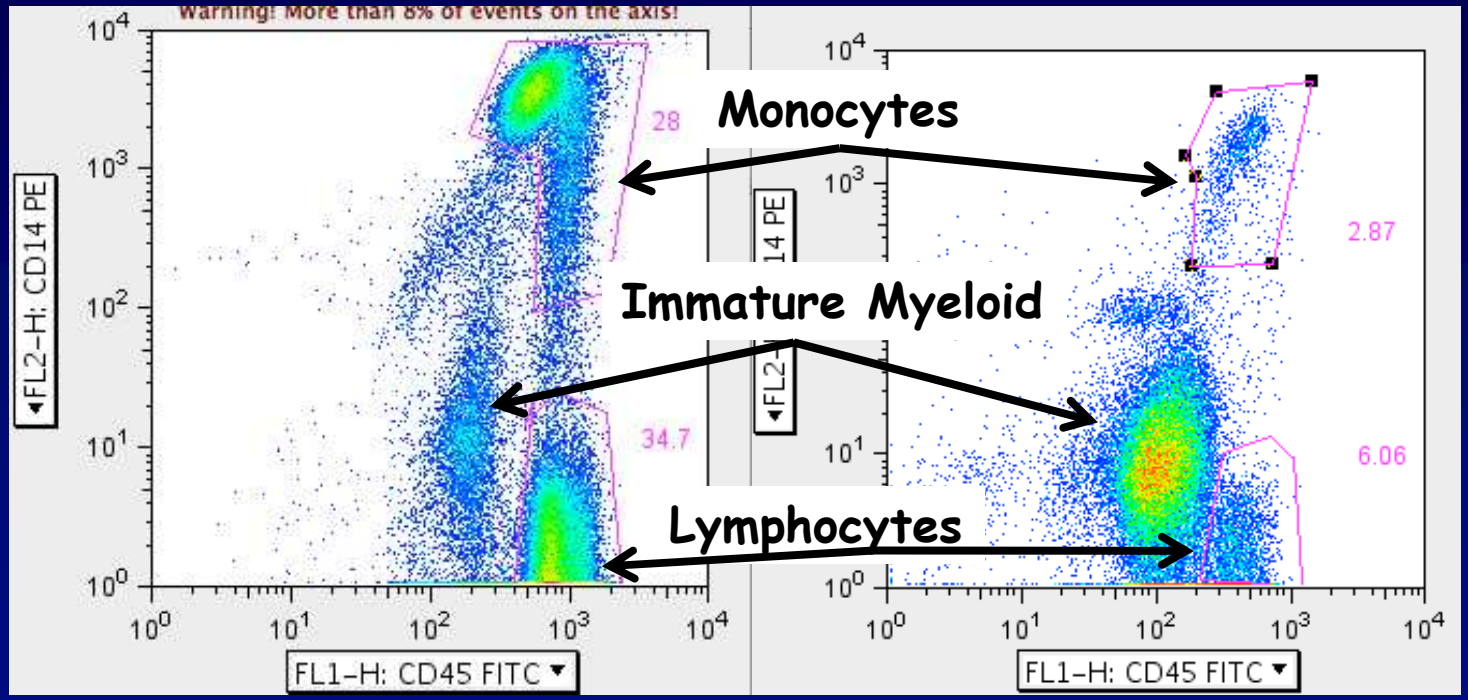
# Mononuclear Cell (MNC) Differentials

- Defined as lymphocytes and monocytes within the Cellular Therapy Product
  - Useful as a surrogate for CD34+ cells, since CD34+ cells have the characteristics of mononuclear cells
  - MNC correlate best with recovery post thawing for HPC(A) products
- Methods of detection
  - Manual Differential- Most common?
  - Flow Cytometry Differential- Accurate and can be performed with CD34-assessment
  - Hematology Analyzer Differential- Poorly correlates with manual method for BM, moderate correlation with HPC(A) products

# MNC Content Vs Thaw Recovery



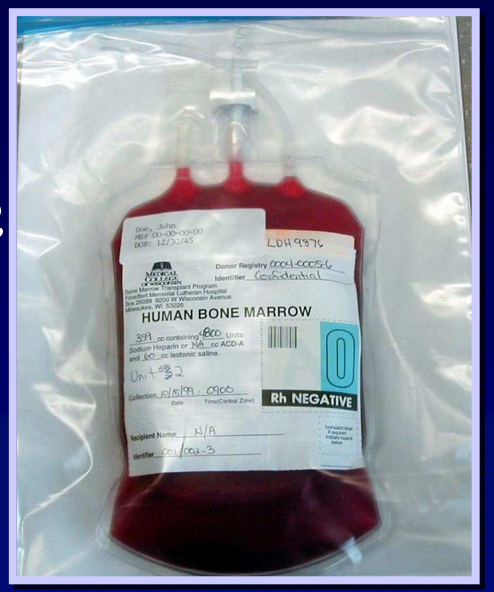
# MNC Assessment-Flow Differential




HPC(A)

HPC(BM)

# Red Blood Cells in HPC Products




- Bone Marrow
  - High RBC content, High Volume
  - Typically give fresh
- Cord Blood
  - High RBC content, Low Volume
  - RBCs lyse at thaw
- HPC, Apheresis Products
  - Low RBC content, typically ≤5%
  - Standard Methods for RBC removal ineffective

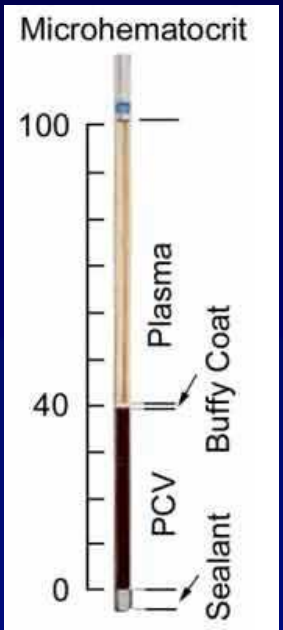
# Red Blood Cell Removal

- Purpose:
  - To reduce the content of donor red blood cells reactive with recipient antibody. MCW limit <20mL total or 0.3 mL/kg.
  - Volume reduction
- Methods For Removal:
  - Gel Sedimentation (Plasmagel, HES). Hct 1-2%.
  - Mononuclear Cell Preparation
  - Density Gradient Method. Hct <0.5%
  - Centrifugation Method. Hct 1-5%

Do not work for PBPC

# RBC Test Methods

- Hematocrit-Ratio of RBC to the total volume
  - Manual Centrifugation Methods
    - Requires specialized centrifuge
    - Can be difficult to distinguish packed RBC layer from WBCs for highly cellular products
  - Hematology Analyzer- Most common method
- RBC Count
  - Manual Method- Uses counting chamber as described for TNC
  - Hematology Analyzer



# Rare Cell Detection-Subset Depletion/Enrichment

- Most Common Procedures
  - CD34+ Cell Enrichment
    - Typically performed for CD3-removal
    - Requires rare event detect of CD3+ cells
  - CD3+ cell Reduction, ± CD19+ cell reduction
    - Detection of CD34+ cells
    - Rare event detection of CD3+ and CD19+ cells
  - CD56+ NK Cell-Enrichment ± CD3-Reduction
    - Rare event CD3+CD56- cell detection

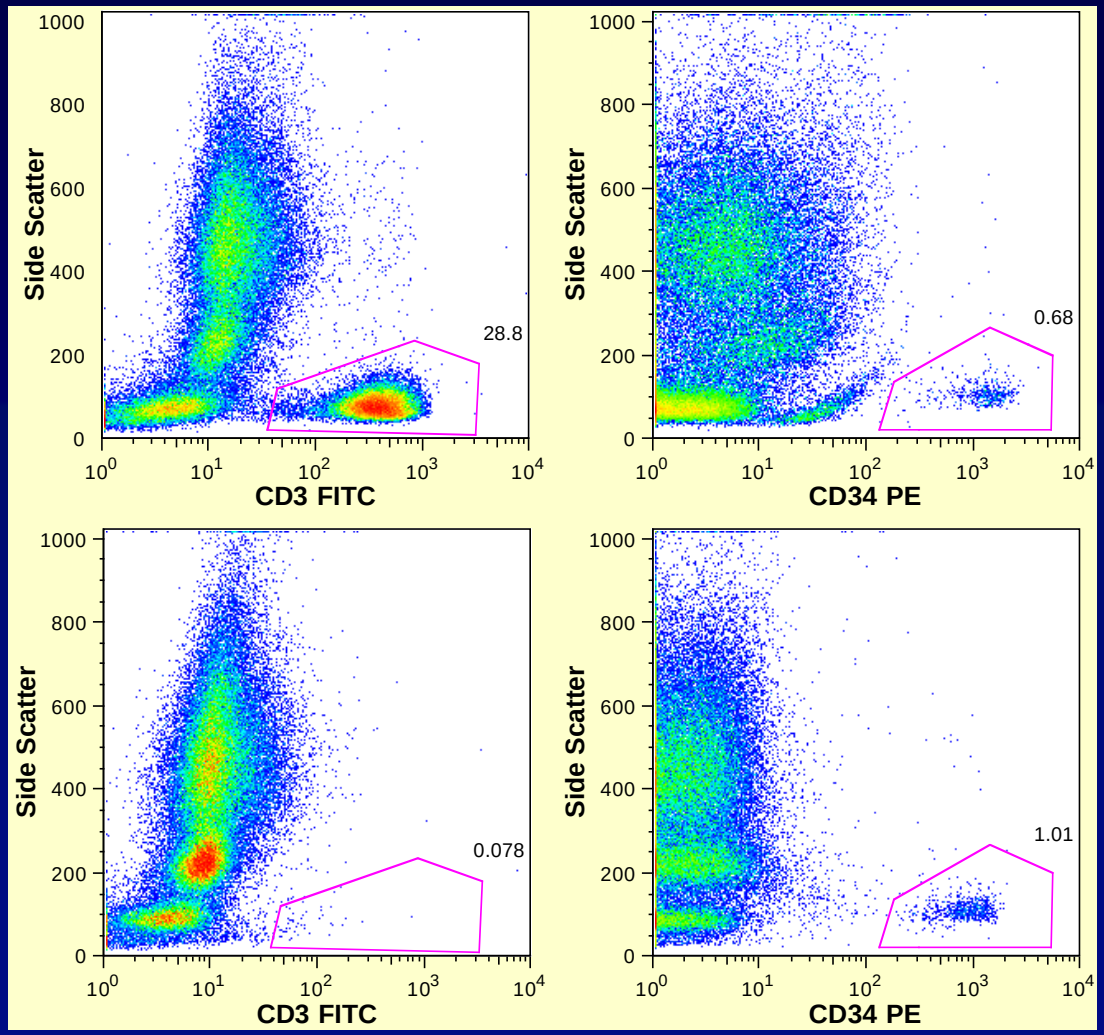
# Rare Cell Detection Methods

- Multi-parameter Flow Cytometry- Only reliable method currently in use
  - Use enriched population to help set gates for reduced population
  - Ensure that antibody used for detection not blocked by antibody used for cell separation
  - Collect sufficient events-Target 100 cells in final analysis gate
  - Use bright fluorochrome for rare events

# CliniMACS CD3-Reduced

Pre Treat

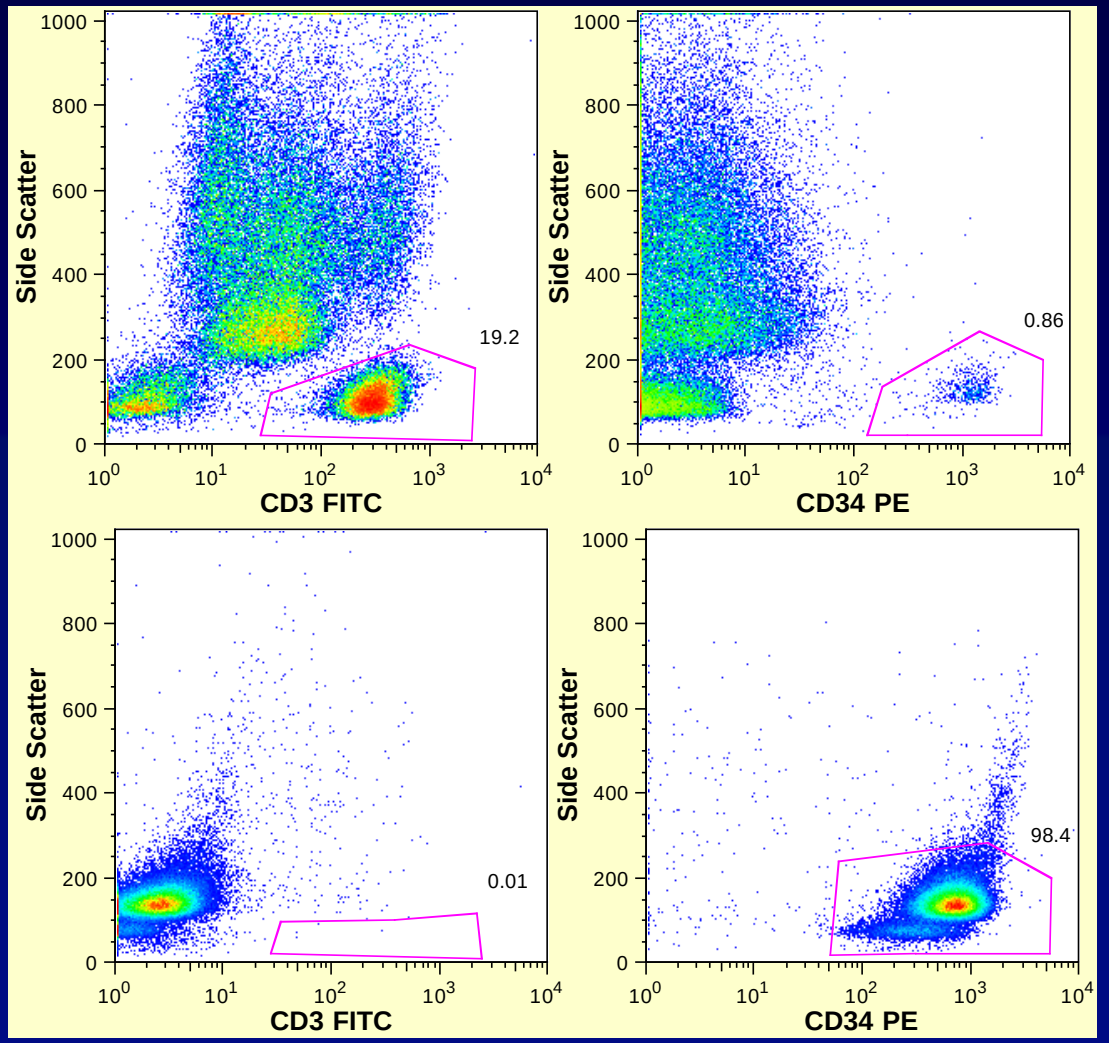
CD3 Reduced



# CliniMACS CD34-Enriched

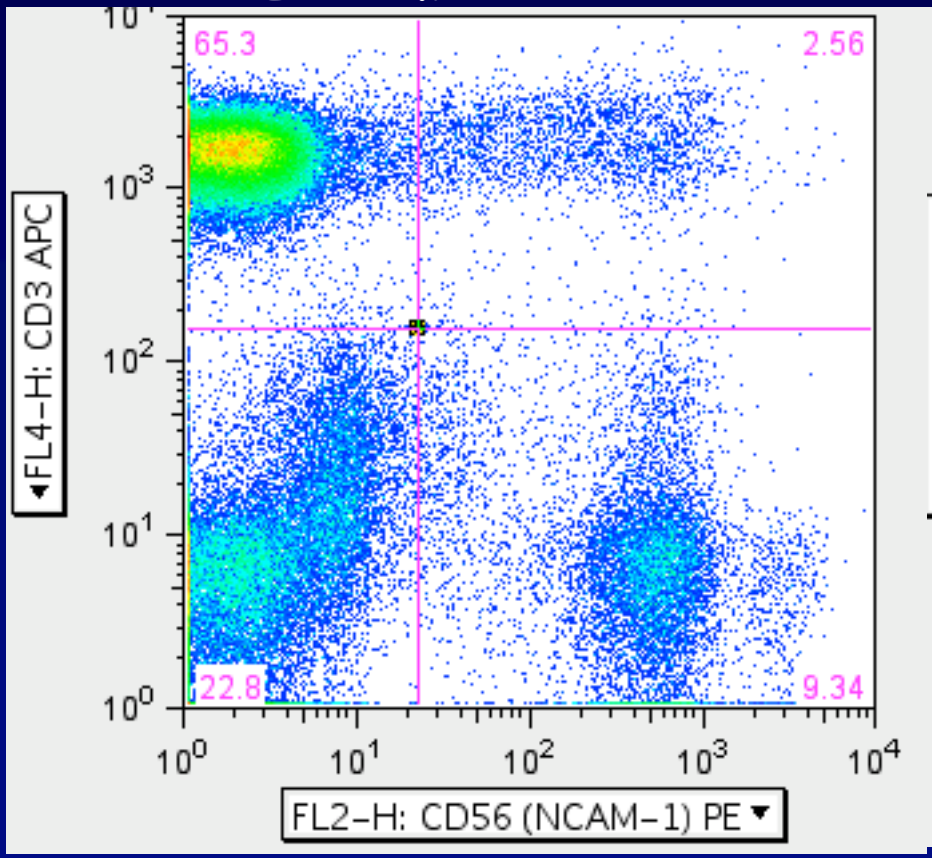
Pre Treat

CD34 Enriched

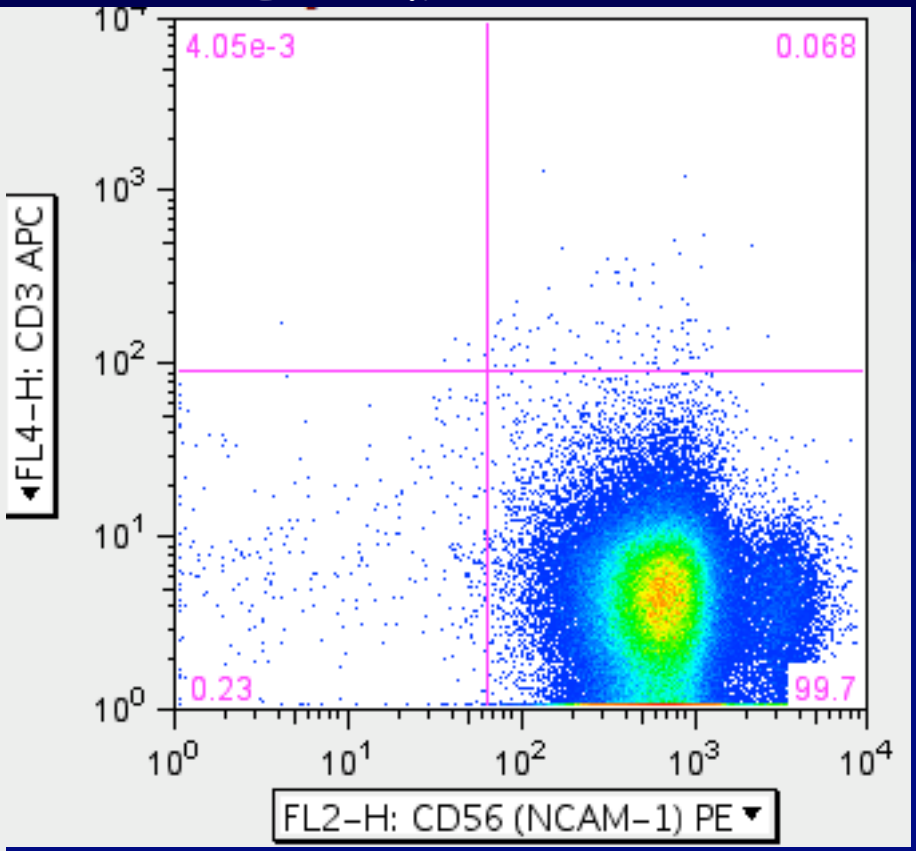


# NK Cell Enrichment

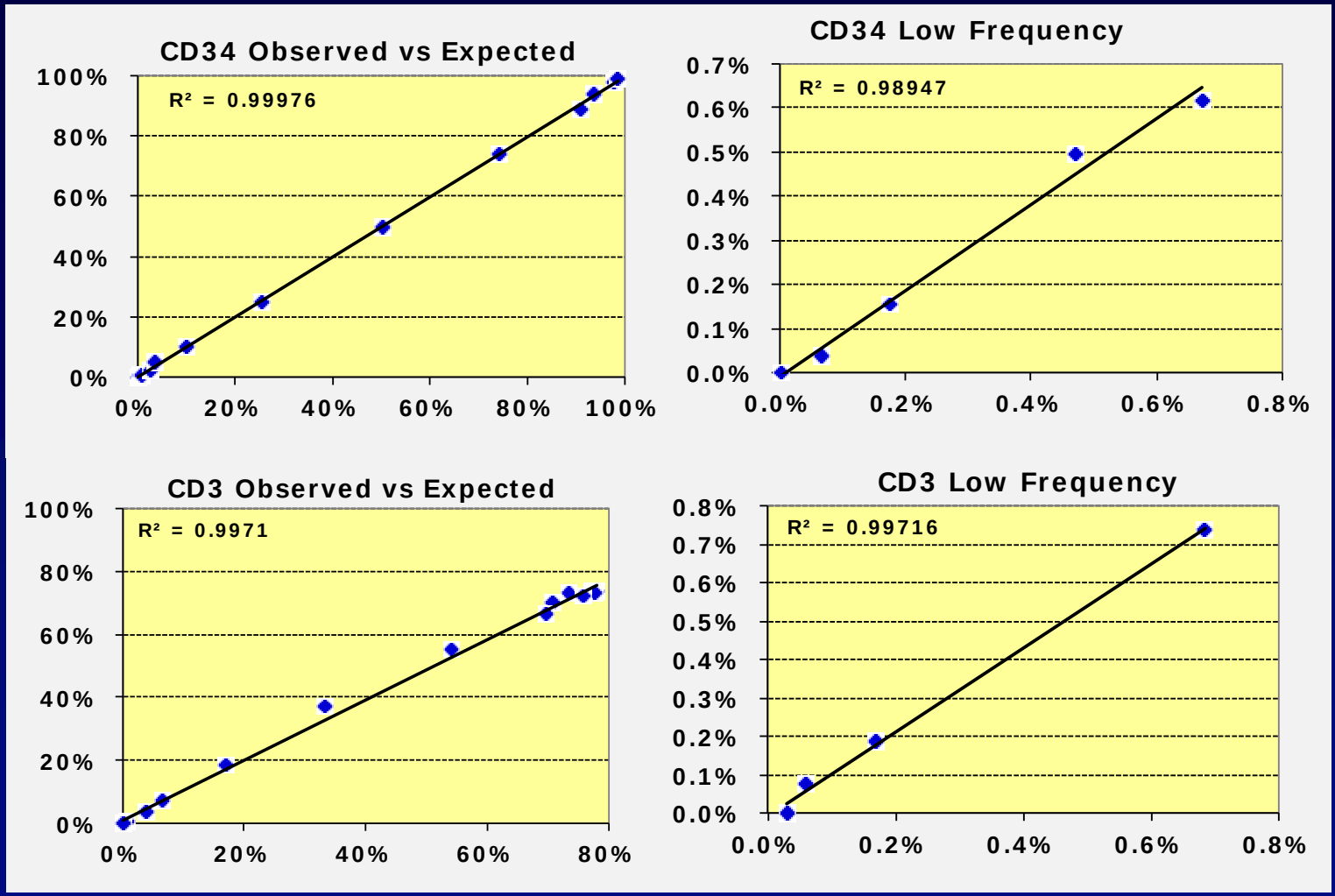
Pre-Enrichment




Post Enrichment



# CD34 & CD3 Detection Limits



# Other Assessments for Release

- Labeling
  - Composition (Cell count, volume, additives)
  - Storage conditions and expiration
  - Patient identification, Unit Identifier
  - Harvest and Processing Center identification
  - Warnings and Precautions
- Product Appearance
  - Appears as expected, no aggregates, clumps, unexpected color or turbidity
  - Container is intact, no evidence of damage or leakage