

Leukoreduction of blood : Techniques, Results and Theoretical Applications to TSE Agents in Blood

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Leukoreduction: the process of reducing the total number of leukocytes in a transfusion component to $<1 \times 10^6$ cells

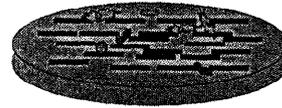
- Methods: filtration apheresis

Concentration and Physical Properties of Human Blood Cells
(Bruil, A. et al. Transf. Med. Rev.2:145-166, 1995)

Cell types	Conc. (uL ⁻¹)	Density (g/cm ³)	Diameter (um)	Deformability	Adhesiveness
RBCs	4-6x10 ⁶	1.09-1.11	7-8	++++	+
Granulocytes	2-6x10 ³	1.08-1.084	5-8	+++	+++
Lymphocytes	1.5-4x10 ³	1.06-1.072	4-8	++	++
Monocytes	2-8x10 ²	1.055-1.062	4-10	++	++++
Platelets	1.5-4x10 ⁵	1.054-1.062	2-3	+	++++

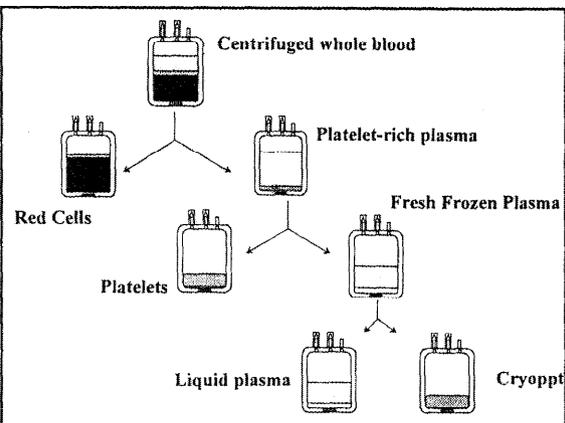
Blood filters

- Materials
 - cellulose acetate
 - polyester
 - microfiber glass
- Mechanisms
 - barrier retention
 - cell adherence
 - cell-cell interaction

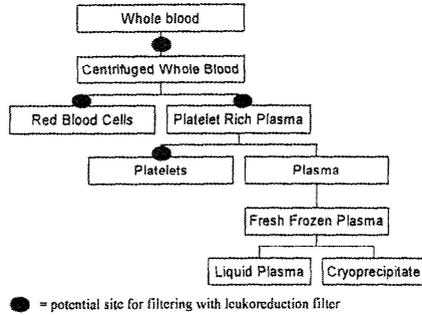


Factors that affect filter efficiency

- temperature
- flow rate
- leukocyte load
- protein content of medium
- age of cellular component
- sickle cell trait



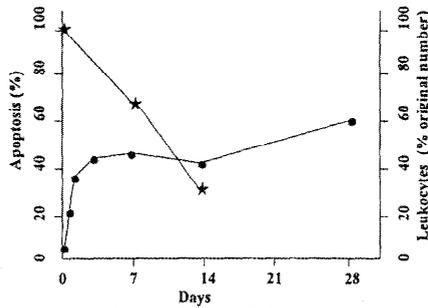
Components Produced From Whole Blood



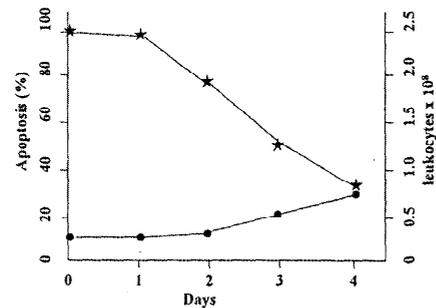
Storage conditions of cellular components

Conditions	RBCs	Platelets	Plasma
Time	42 days	5 days	1 year
Temperature	1-6° C	22° C	-20° C
Leukocyte load/ unit	1x10 ⁹ cells	1x10 ⁹ cells	1x10 ⁸ cells
Leukocyte Status	dormant	active	frozen
Leukocyte Fate	Apoptosis/necrosis 3-5 days	Apoptosis/necrosis 3-5 days	Cells lysis

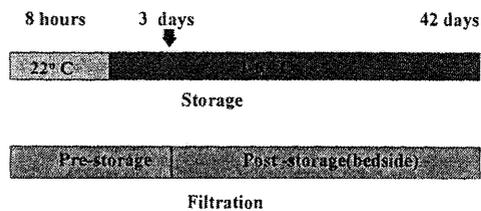
Leukocyte Apoptosis and Number in Packed Red Cells
Frabetti et al. Transfusion 38:1082-1089, 1998



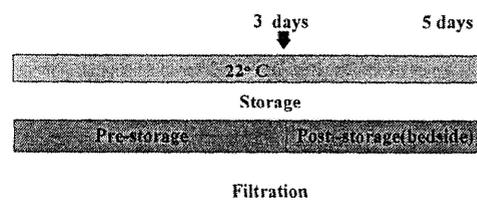
Leukocyte Apoptosis and Number in Stored Single-Donor Platelet Concentrates
Frabetti et al. Transfusion 40(2): 160-168, 2000



Timing of filtration for RBCs



Timing of filtration for Platelets



Timing of filtration

- Pre-storage
 - performed under GMPs
 - quality control
 - removal of leukocytes prior to breakdown
 - prevention of cytokine production during storage
- Post storage (bedside)
 - no quality control
 - leukocytes disintegrated, filtration may not remove cell fragments or cell associated pathogens
 - cytokine build-up
 - hypotensive episodes (bradykinin)

Separation of blood components by apheresis

Apheresis = selective removal of one or more components of whole blood and return of the remaining components to the donor

Components collected by apheresis

- Red cells
- Platelets*
- Granulocytes
- Peripheral Blood Stem Cells
- Monocytes
- Plasma
 - * leukoreduced with some instruments

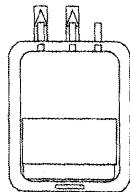
Failure rates (>5 x 10⁶ WBC/unit) for filtered products

Kao, K.J et al (TRAP Study Group), Transfusion, 35:13-19, 1995

- Platelets
 - 7% apheresis
 - 5% pooled
- Red Cells
 - 2.7%

Plasma

- "Acellular" portion of blood
- involves hard spin step to prepare
- not cell free
- ~1x10⁴ leukocytes/unit
- not routinely filtered



Leukoreduction as means to reduce TSE infectivity-Theoretical Aspects

- Positive
 - Remove infectious agent
 - Remove cells that carry infectious agent
 - Remove cells that support peripheral propagation of infectious agent
- Negative
 - Rupture cells that carry infectious agent and release agent
 - Remove cells that could neutralize infectious agent

Leukoreduction as means to reduce TSE infectivity-Theoretical Aspects cont.

- Concerns:
 - do rodent leukocytes adequately model human leukocytes
 - do same cell types carry infectivity in rodent and human blood
 - do rodent leukocytes have similar physical characteristics so that cells will follow same isolation pattern as humans
 - will filters optimized for human blood be effective in rodent blood

Summary

- Cellular blood products (RBCs, Platelets) contain substantial amount of leukocytes
 - (1×10^9 leukocytes/component)
- Leukoreduction reduces the load
 - ($< 1 \times 10^6$ leukocytes/component)
- Pre storage filtration is better than bedside filtration
- Plasma
 - $\sim 1 \times 10^4$ leukocytes