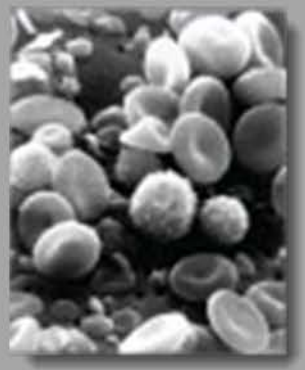


12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

Canadian Blood Services
it's in you to give

SHARE YOUR HEALTH AND VITALITY WITH SOMEONE IN NEED

CBS & Plasma Protein Products:

Where do they come from….

September 13, 2014

Canadian Blood Services
it's in you to give

Objectives

- Discuss the various sources of plasma for fractionation
- Describe the fractionation process for plasma derived factors
- Explain how products are brought to market in Canada - licensing/regulated HC,FDA, CE

Setting the stage

- Plasma fractionation started in the 1940s Cohn-Oncley method
- 1965 - Australia Antigen found (HBV)
- 1968-69 first FVIII & FIX concentrates
- 1983-84 HIV (HTLV-III) and 1989 HCV
- Mid-late 1980's dedicated viral reduction strategies added
- Recombinant manufacture started to be available in 1990s

Plasma Fractionation

Plasma Donation

Recovered

- Whole blood
  - Volume removed from whole blood donation
  - Includes FFP, FP, CSP from whole blood donation labelled as recovered plasma
  - Same donor screening as whole blood donations
  - US market – bought and sold as Plasma Normal

Source

- Apheresis
  - Larger donation based on donor weight
  - More frequent ~weekly up to ~31 L/yr (more or less depending on weight)
  - Additional donor screening Total protein, immunoglobulin quantitation and annual physical exam.
  - Some donors sensitized to increase antibody levels
  - May be recompensed

Typical Characteristics of a Unit of Plasma

Characteristic	Recovered plasma	Source plasma
Volume, mL	100-260	450-880
Protein content, g/L (each donation)	≥ 50 (but typically greater than in source plasma)	≥50
Factor VIII, iu/mL (average)	≥ 0.7 (but typically less than in source plasma)	≥ 0.7
Anticoagulant concentration	Variable, according to donation size (volume of anticoagulant is fixed for a given pack type; the acceptable blood volume range should be specified)	Constant (metered into donation)

Adapted from WHO Recommendations for the production, control and regulation of human plasma for fractionation.

Plasma Pooling

- **Plasma**
  - **Recovered -** from whole blood donations
  - **Source** - from Apheresis donations
- **Donors**
  - Screened – meet FDA and/or local regulations
  - Tested for TD markers – source not the same as for Whole Blood (HTLV I/II, anti-HBc, WNV)
- **Pools of 5,000-30,000+L**
  - Pooled from 10,000-50,000 donations
- **Other :**
  - **In-process testing:** eg. B19, (HAV), pyrogens
  - **Plasma hold:** 6 month

Factors Claimed to Affect FVIII Yield in Fractionated Concentrates

- Anticoagulant
- Collection method
- Time/temperature to separation/freezing
- Freezing rate
- Storage conditions of frozen plasma
- Thawing conditions
- Purification chemistry
- Viral inactivation

Blood/plasma centre

Fractionator

Adapted from Farrugia A: Presented at the World Federation of Hemophilia Global Forum, Montreal, 2005.

Plasma Pool Testing

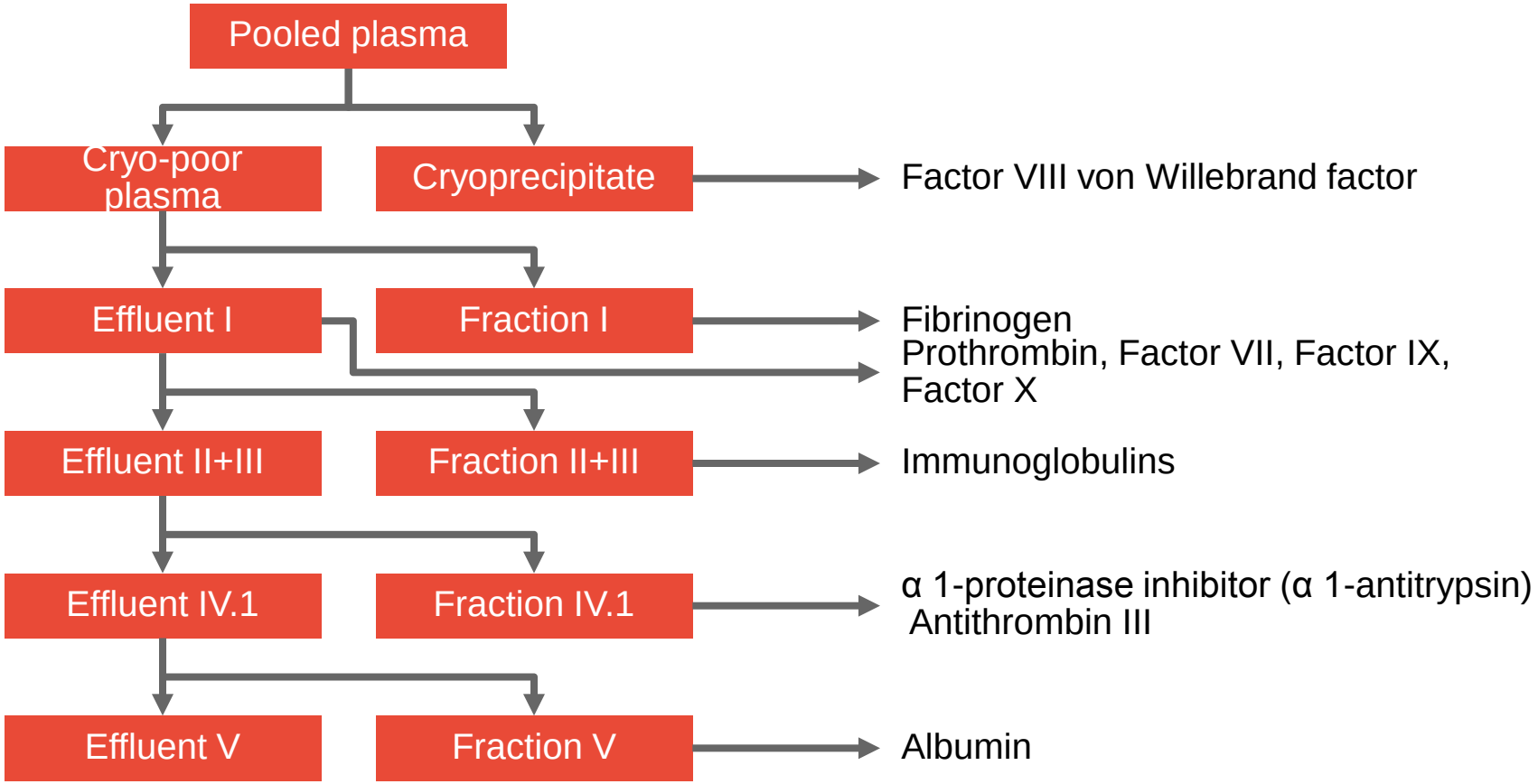
Plasma Manufacturing Pools

- Serologic testing
  - HBV surface antigen
  - HIV-1/HIV-2 antibody
- NAT
  - HIV-1
  - HBV
  - HCV
  - High titer B19V
  - HAV by some

HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; B19V = parvovirus B19. HAV= hepatitis A virus

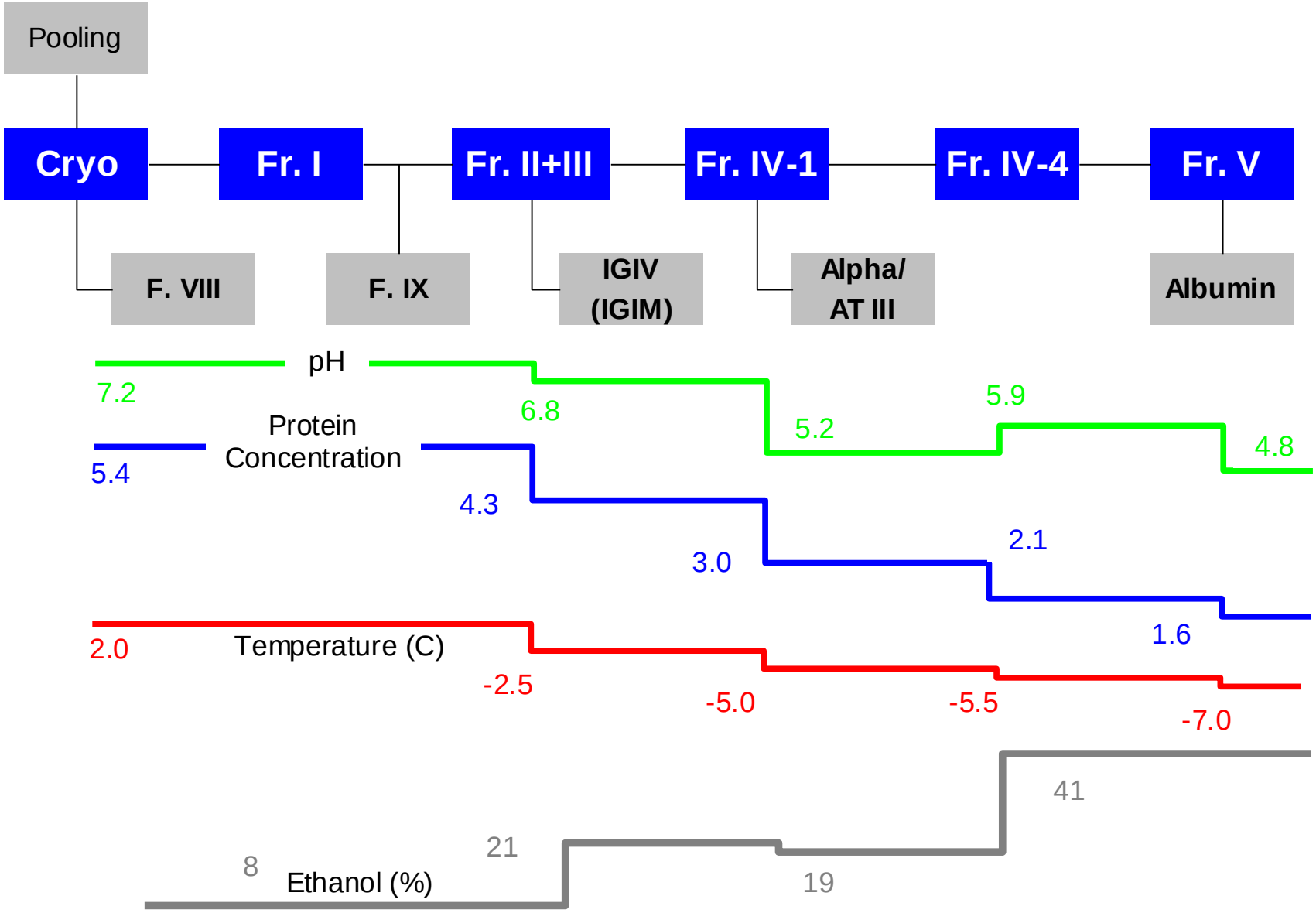
Reference: . *Trejo et al., Vox Sanguinis 2003*

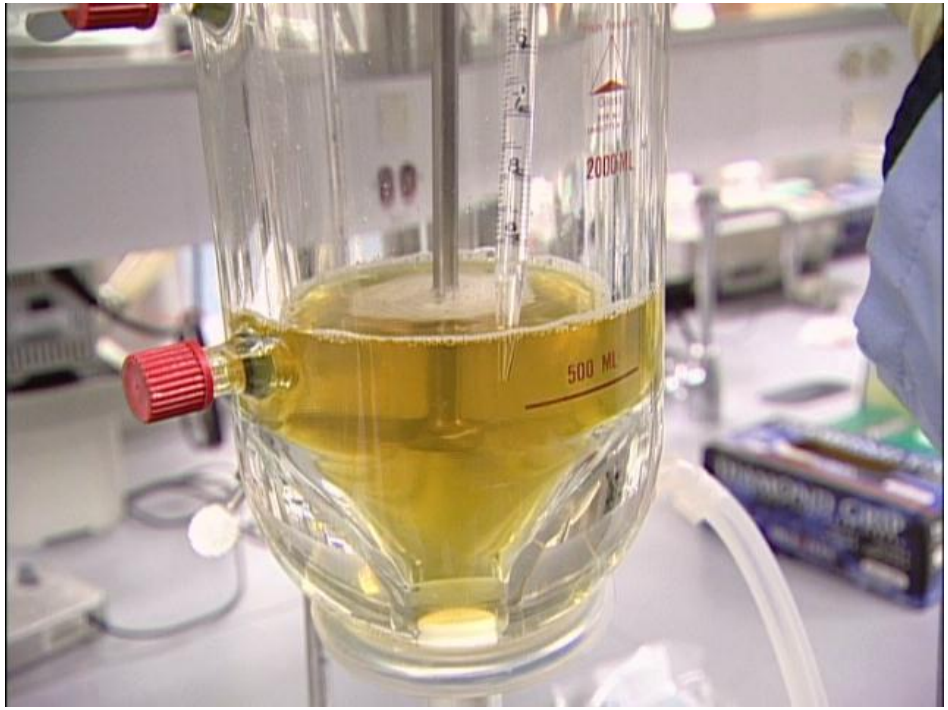
Example of a Plasma Fractionation Method (Cohn Method)



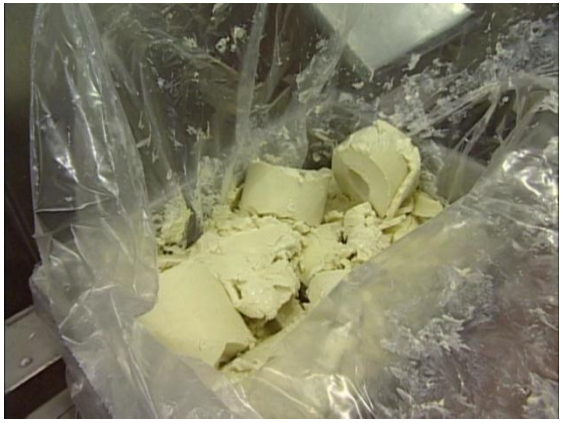
Adapted from Ofosu FA, *et al: Thromb Haemost* 2008; 99(5):851-62.

Fractionation Cohn (1946)





Temperature, pH, and ethanol concentration altered to selectively precipitate proteins of interest



Intermediates steps come off as paste that can be sent for further manufacture

Additional Separation Steps

- **Chromatography**: collective term for a set of laboratory techniques for separating mixtures
- Separate proteins in a mixture due to differential interaction with a solid support (column)
- **Ion exchange**: separate proteins based on their electric charge with charged support column
- **Affinity**: separate proteins based on interactions with support column that includes a ligand (e.g. heparin for ATIII) or an antibody (e.g. FVIII)

Pathogen Removal

- Bacteria / parasites not transmitted by PP
  - Destroyed by freeze – thaw
  - Removed by filtration (0.2 -1 µm)
- Viruses removed in chromatographic steps
  - Viral proteins have different affinities for chromatographic columns than PP
- Removed by nanofiltration (e.g. 20 nm)
  - Virus - antibodies complexes

Transfus Med Rev. 2007 Apr;21(2):101-117

Pathogen Inactivation

- **Solvent – Detergent Treatment**
  - Lipid enveloped viruses inactivated
  - Labile proteins mostly unaffected
  - Non-enveloped viruses unaffected (HAV, PVB19)
  - Removed by subsequent chromatographic steps
- **Pasteurization**
  - Treatment with liquid heat (60 C) for 10 hours
  - Presence of protein stabilizers (stabilize viruses)
  - Removed by subsequent chromatographic steps
  - Kills enveloped and non-enveloped viruses
  - Can be done by vapor heat as well

Pathogen Inactivation

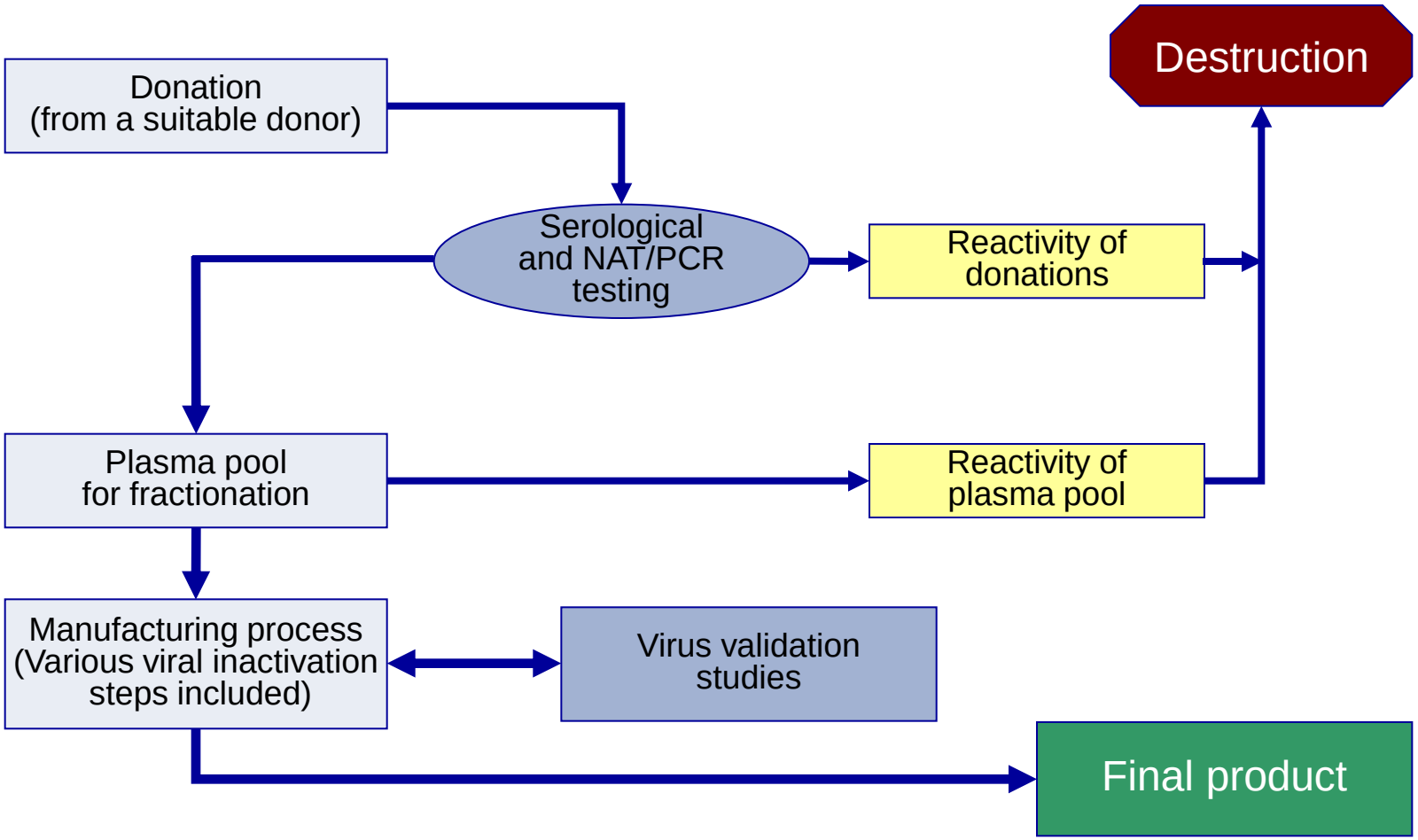
- **Low pH incubation**
  - pH of 4 at 30 – 37 C for 20 hours
  - Inactivates enveloped viruses
- **Caprylate**
  - Fatty acid of plant origin, used as stabilizer for albumin
  - Disrupts lipid envelope
  - Precipitates contaminating proteins (viral particles)
- **Terminal Pasteurization**
  - Dry heat 80 C for 72 hours, 100 C for 30 minutes (coagulation factors)
  - Liquid at 60 C for 10 hours (gold standard for albumin)

TSEs

- No laboratory test to screen for infected donors
- Donor screening questions in place
- Inactivation of TSE proteins (oxidation, strong base, extreme heat) destroys plasma proteins
- Filtration and chromatographic evaluated with scaledown models show effective removal
- TSEs can complex = removal with nanofiltration
- No known cases of transmission

Transfus Med Rev. 2006 Jan;20(1):57-62 Vox Sanguins, 2003;84:176-187

Example of Virus Safety Procedure for Plasma Products



Adapted from Gröner A, *et al*: *Haemophilia* 2008; 14 Suppl 5:54-71.

Table 1. Most Relevant Measures Taken to Prevent the Transmission of Plasma-Borne Infectious Agents by Fractionated Plasma Products

	Blood establishment		Plasma fractionator				
Infectious agent	Donor screening (exclusion criteria)	Individual serologic testing	Mini-pool NAT*	Manufacturing pool testing†	Viral inactivation treatments	Removal by purification steps‡	Removal by nanofiltration§
HIV I and II	Questionnaire	Anti-HIV 1 and 2	Yes	Anti-HIV 1 and 2; HIV NAT	+	(+)	+
HBV	Questionnaire	HBsAg	Yes	HBsAg; HBV NAT	+	(+)	+
HCV	Questionnaire	Anti-HCV	Yes	Anti-HCV; HCV NAT	+	(+)	+
Hepatitis delta virus	(questionnaire)	ND	ND	ND	+	(+)	+
HAV	ND	ND	Yes	HAV NAT	±	(+)	+
Hepatitis E virus	ND	ND	ND	ND	+	(+)	+
Hepatitis G virus	ND	ND	ND	ND	+	(+)	+
TT virus	ND	ND	ND	ND	+	(+)	+
B19	ND	ND	Yes	B19 NAT	±	(+)	+
WNV	ND	ND	ND	ND	+	(+)	+
vCJD	Questionnaire	ND	Not relevant	ND	Not relevant	(+)‖	(+)‖

+ Indicates major contribution to safety; ±, contribution depends on type of treatment; ND, not done (or test not available).

*Performed by most fractionators.

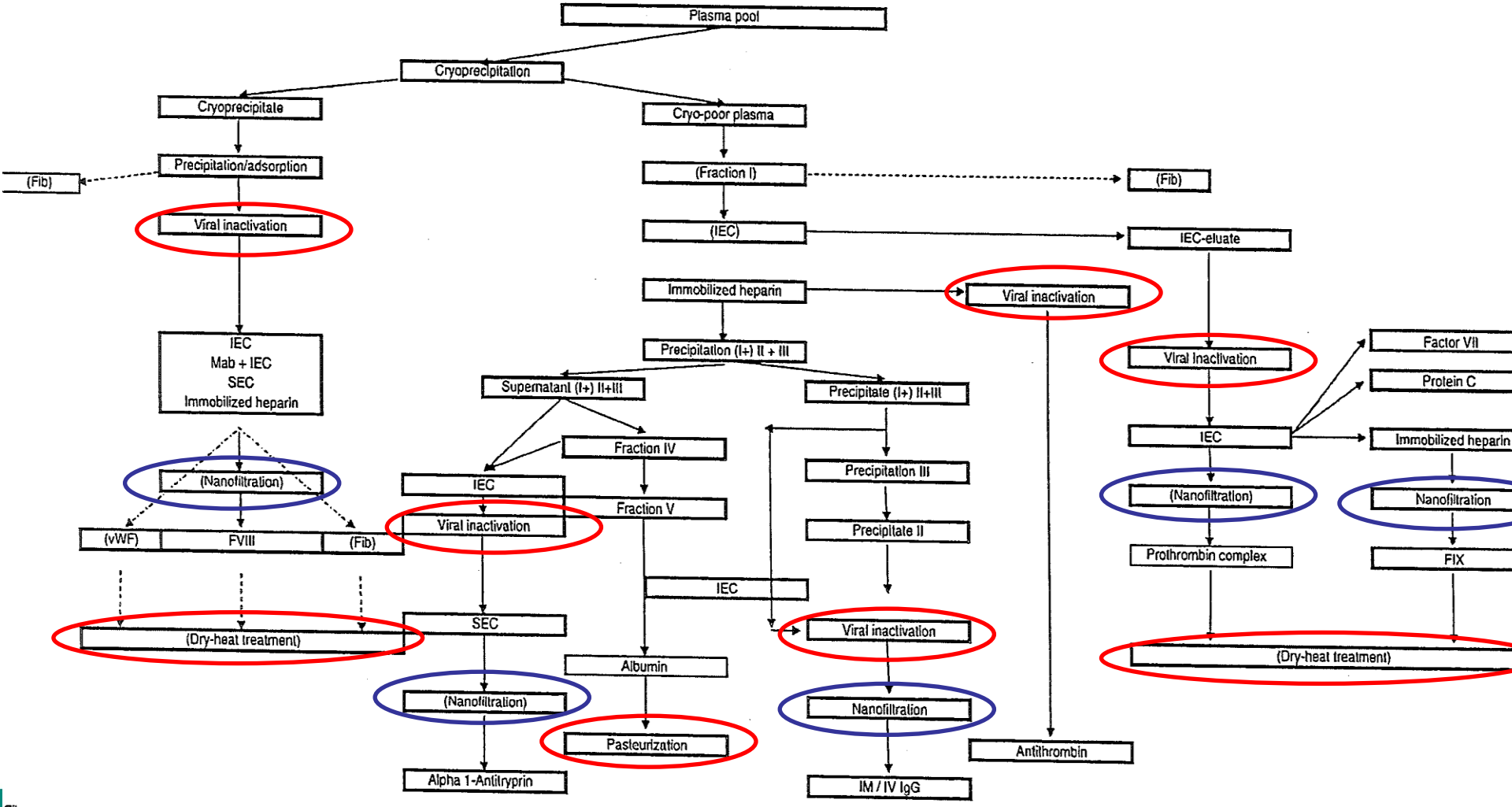
†Mandatory in Europe for HCV.

‡May contribute to viral clearance but does not necessarily result in robust and consistent removal.

§For small viruses, robust removal is achieved by narrow pore size membranes (≤20 nm).

‖Expected contribution based on experimental studies using spiked TSE agents, in the absence of information of the biological nature of the TSE-human plasma associated agent.

Typical Modern Fractionation Process



Fib Fibrinogen
IEC Ion exchange chromatography
Mab Monoclonal antibody chromatography
SEC Size exclusion chromatography
() Indicates optional treatment

T. Burnouf Trans Med Rev 2007,Vol.21,No 2, p101-117: Modern Plasma Fractionation

Viral Clearance

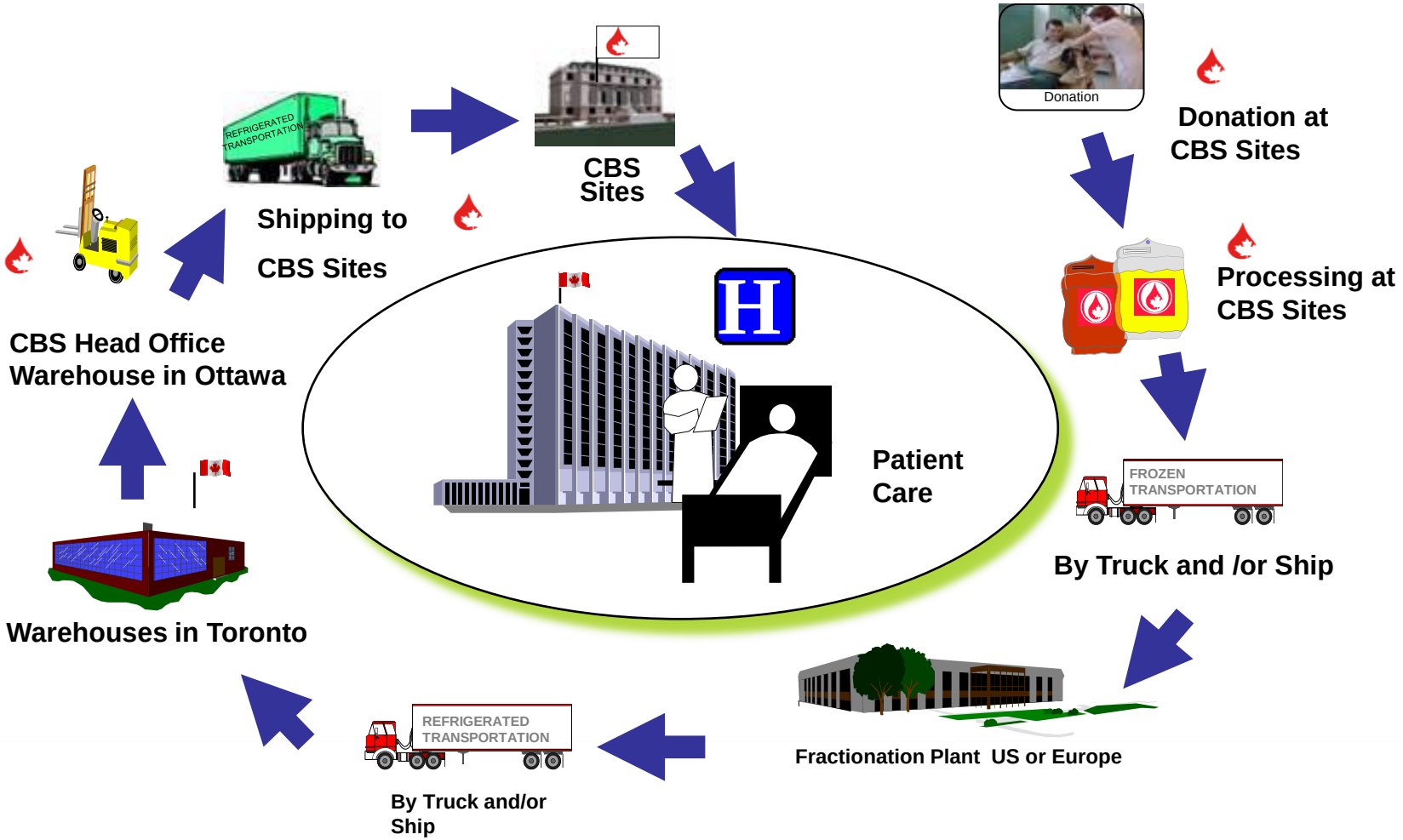
Process step	HBV		Log_{10} virus reduction			B19
	HIV	PRV	BVDV	Reo	HAV	PPV
Caprylate incubation	> 4.5	> 4.6	> 4.5			
Caprylate precipitation & depth filtration			2.7	> 3.5	> 3.6	4.0
Column chromatography	> 3.0	> 3.3	> 4.0	> 4.0	> 1.4	4.2
Low pH incubation	> 6.5	> 4.3	5.1			
Global reduction	> 14.0	> 12.2	> 16.3	> 7.5	> 5.0	8.2

LRV	Infectivity reduction
4.0	99.99%
6.0	99.9999%
≥6.0	≥99.9999%, reduced to the limit of detection

Vox Sanguins, 2003;84:176-187

Gamunex/IGIVnex prescribing information (rev. Oct 2010)

Manufacturing Cycle



Canadian Self Sufficiency

- 30% IV Ig from Canadian sourced plasma:
  - Recovered Plasma from Whole blood collections
  - Apheresis collections
  - Purchased recovered plasma open market
- Custom fractionated by:
- CSL or Grifols:
  - Albumin, IVIg, FVIII/vWF
  - Perhaps others in future

CBS Products Plasma Derived

- **Albumin**
  - Plasbumin 5% & 25%
  - **Albumin 25%**
  - Alburex 5% & 25%
- **Antithrombin III**
- **C1 Esterase Inhibitor**
  - Berinert P
- **FVII**
  - * Factor VII
- **FVIII**
  - **Humate-P (FVIII/vWF)**
  - Wilate
- **FIX**
  - Immunine
- **FXI**
  - ***** Factor XI concentrate
- **FXIII**
  - Corifact
- **FEIBA**
- **Fibrinogen**
  - RiaSTAP
- **Hyperimmune Globulins**
  - CytoGam (anti-CMV)
  - GamaSTAN S/D (anti-HA)
  - HepaGamB (anti-HB)
  - HyperHEP B S/D (anti-HB)
  - VariZIG (anti-Varicella Zoster)
  - WinRho SDF (anti-D)
- **IGIV and SCIG**
  - **IGIVnex 10%**
  - Gamunex 10%
  - Gammagard Liquid
  - Privigen
  - Gammagard-SD
  - Hizentra
  - Octagam
- **Protein C**
  - -* Ceprotin
- **Prothrombin Complex**
  - Octaplex
  - Beriplex
- **SDP**
  - Octaplas

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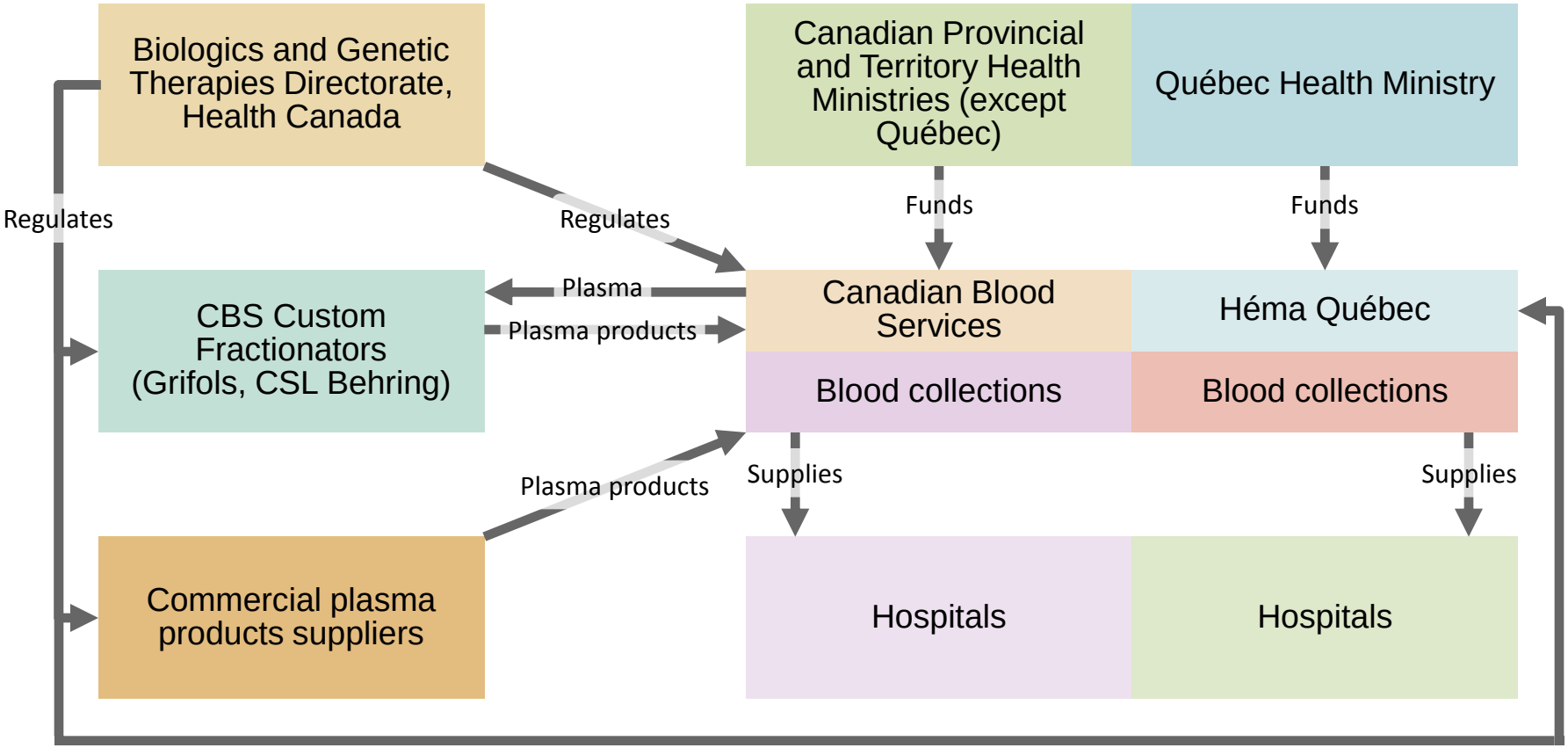
* These products are not licensed by Health Canada and require Special Access Program authorization

CBS Products Recombinant

- rVIIa
  - Niastase
- rVIII
  - Advate
  - Kogenate
  - Xyntha
- rIX
  - Benefix
- rXIII
  - Tretten

Licensing & Product Selection

Blood and Plasma Supply System in Canada



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Adapted from Review of Australia's Plasma Fractionation Arrangements: Chapter 4: How other countries meet their needs

Licensing

- Plasma products are **Drugs**
- Licenced as any drug
- Adverse events are handled as any adverse event from a drug as far as reporting to authorities (HC)
- All are licensed somewhere in the world even if not licensed in Canada
- Unlike drugs –plasma products paid for directly by the provinces (tax dollars) = **blank cheque**

CBS Product Selection Process

- CBS is the distributor for Plasma products:
  - More than one supplier where possible
- New brand versus new category
  - Request for proposal (RFP)
  - Medical review – includes treaters and users
  - Economic- cost benefit and alternatives
- EMT (CBS) approval and P/T approval – especially if economic impact

Acknowledgements

- Alan Lazarus
- Mathias Haun
- Joanne Cybulski
- Plasma Protein Staff
- Robert Skeate

References and Resources

- T. Burnouf; Modern Plasma Fractionation; Trans. Med. Rev. 2007 Vol 21 No. 2 p. 101-117
- T. Burnouf: Recombinant Plasma Proteins; Vox sanguinis 2011; 100,p.68-83
- Specific Product monographs
- www.who.int/bloodproducts
- www.Blood.ca
- Clinical Guide to Transfusion:

  Chapters 5 & 7

Questions?