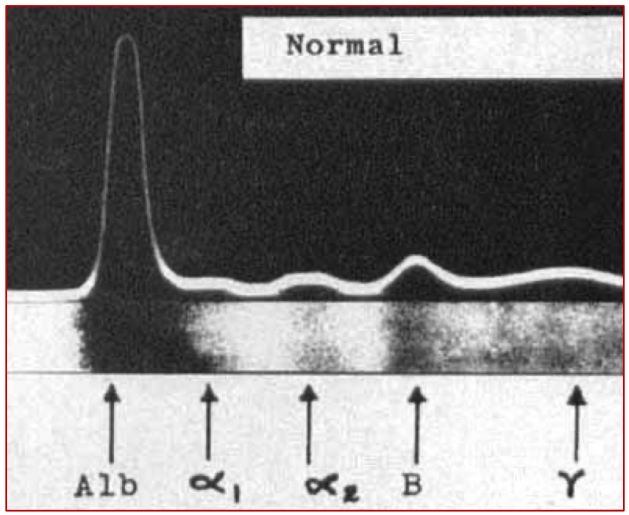
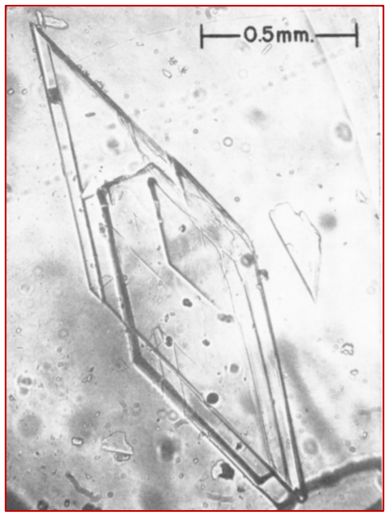
Fractionation in a nutshell

John Curling, JCC AB, Uppsala, Sweden



Tiselius *et al.*, 1937



Cohn *et al.*,1947

Octapharma/ZETA, 2022

Key figures for fractionated, plasma-derived products

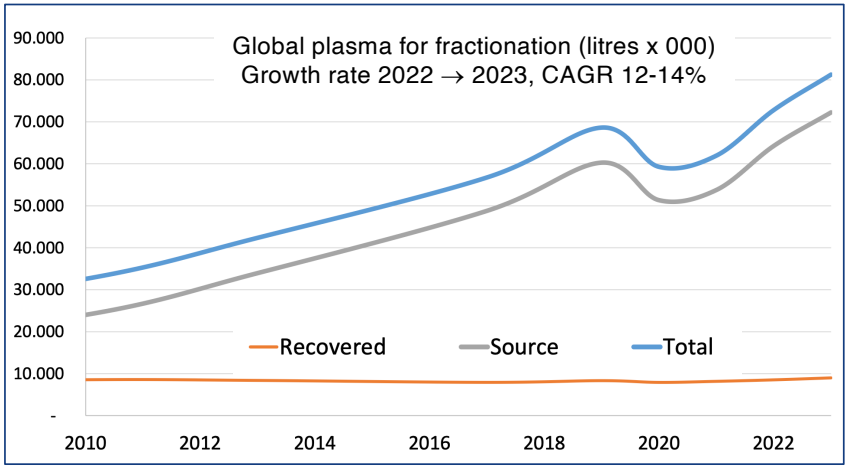
Australian Red Cross Lifeblood

Global use of plasma-derived products, 2022

- **16.5 million people received a plasma-derived product**
- **12.5 million were treated with a hyperimmune product** (6.5 million in China)
- **IgG:** ~ 1.0 million were prescribed polyvalent IgG (300,000 in the USA)
- **Albumin**: ~ 1.9 million were administered albumin (almost 50% in China), mostly for acute conditions
- **Coagulation factors:** ~ 100,000 patients were treated with FVIII, FIX, VWF complex or another P-D factor.
- **Other PDMP's**: ~ 1.0 million patients received another PDMP (6% of the total)

Global forecasts for 2026

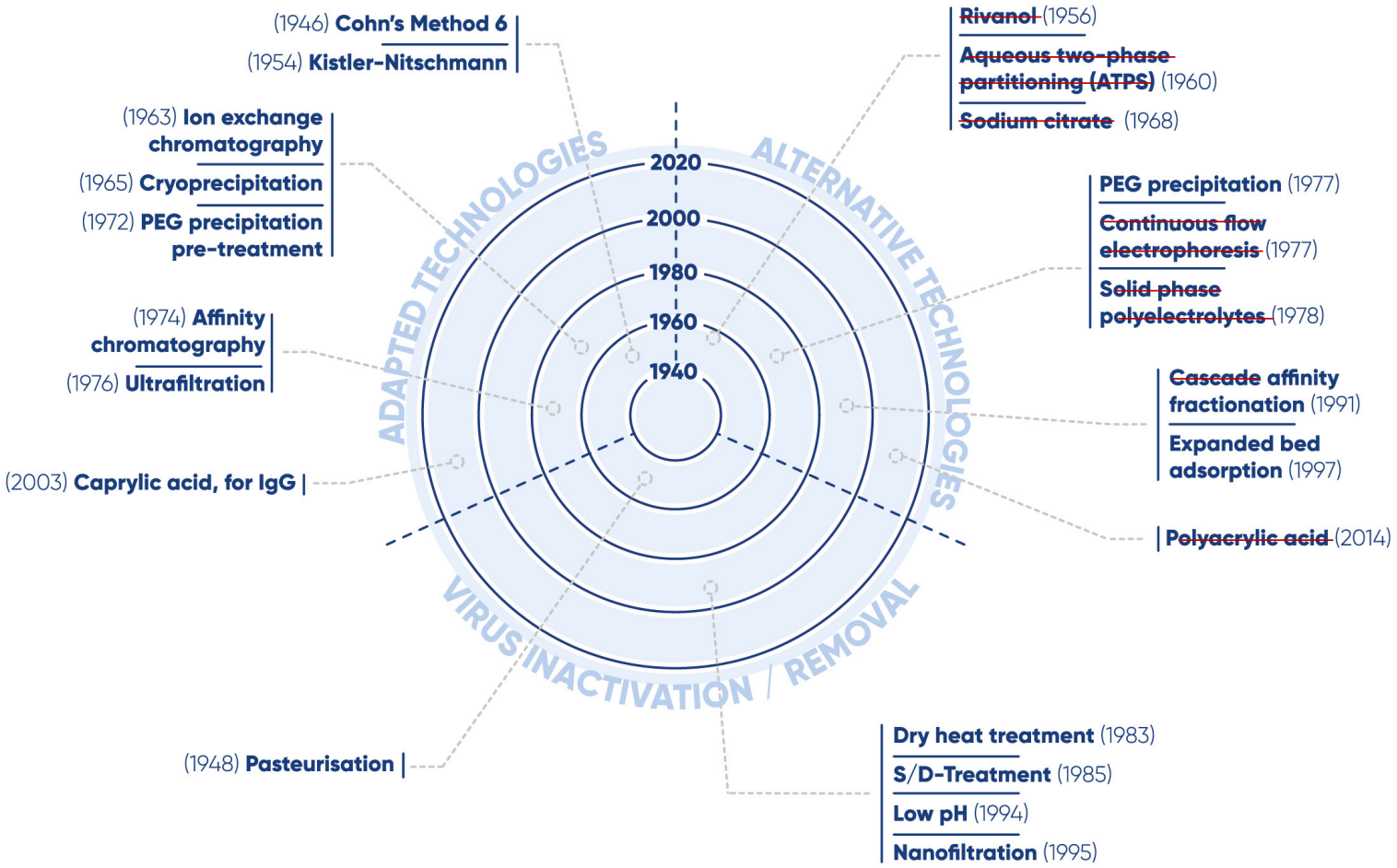
Plasma for fractionation	**90 million litres***
Immunoglobulin	360 tonnes ↑
Albumin	1,400 tonnes ⇅ ?
P-D Factor VIII	14 Billion units ↓



* Four fractionators command 75% of the $35 Billion market (2024)

Robert, 2020, 2024, Statista, 2025

Eight decades of innovation: integrated fractionation technologies



Curling *et al.*, 2025

Separation and purification: science + technology/technical know-how

- **Cryoprecipitation**, coagulation factors
- **Ethanol precipitation**, bulk products, HSA and IgG
- **Caprylic acid precipitation**, IgG
- **Depth filtration + filter aid**, silica, Diatomaceous earth, Bentonite, cellulose
- **Centrifugation**, cryoprecipitate separation
- **Ion exchange chromatography**, all proteins
- **Hydrophobic interaction chromatography**, C1H etc
- **Affinity chromatography**, ceruloplasmin, anti-A/Anti-B, plasminogen, etc
- **Reverse phase chromatography**, S/D removal
- **Ultrafiltration**, concentration, removal process reagents
- **Diafiltration**, buffer change
- **S/D incubation**, lipid envelope virus (LEV) reduction
- **Caprylic acid incubation**, lipid envelope virus reduction
- **Pasteurization**, virus reduction
- **Dry heat/vapour heat**, virus reduction
- **Nanofiltration**, non-LEV reduction
- **Sterile filtration**, sterility
- **Product hold at elevated temperature**, security

Robotics

Digital twin designed precipitation

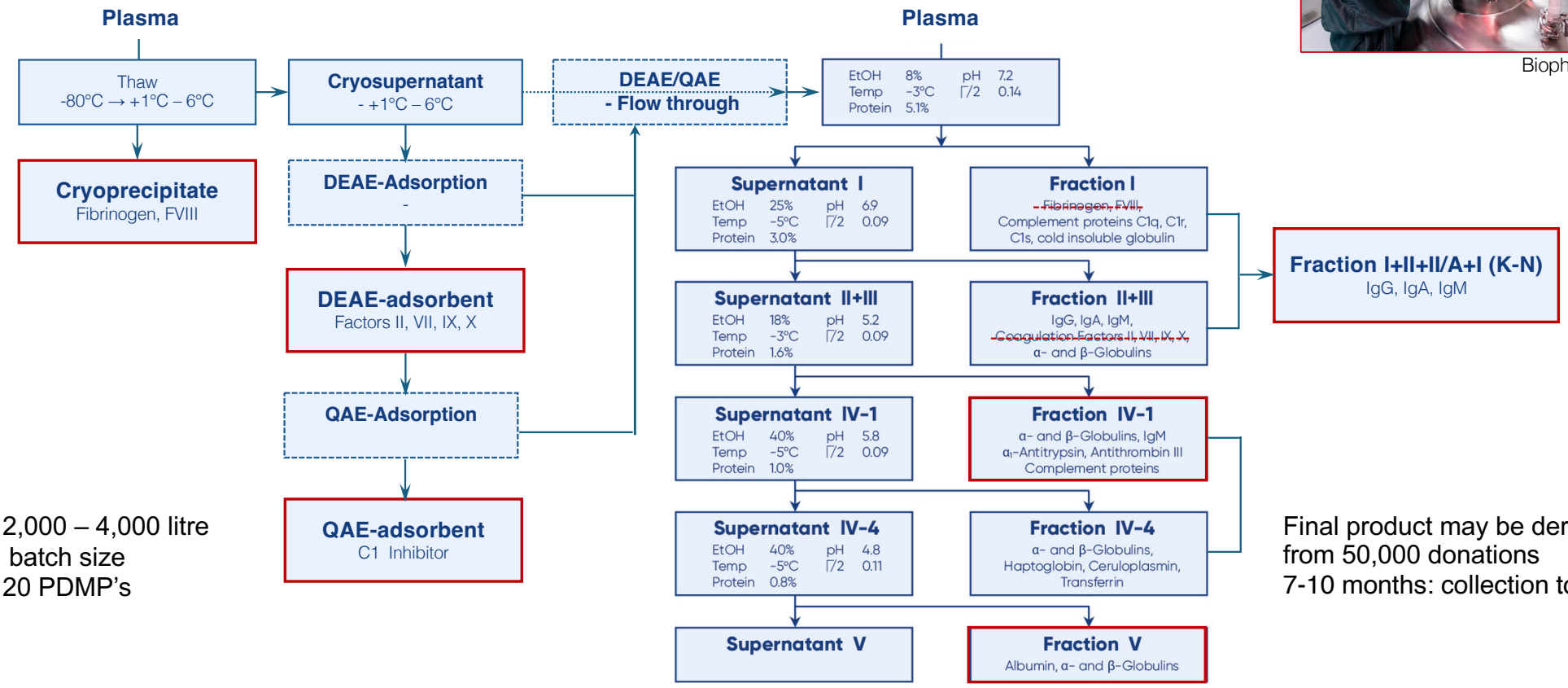
Chromatography

Filter press separation

Grifols, CSL, Takeda

Cohn/Kistler-Nitschmann – Generalised hybrid fractionation


Biopharma

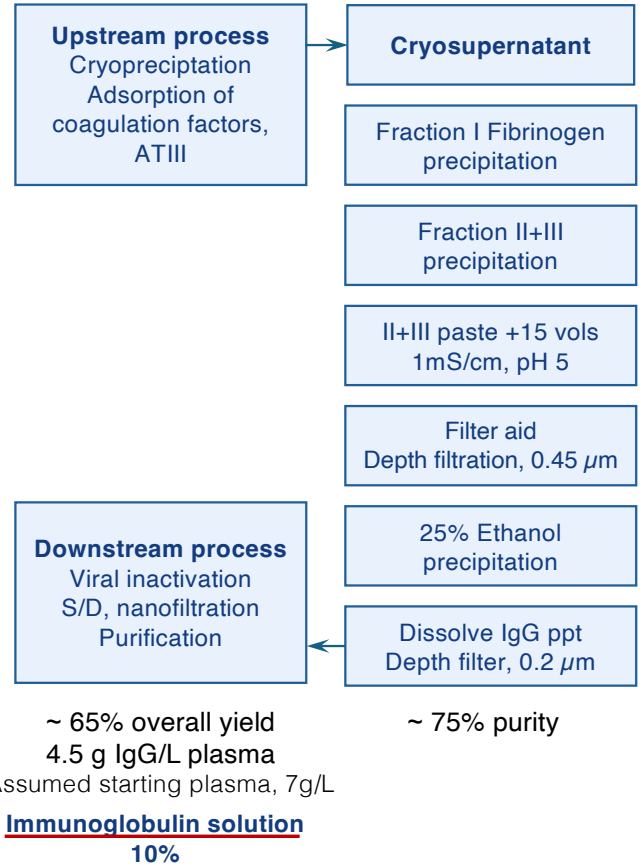


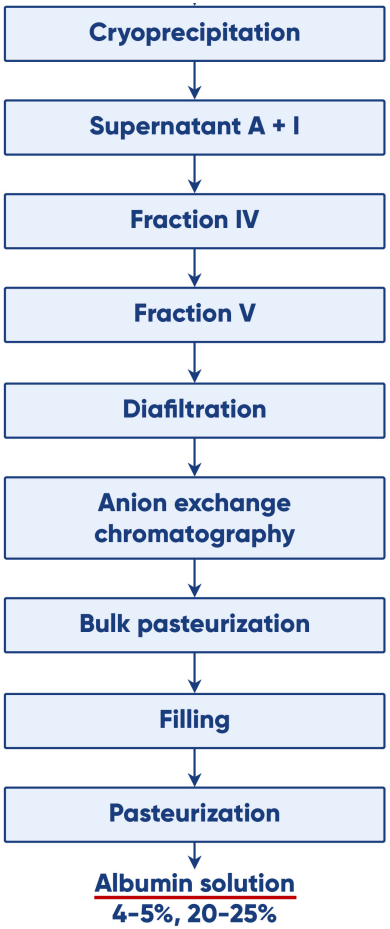
~ 2,000 – 4,000 litre batch size
~ 20 PDMP's

Final product may be derived from 50,000 donations
7-10 months: collection to use

Adapted from Curling *et al.*, 2025

Ethanol precipitation





Virus and prion removal capacity of the cold ethanol fractionation/depth filtration process

Table 5 Virus and prion removal capacity of the upstream cold ethanol fractionation/depth filtration process

Virus/prions	pH	Reduction factor (log_{10})[a,b]
HIV-1	Min	> 5·2
	Max	> 5·1
BVDV	Min	0·8
	Max	1·9
PRV	Min	> 5·0
	Max	> 4·8
HAV	Regular	3·9
EMCV	Min	3·8
	Max	> 4·4
B19V	Regular	5·8
MMV	Min	4·7
	Max	> 5·1
PrP^{TSE} (crude brain homogenate)	Max	≥ 3·6
	Max	≥ 3·7
PrP^{TSE} (microsomal fraction)	Max	≥ 2·1
	Max	≥ 3·3

Max, maximum; min, minimum.
[a]Viral load determined by infectivity assays all samples except B19V, for which PCR was used.
[b]The '>' and '≥' symbols indicate complete removal.

Teschner *et al.* 2006, Poelsler 2008, Matejtschuk *et al.* 2000

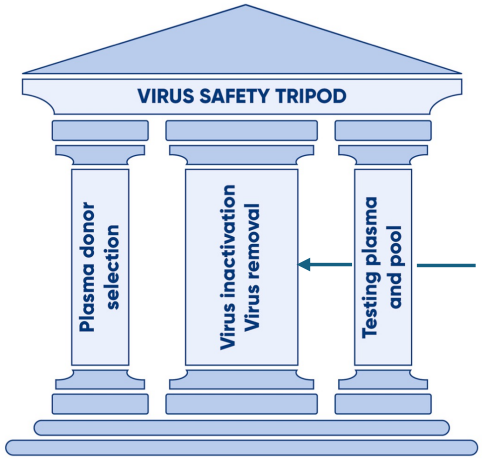
Downstream purification and viral reduction/inactivation

CSL

Product example	Plasma intermediate	Purification sequence
Factor VIII LFB, Factane	Cryoprecipitate	Re-suspend → $Al(OH)_3$ adsorption → centrifugation → filtration → **S/(D treatment** → anion exchange chromatography → FVIII/VWF dissociation ($CaCl_2$) → **35nm,15nm nanofiltration** → re-association → diafiltration → lyophilization
Fibrinogen Intas, Fibrogen-I	Cryoprecipitate	Re-suspend → $Al(OH)_3$ adsorption → glycine precipitation → **S/D treatment** → depth filtration → anion exchange chromatography → diafiltration → lyophilization → **dry heat treatment, 100°C/30 min**.
PCC Octapharma, Octaplex	Cryosupernatant	Heparin/pH adjustment → anion exchange adsorption → **S/D treatment** → anion exchange chromatography → **nanofiltration** → DF/UF → Heparin/pH adjustment → lyophilization
Factor IX Octapharma, Octanine F	PCC	Anion exchange chromatography → **S/D treatment** → removal of S/D reagents → heparin affinity chromatography → **nanofiltration** → UF/DF → lyophilization
C1 esterase CSL	PCC supernatant	$AmSO_4$ precipitation → **pasteurization** → $AmSO_4$ precipitation → hydrophobic interaction chromatography → **nanofiltratio**n → DF/UF → lyophilization
AAT Grifols, Prolastin	Fraction IV 1,4	Re-suspend → PEG precipitation → **S/D treatment** → anion exchange chromatography → UF//DF → cation exchange chromatography → **nanofiltration** → UF/DF → lyophilization
IgG GC Biopharma, ALYGLO	Fraction I+II+III→ II	Re-suspend → depth filtration → diafiltration → anion exchange chromatography → **S/D treatment** → depth filtration → cation exchange chromatography → UF/DF → **nanofiltration, 20 nm** → UF
Albumin Kedrion (BPL) Zenalb	Fraction V	(Initial plasma pool treated with diatomaceous earth) Re-suspend → diafiltration → anion exchange chromatography → **bulk pasteurization** → **post-filling pasteurization**
Hyperimmune IgG (Cangene)	Hyperimmune plasma	Thaw and dilute plasma → Lipid precipitation → clarification/dilution → anion exchange chromatography → **nanofiltration, 20nm** → ultrafiltration → **S/D treatment** → reverse-phase chromatography → UF/DF→ formulation

Adapted from Curling, 2025

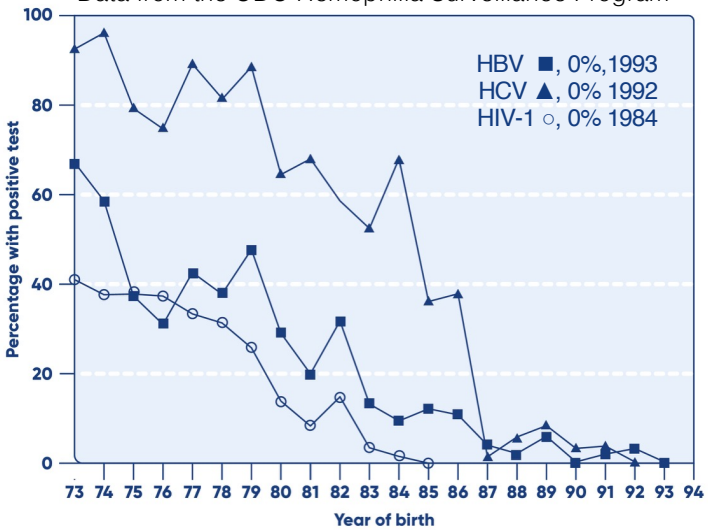
Virus safety steps



- At least two orthogonal viral inactivation steps
- Dedicated, claimed
- Reduction factor ranges from >10 $_{log10}$ to >12 $_{log10}$
- Total clearance >14 $_{log10}$ to >18 $_{log10}$
- ($_{>10\ log10}$ considered effective)
- < 10^{-6} virus particles in final product

- S/D, Caprylic acid incubation
- Nanofiltration
- Pasteurization
- Dry heat/Vapour heat
- pH 4 for IgG
- Partition effects
  - Precipitation
  - Chromatography
  - Depth filtration

Data from the CDC Hemophilia Surveillance Program



Prevalence of blood-borne infections in haemophilic birth cohorts in the United States. Based on the results of laboratory testing for HBV (■), HCV (▲), and HIV-1 (○). The proportion was zero for HIV after 1984, for HCV after 1992, and for HBV after 1993.

"Overall, the experience gained in the past decades has resulted in an absence of pathogen transmission from the current generation of plasma derivatives, but maintaining vigilance, and the surveillance of the emergence of infectious agents, is vital to ensure the continued efficacy of the measures in place and the development of further interventions aimed at obviating safety threats" Farrugia A, 2023

Farrugia, 2023, Turecek *et al.* 2022, Dichtelmüller, 2013, Soucie, 2001

Virus reduction

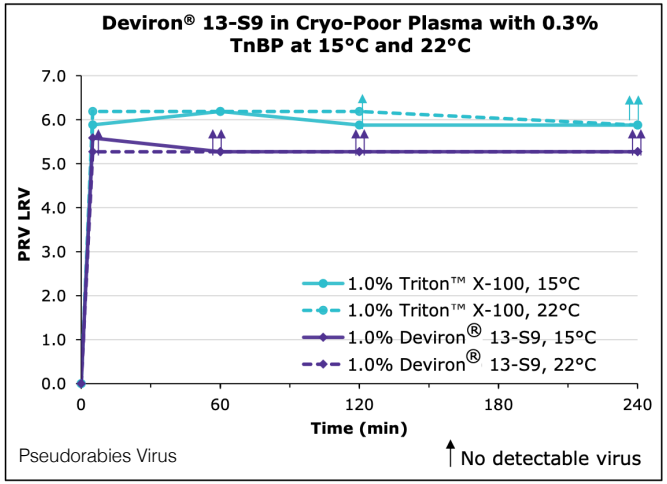
NBI

Solvent/detergent inactivation

Inactivation of viruses in labile blood derivatives. I. Disruption of lipid-enveloped viruses by tri(n-butyl) phosphate detergent combinations
Horowitz *et al.* New York Blood Center, 1985

Classical combination: TNBP/Triton X-100
REACH* classification of Triton X-100: ecological toxicity
Alternative: Deviron 13-S9 (non-ionic C11-C15 alcohol)
Eliminated in downstream process (ICH Q6B)
Biodegradability demonstrated (Sustainability)

*REACH: EU Registration, Evaluation, Authorisation and Restriction of Chemicals



Sodium caprylate inactivation

Example from IVIG manufacturing

Process step	Log_{10} virus reduction HIV-1	PRV	BVDV	Reo	HAV	PPV
Caprylate precipitation/ cloth filtration	ND	ND	2·4 ± 0·3	2·1 ± 0·4	2·6 ± 0·2	2·2 ± 0·1
Caprylate incubation	≥ 4·5	≥ 4·6	≥ 4·5	ND	ND	ND
Depth filtration	ND	ND	ND	≥ 4·3	≥ 2·0	3·3 ± 0·3
Column chromatography	≥ 3·0	≥ 3·3	4·0 ± 0·3	≥ 4·0	≥ 1·4	4·2 ± 0·2
Low-pH incubation	≥ 6·5	≥ 4·3 ± 0·3	3·5 ± 0·4	ND	ND	ND
Global reduction[a]	≥ 14·0	≥ 12·2	≥ 14·4	≥ 6·1	≥ 3·6	6·4

Data are expressed as mean ± SD log_{10} virus inactivated or partitioned.
[a]Depth filtration not included (see ref. 35).
BVDV, bovine viral diarrhoea virus; HAV, hepatitis A virus; HIV-1, human immunodeficiency virus 1; ND, not done; PPV, porcine parvovirus; PRV, pseudorabies virus; Reo, reovirus type 3.

Nanofiltration

Example for Planova 20nm

Virus removal for IgG

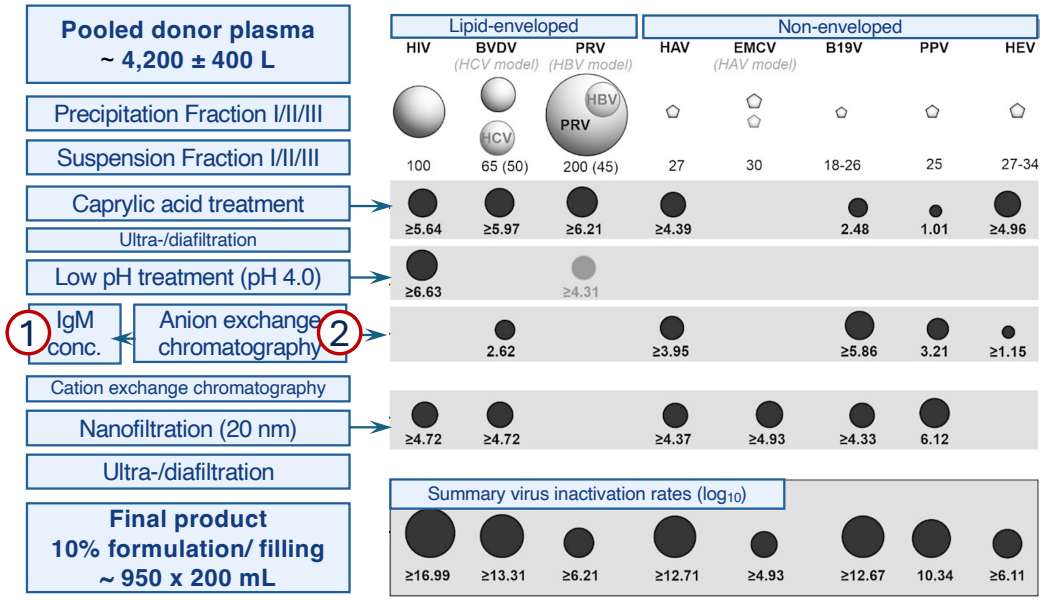
Filtration to 150 L/m² of1 mg/mL h-IgG solution in 100 mM NaCl on Planova S20N filters at 196 kPa. Process pause for 30 min, additional 15 L/m² filtration
(Data from Asahi)

Virus	Size (nm)	LRV*
Minute virus of mice, MVM (N)	18-24	≥ 5.3
Porcine parvovirus, PPV (N)	18-24	≥ 5.8
Pseudorabies virus, PRV (E)	120-200	≥ 5.4
Encephalomyocarditis virus, EMCV (N)	25-30	≥ 5.9
Bovine diarrrhoea virus, BVDV (E)	50-70	≥ 5.9
Human immunodeficiency virus, HIV (E)	80-120	≥ 4.1

* Total pool virus LRV after 30 min process pause. LRV =average of duplicate runs

Banerjee *et al.* 2025, Antonello 2025, Lebing 2003, Hongo-Hirasaki *et al.* 2025

Immunoglobulin process example: virus and prion clearance

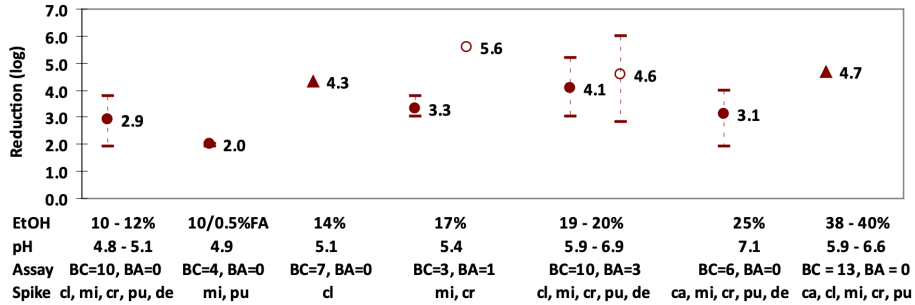


1. ~ 21% of protein load
2. ~ 78% of protein load

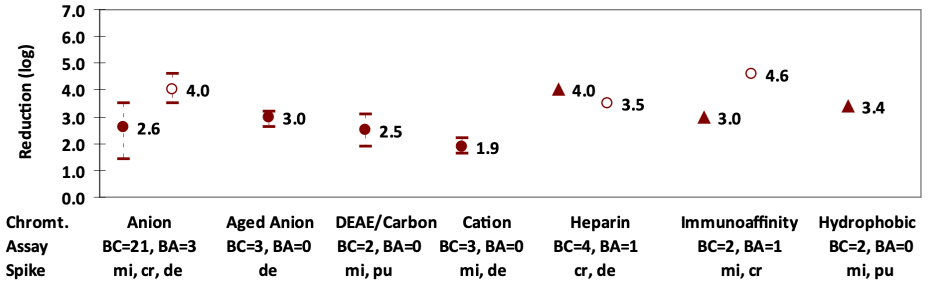
Viral clearance for an immunoglobulin process (EU approval 2022, US approval 2024)

Prion clearance estimate ≥10.45 $_{log10}$ to ≥ 12.59 $_{log10}$

Prion clearance by ethanol precipitation (relative to supernatant)



Prion clearance by chromatography (target protein bound)



Poelsler *et al.* 2025, Cai *et al.* (PPTA) 2013

Manufacturing scale: an example

Biotest project
VTU Engineering (+ >100 contractors)

- 1.4 Million litres plasma
- Albumin, IgG, IgM, Fibrinogen

- Approx.1,000 process devices and equipment units
- 160 tonnes steel construction and brackets
- 35 km of pipes, approx. 41,000 pipe components
- 6,000 valves
- 250 control cabinets
- 241 P&ID

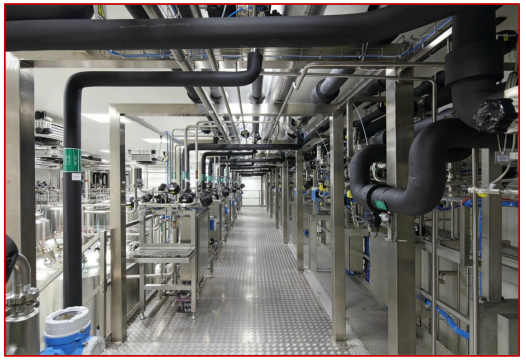
- Production building:
- Dimension: 60 x 100 m = 6,000 m²
- Building height: 32 m from ground level
- Gross floor area (GFA): 31,750 m²
  (= 4.5 football pitches)
- 300 new positions

Core engineering: 50
Total project: 180 engineers

- Plasma cold storage
- Defrosting area incl. cutting area
- Filter aids / Weighing
- Ultra pure media (AP, WFI, ultra pure steam)
- Preparation of CIP solutions
- Preparation of buffer solutions

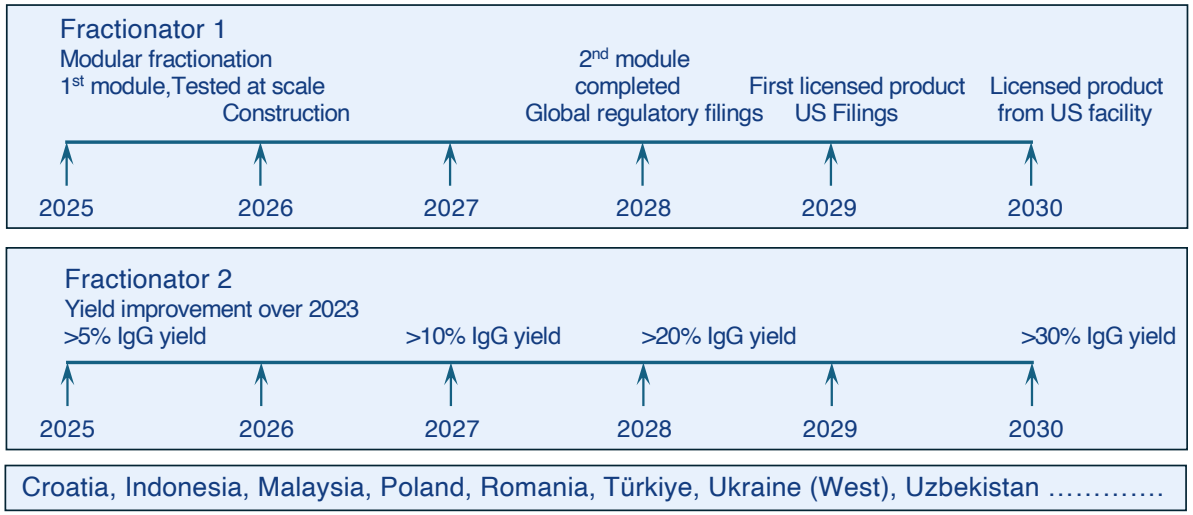
- Fractionation

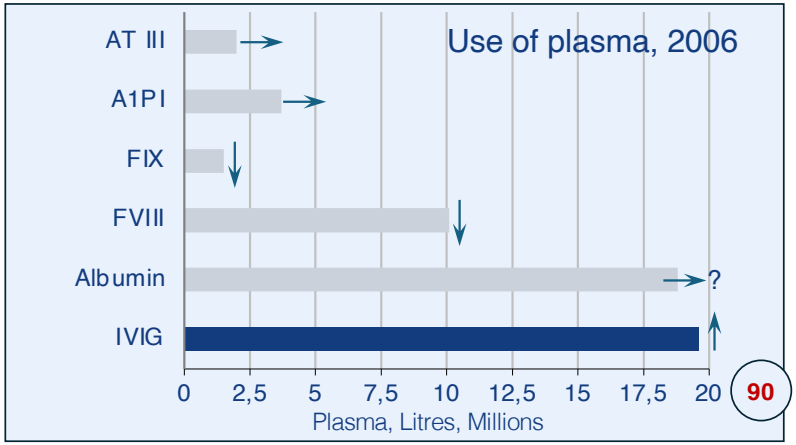
- Heating / cooling media, heating steam
- Combined Heating and Power units (Sustainability)
- Compressed air
- Tank farm incl. tanking (solvent, acid, lye, heating oil)
- Waste water collection and treatment (Sustainability)
- Process exhaust air
- Support area (cleaning, autoclaving)



Biotest/VTU

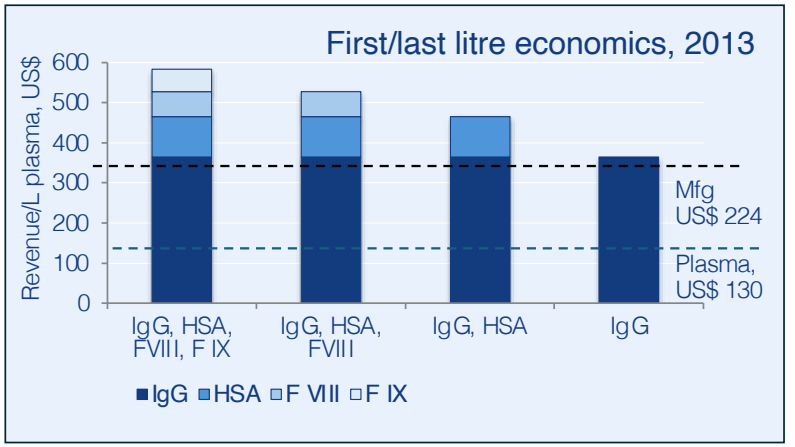
Future plasma fractionation





- Bioprocessing 4.0
- Digitalization
- Sustainability

"The realization of Industry 4.0 promises digitally integrated facilities with fully automated manufacturing, real-time traceability, standardized procedures and agile processes."



Grifols 2005/6, Goss & Curling 2013, von Bonsdorff *et al.* 2025, Farid *et al.* 2023

Fractionation in a nutshell

John Curling, JCC AB, Uppsala, Sweden

IPFA/EBA, Leuven 2026, Reference List

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IPFA, PPTA, friends and colleagues of the plasma world

“We might be lying in the gutter, but we are looking at the stars”

Oscar Wilde