Domestic Scalable Plasma processing: technological approaches and solutions for LMIC

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ISBT Workshop
September, 2021

Disclaimer:

Views expressed in this talk constitute our professional opinion while being Merck Employees

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Outline

1. **Overview on Plasma production**
2. **Safe plasma from safe processes- case study sharing**
3. **Proposals for strategic approach to improve domestic plasma production in LMIC**
4. **Take-home messages**

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Immunoglobulin G

Prosperous market outlook however unbalance supply for LMIC

How to implement pragmatic and scalable technologies to make safe products to treat patients in need?

Wastage esp. in LMIC

Shortage

objectives

Approaches to make safe plasma & avoid plasma wastage in LMIC

Intensified purification of Plasma IgG

How novel technologies helps to make safe PDMPs efficiently

Scalable process –every drop can be used

How to design a process from small to large scale

Process technology proposals

Single-use technologies accelerate flexible manufacturing

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From Plasma donation to patient adminstration

Case study from public-private collaboration

Blood Transfusion 2021;
DOI 10.2450/2021.0159-21

BLOOD TRANSFUSION

Home Aims and Scopes Affiliated societies Editorial board

Process steps for the fractionation of immunoglobulin (Ig) G depleted of IgA, isoagglutinins, and devoid of in vitro thrombogenicity

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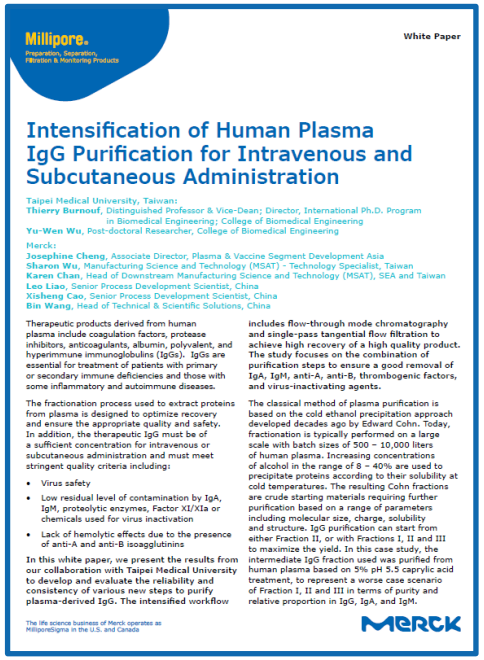
Preview

ABSTRACT

Background - Plasma-derived immunoglobulins (IgG) are essential medicines that are in worldwide shortage, especially in low- and middle-income countries. Optimised manufacturing processes can increase supply. We evaluated various new process steps for IgG fractionation.
Material and methods - A crude, worst-case, IgG intermediate obtained by caprylic acid fractionation of cryoprecipitate-poor plasma was used as starting experimental material. It was processed inline by Fractogel® (Merck)TMAE anion-exchanger to deplete IgA and IgM, Eshmuno® P (Merck) anti-A and anti-B affinity chromatography to remove anti-A and anti-B isoagglutinins, 0.3% TnBP-1% Triton X-100 (S/D) treatment, C18 chromatography for removal of S/D agents, and single-pass tangential flow filtration (SPTFF) concentration to 20%. Quality, safety, and recovery were evaluated at small and pilot scales to assess purity, removal of IgA, IgM isoagglutinins, S/D agents, thrombogenic factors, and lack of toxicity in a cell model.
Results - The starting IgG intermediate contained approximately 90% IgG, IgA, and IgM and 10% albumin. Fractogel® TMAE, equilibrated in 25 mM sodium acetate-pH 6.0 and loaded with up to 225 mg of IgG/mL, could remove IgA and IgM, with over 94% IgG recovery with preserved sub-class distribution in the flow-through. Sequential Eshmuno®-P anti-A and anti-B columns efficiently removed isoagglutinins. The C18 packing, used at up to 17 mL of S/D-IgG solution per mL, removed TnBP and Triton X-100 to less than 1 and 2 ppm, respectively. The 20% purified IgG was devoid of activated factor XI and thrombin generation activity.
Discussion - This purification sequence yields a >99% pure, 20% (v/v) IgG product, depleted of IgA, isoagglutinins, and thrombogenic markers, and should be implementable on various IgG intermediates to help improve the supply of immunoglobulins.

Keywords: *plasma, IgG, IgA, anti-A, anti-B, factor XI, solvent-detergent.*

Merck White Paper



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White Paper

Intensification of Human Plasma IgG Purification for Intravenous and Subcutaneous Administration

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Therapeutic products derived from human plasma include coagulation factors, protease inhibitors, anticoagulants, albumin, polyvalent, and hyperimmune immunoglobulins (IgGs). IgGs are essential for treatment of patients with primary or secondary immune deficiencies and those with some inflammatory and autoimmune diseases.

The fractionation process used to extract proteins from plasma is designed to optimize recovery and ensure the appropriate quality and safety. In addition, the therapeutic IgG must be of a sufficient concentration for intravenous or subcutaneous administration and must meet stringent quality criteria including:

- Virus safety
- Low residual level of contamination by IgA, IgM, proteolytic enzymes, Factor XI/XIa or chemicals used for virus inactivation
- Lack of hemolytic effects due to the presence of anti-A and anti-B isoagglutinins

In this white paper, we present the results from our collaboration with Taipei Medical University to develop and evaluate the reliability and consistency of various new steps to purify plasma-derived IgG. The intensified workflow includes flow-through mode chromatography and single-pass tangential flow filtration to achieve high recovery of a high quality product. The study focuses on the combination of purification steps to ensure a good removal of IgA, IgM, anti-A, anti-B, thrombogenic factors, and virus-inactivating agents.

The classical method of plasma purification is based on the cold ethanol precipitation approach developed decades ago by Edward Cohn. Today, fractionation is typically performed on a large scale with batch sizes of 500 – 10,000 liters of human plasma. Increasing concentrations of alcohol in the range of 8 – 40% are used to precipitate proteins according to their solubility at cold temperatures. The resulting Cohn fractions are crude starting materials requiring further purification based on a range of parameters including molecular size, charge, solubility and structure. IgG purification can start from either Fraction II, or with Fractions I, II and III to maximize the yield. In this case study, the intermediate IgG fraction used was purified from human plasma based on 5% pH 5.5 caprylic acid treatment, to represent a worse case scenario of Fraction I, II and III in terms of purity and relative proportion in IgG, IgA, and IgM.

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada

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Facilitating towards safe & efficient Plasma production

A generic, easy-to-operate, flowthrough-mode purification process that provides scalable & robust purification with enhanced productivity and quality IGG fitting for therapeutic usage.

Quality criteria:
- Virus safety
- Low IgA & IgM contamination
- Low FXI/XIa
- Lack of Hemolytic effect
- Lack of chemicals used for virus inactivation

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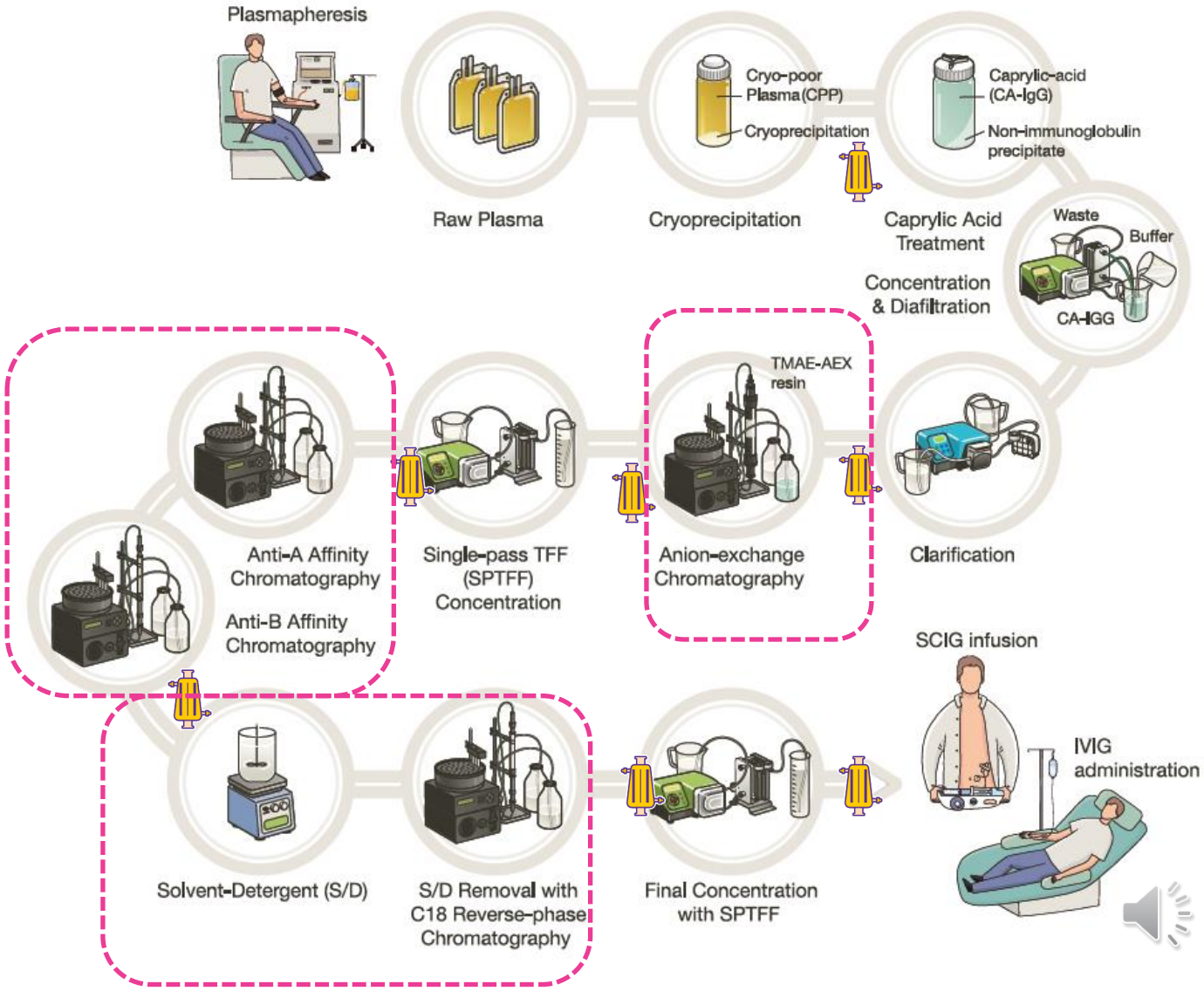
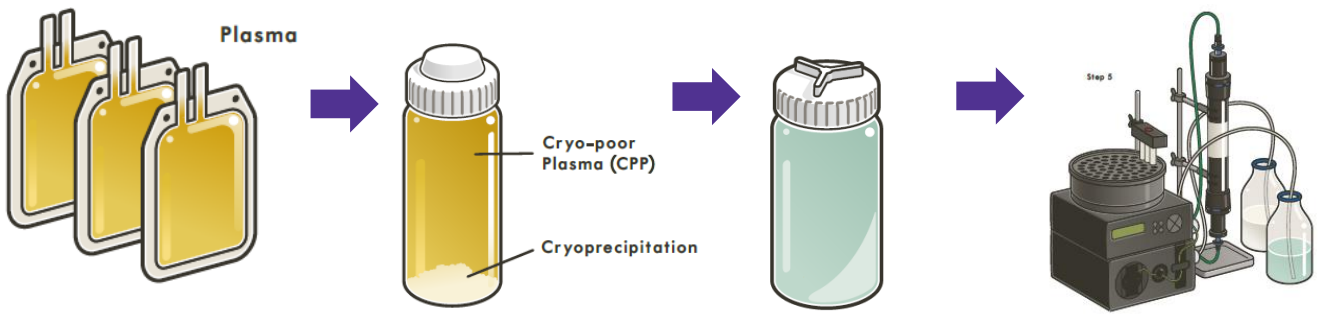
Solid proof points for intensified IgG purification readily scalable for small-large-scale manufacturing

- ✓ FT-chrom for primary purification 82%→ 99%, mainly reduce IgA and IgM
- ✓ Eshmuno P reduced 8-32X anti-A/ anti-B isoaglutinins
- ✓ Triton-X 100/ TnBP provides strong Virus inactivation as soon as 5 minutes, and removal of S/D with Licroprep C18 resin in FT mode is effective (bdl)
- ✓ SPTFF technology (data not shown) provides a gentle way of inline/ final concentration, reaching 20% final target for SCIg purpose.
- ✓ All Aseptic filters/ prefilters showing robust filtration results and recovery (~ 100%)
- ✓ Overall process can be sliced & diced fitting the target end product(s), and can be easily incoporated a second virus removal method eg. Virus filtration, to meet regulatory requirement.
- ✓ All steps are readily scalable & implementable

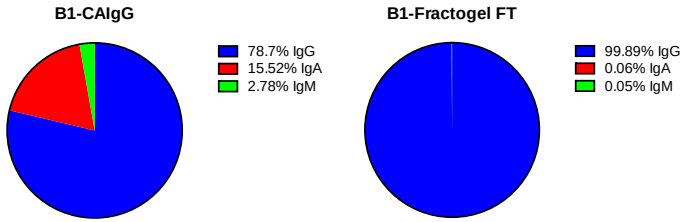


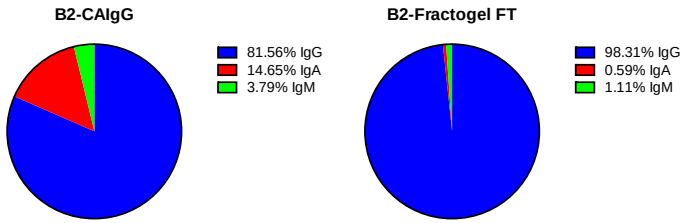
Proposal #1:

Flowthrough one-step to remove IgA & IgM

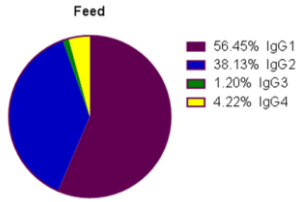


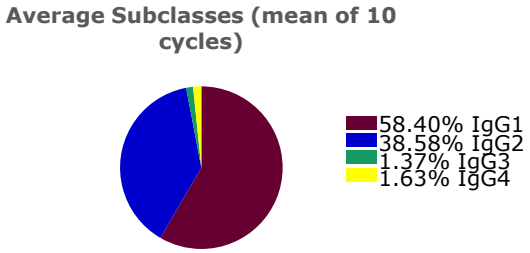
Pilot scale in 2 batches IgG/IgA/IgM





Small scale in 10 cycles IgG1/IgG2/IgG3/IgG4





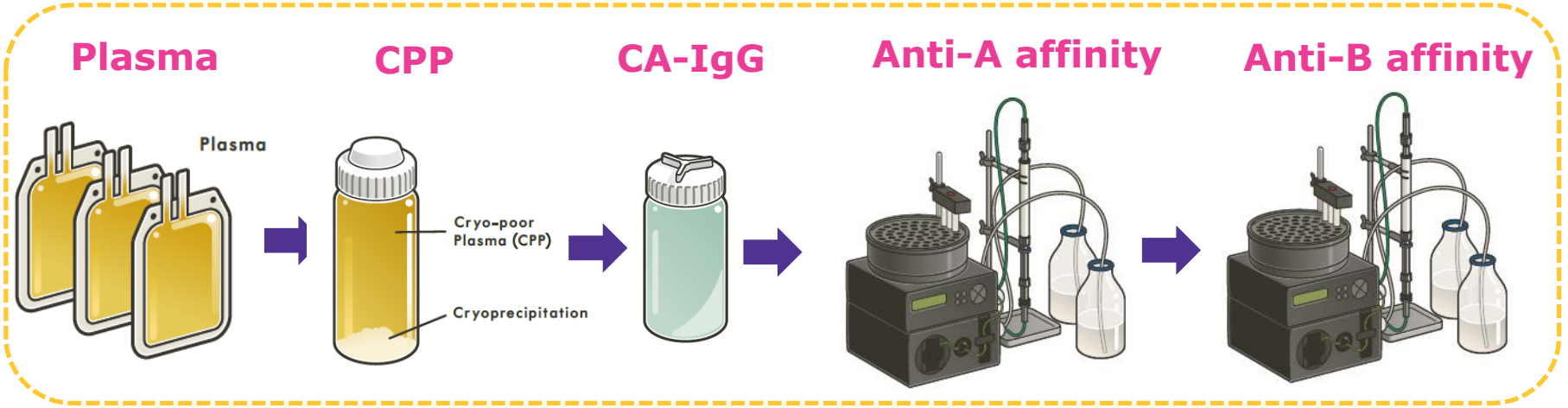
Summary #1 with AEX step:

1. Flow through one-step IgA/IgM removal
2. Purity IgG avg. 82% to 99% in small and pilot scale.
3. 200 cycles test
4. No changes in IgG subclasses.
5. No thrombogenicity activity detected

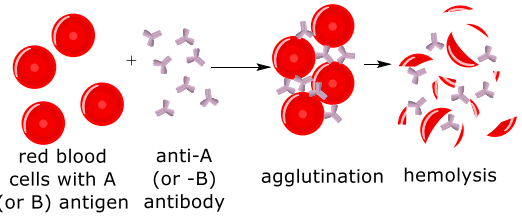
Learn More with our webinar:
Chromatography: Chromatographic strategies for IVIG purification - Part 2

Proposal #2:

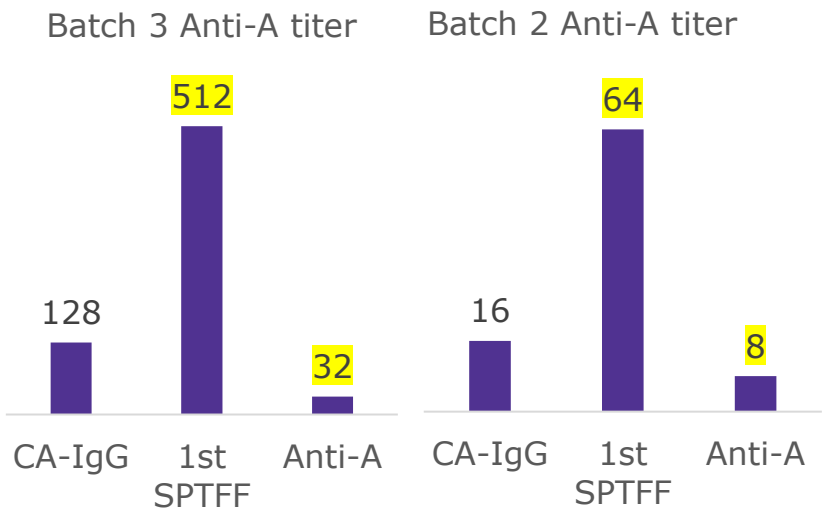
Robust reduction of the blood-type specific isoagglutinins



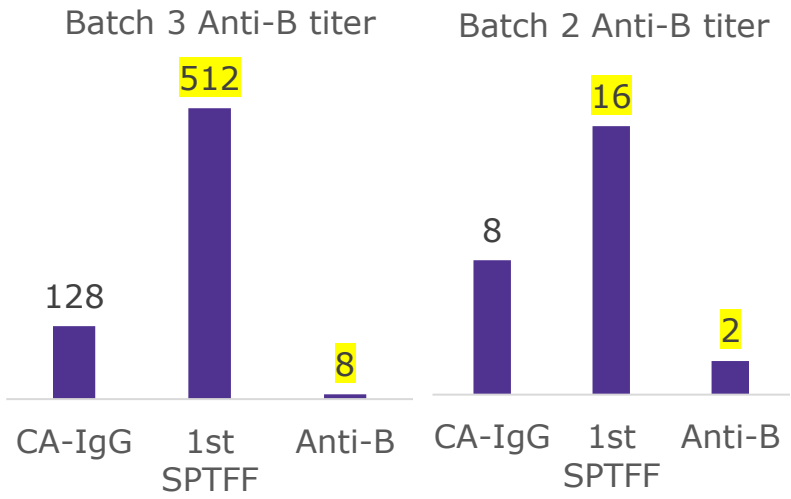
	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B



Eshmuno® P Anti-A (FT)



Eshmuno ® P Anti-B (FT)

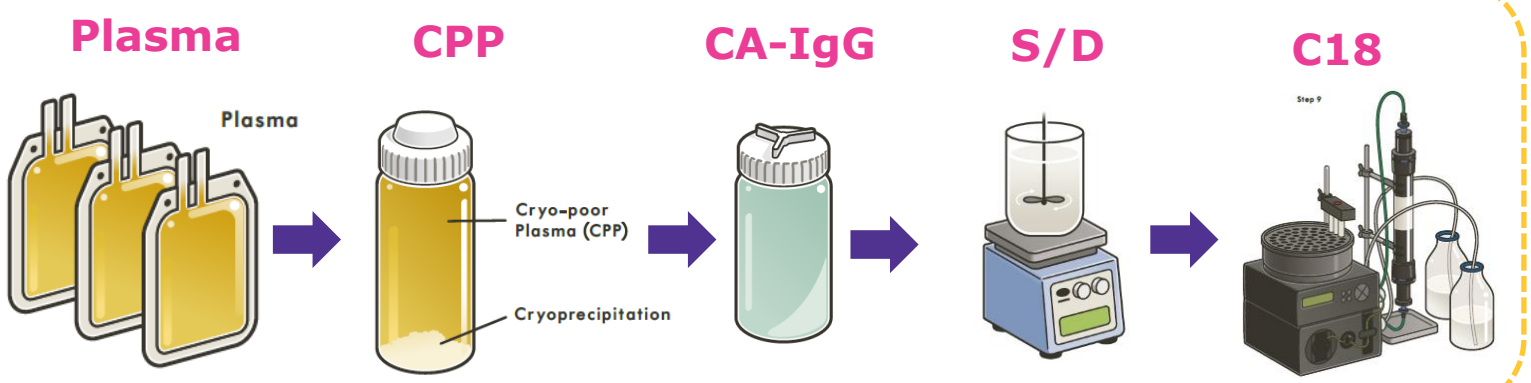


- **8 to 16 times reduction in Anti-A titer**
- **16 to 32 times reduction in Anti-B titer**

*samples tested at 30mg/ml concentration from 1st SPTFF (6X) step to the last step.

Proposal #3:

Second Virus inactivation step with S/D and removal by Licroprep C18 (40 – 63um)



Key points:

A. Classical TnBP/Triton X-100 provides > 4-5 LRV in time as short as 5 minutes.

B. Typical chromatography for FT mode S/D-IGG running through C18 column, residual of S/D tested as low as 1ppm and 2ppm, respectively.

A.

Human IgG : 0.3% TnBP + 1% TX100 LRV Results					
Virus	Device	LRV at Incubation Time (min)			
		5	30	60	360
XMuLV	Mobius 1	≥5.5	≥5.3	≥5.3	≥5.4
	Mobius 2	≥5.5	≥5.3	≥5.3	≥5.5
BVDV	Mobius 1	≥4.5	≥4.4	≥4.6	≥4.5
	Mobius 2	≥4.4	≥4.6	≥4.4	≥4.5

B.

Residual TnBP of SD-IgG (Ratio of resin and loaded IgG)	Batch 3 (1mL:6mL)
C18	<1 ppm
SPTFF-5X	<1 ppm

Residual Triton X-100 of SD-IgG (Ratio of resin and loaded IgG)	Batch 3 (1mL:6mL)
C18	<2 ppm
SPTFF-5X	<2 ppm

Learn More with our webinar:
Solvent Detergent Viral Inactivation using S.U Technology in Blood Fractionation Processes

*Table A source: Hsieh YT, Mullin L, Greenhalgh P, Cunningham M, Goodrich E, et al.: Single-use technology for solvent/detergent virus inactivation of industrial plasma products. *Transfusion* 2016; 56: 1384-93.

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A pragmatic approach

Establishing domestic capability on plasma processing

Understand cost structure

Think scalable from the start

Incorporate single-use to facilitate competency

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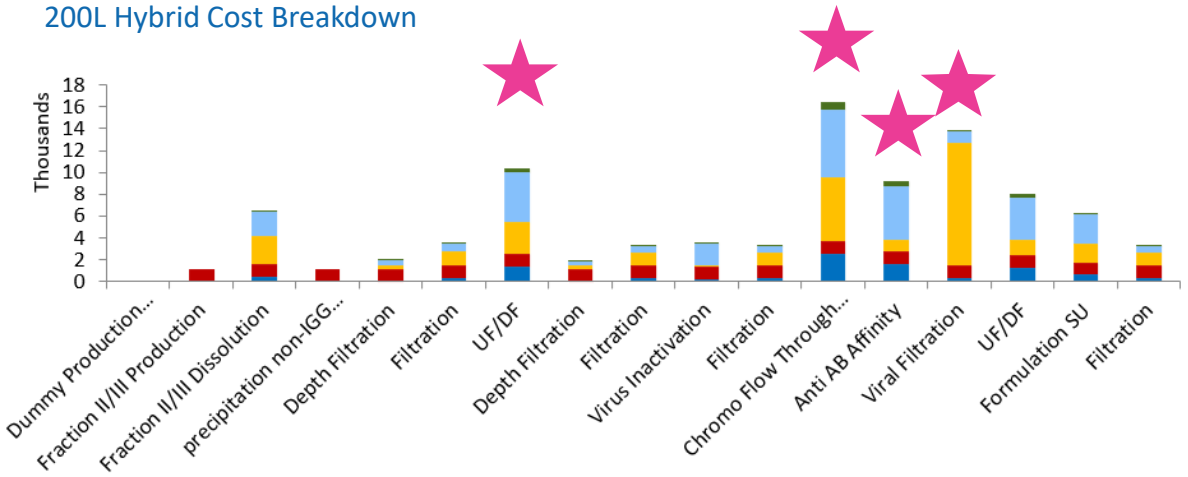
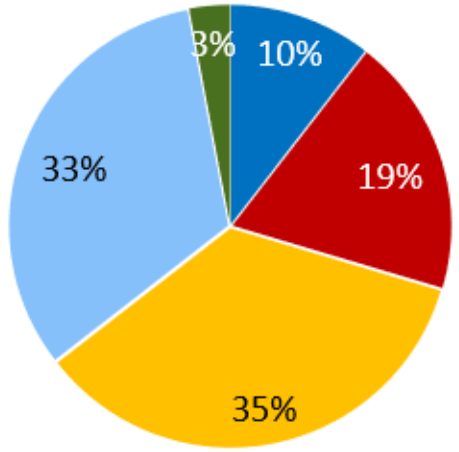
Cost Analysis

Understanding the cost structure in IgG processing

Capital
Materials
Consumables
Labour
Other

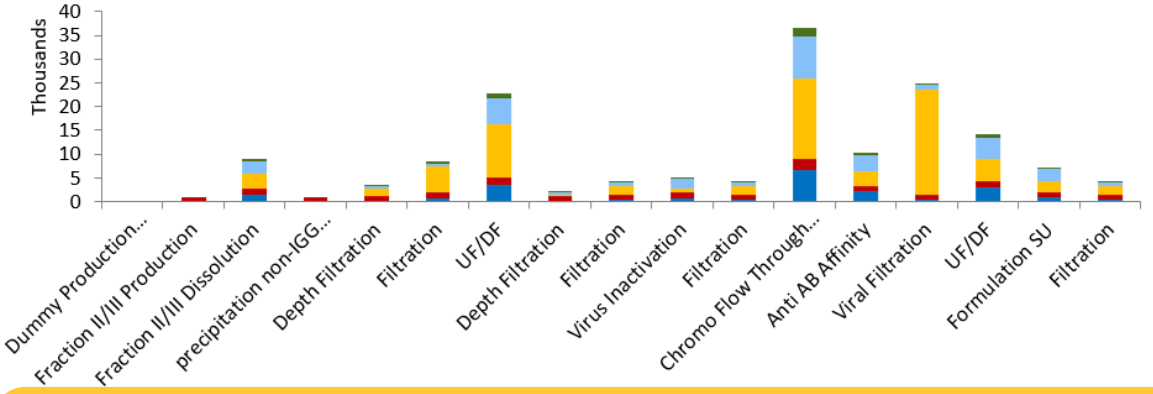
200L Hybrid Cost Breakdown

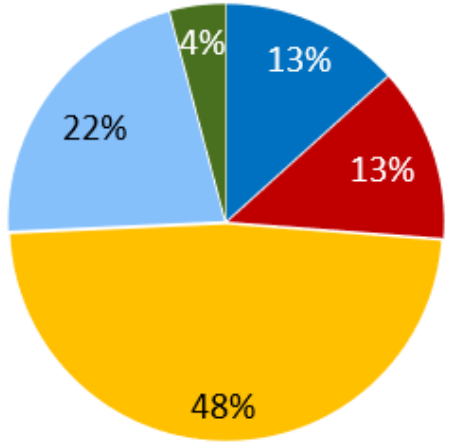




Capital	11.28 USD Million
Cost of Goods	108.1 USD/g
PMI	8,165
Capacity	198.0 kg/yr
Doses per Year	7.9E+04

2000L Hybrid Cost Breakdown





Capital	24.21 USD Million
Cost of Goods	18.3 USD/g
PMI	3,905
Capacity	1979.9 kg/yr
Doses per Year	7.9E+05

- Costly steps are IEX, AC, VF, and UF.
- In a hybrid (SU-SS) process, labor cost ~20-30% depending on scale.

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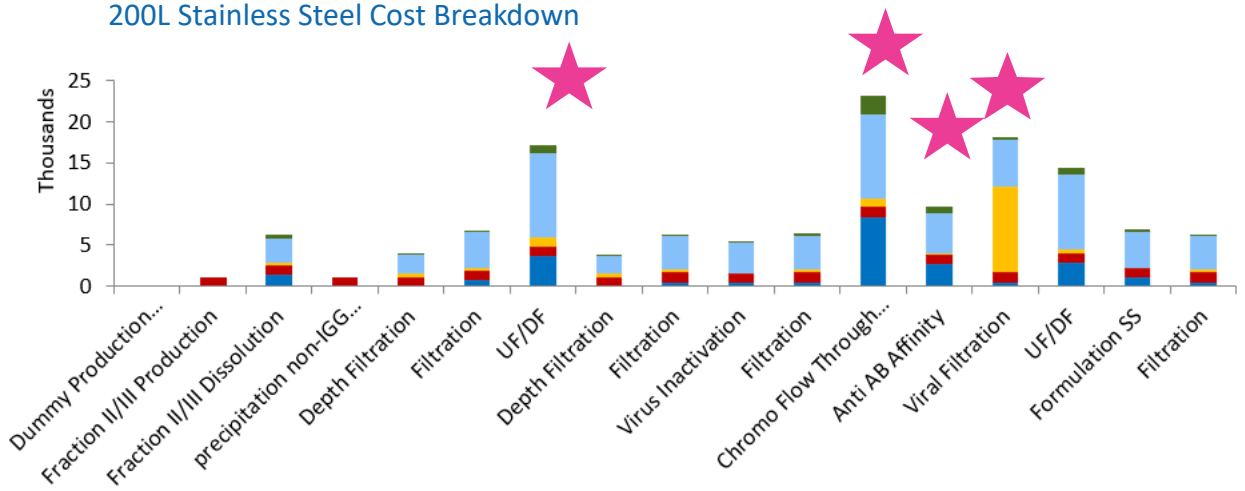
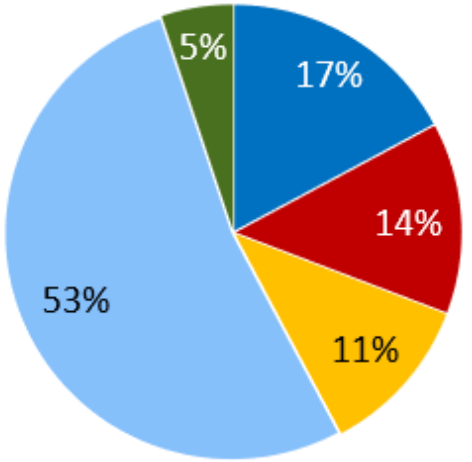
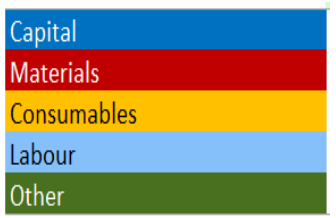
Understanding the cost structure in IgG processing

200L Stainless Steel Cost Breakdown

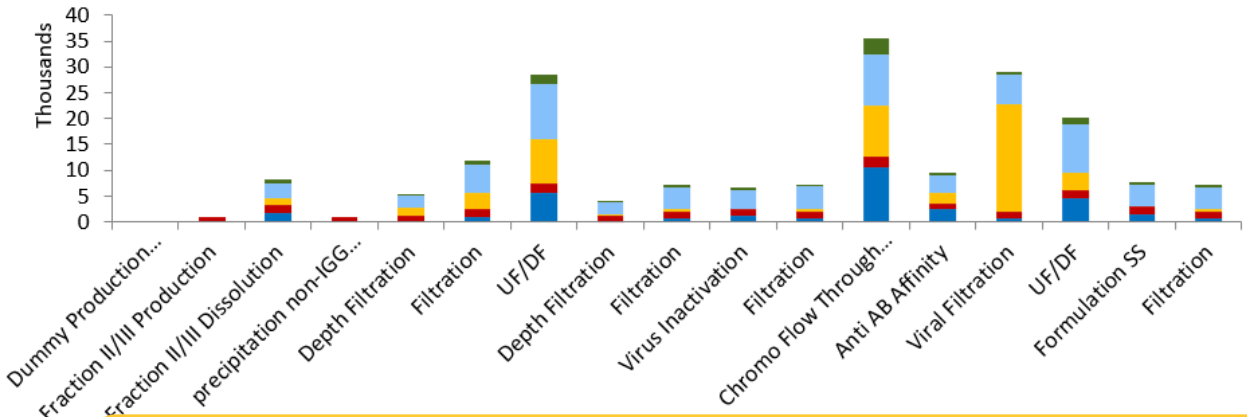


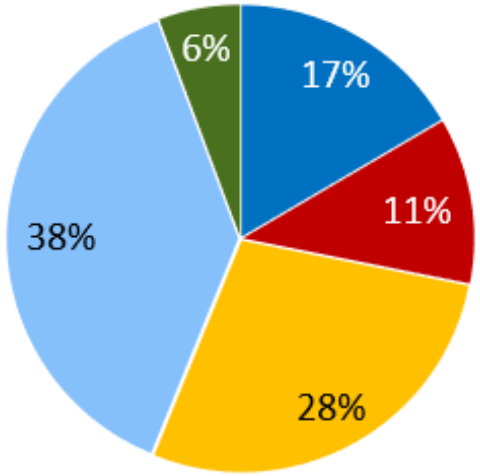




Capital	25.91 USD Million
Cost of Goods	157.6 USD/g
PMI	33,796
Capacity	191.0 kg/yr
Doses per Year	7.6E+04

2000L Stainless Steel Cost Breakdown





Capital	34.77 USD Million
Cost of Goods	21.9 USD/g
PMI	8,991
Capacity	1910.4 kg/yr
Doses per Year	7.6E+05

- Costly steps are IEX, AC, VF, and UF.
- In a non-SU process, labor cost ~40-50% depending on scale.

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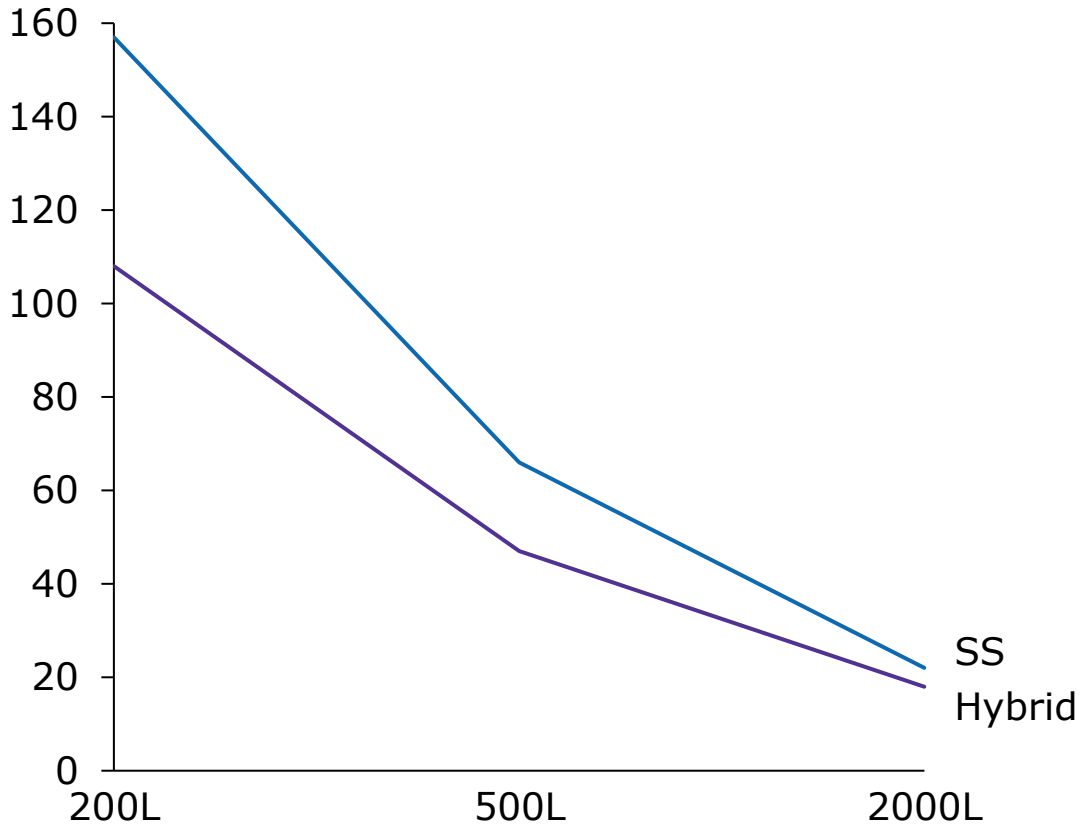
Observations from cost analysis

Cost per dose at different production scale (USD/g) reduces when scale increases



- Unit operations in a IGG purification process which require the most cost are IEX, AC, VF, and UF. Optimizing these steps can make the most impact on reducing the overall cost.
- Incorporating Single-use technologies reduce the Capex and Labour cost, mainly due to the elimination of large systems, and cleaning/validation time.
- Though comsumable cost will be higher, the overall cost of including SU technologies can help to accelearate time of establishment, time to train employee, reduced footprint needed, and eliminate risk of human error related contamination.

Incoporating Single-use technologies

WHY Single-Use?

Examples in single-use

Mixing

Assemblies, Connectors, Samplings

Storage & Transport

Final Filling

Imaging a site with simplicity

Stainless Steel

vs

Single-Use

Flexible & Next generation manufacturing taking vaccine as example

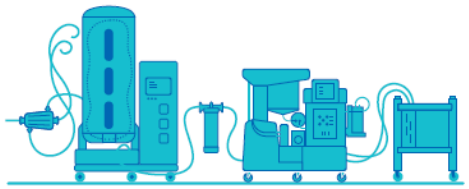
Implementation of Single use in Final filling – GSK case study H1N1, 2009

	Traditional	Single use
Clean and set-up	14 Hrs	<1 Hr
Cleaning validation	Extensive	Zero
Filling time	24 hrs	10 hrs
Average vials/hr	3,000	10,000
Aseptic connections	50	0
Operator training	2 weeks	2 days
Equipment utilization	35%	82%
Total time	38 hrs	12 hrs

Faster deployment
Flexibility to change scale or process
Reduces time to market
Accelerates response to high surge of vaccines: this can well apply for PLASMA

Traditional large vaccine manufacturing facilities

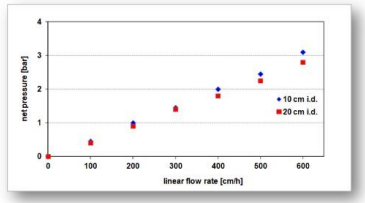
Manufacturing facility using single-use technologies

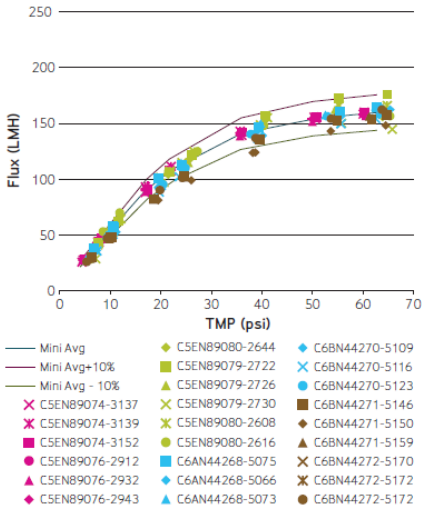


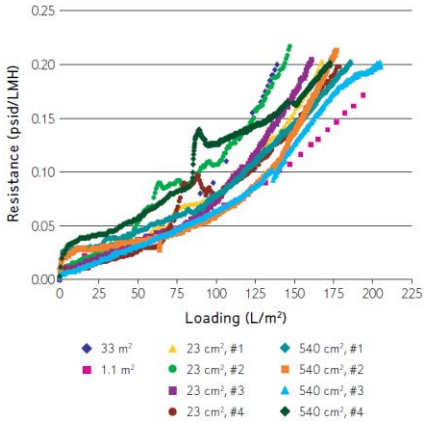
	Traditional stainless facility	Single-use facility
Capex required	~$500M to $1B	$20-100M
Time to construct	5-10 years	1.5 years
Change over time	4 weeks	0.5 days
Footprint	~>70,000 m²	~11,000 m²

Scalable process eliminates all kinds of process upsets and surprises

Scalability







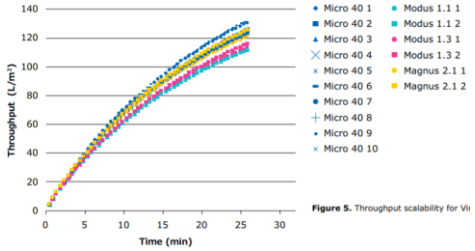
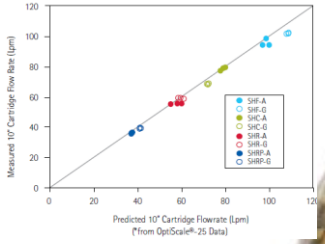


Figure 5. Throughput scalability for Vi

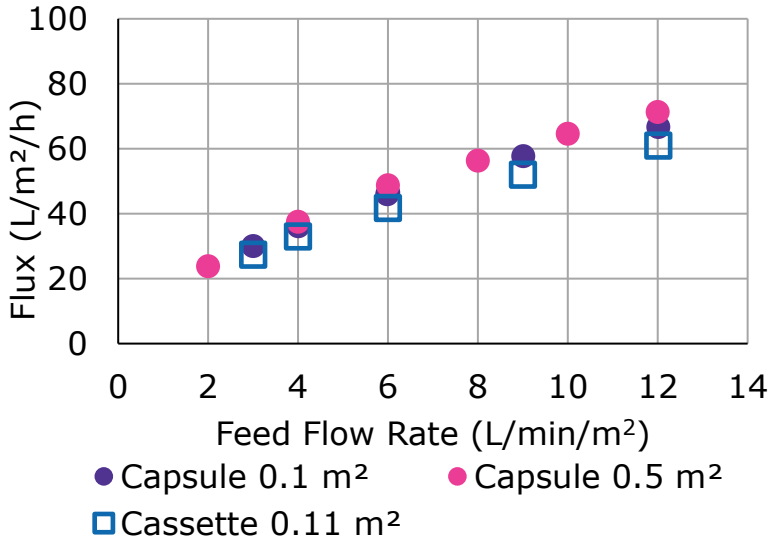


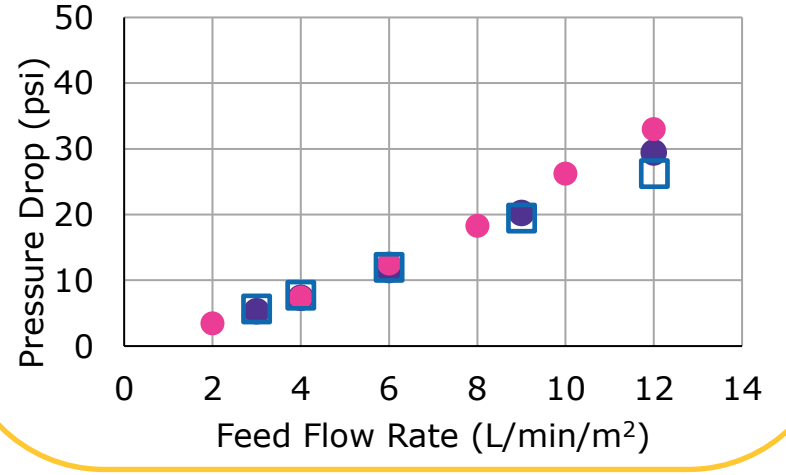
Does Single-use scale up well?

Performace comparison

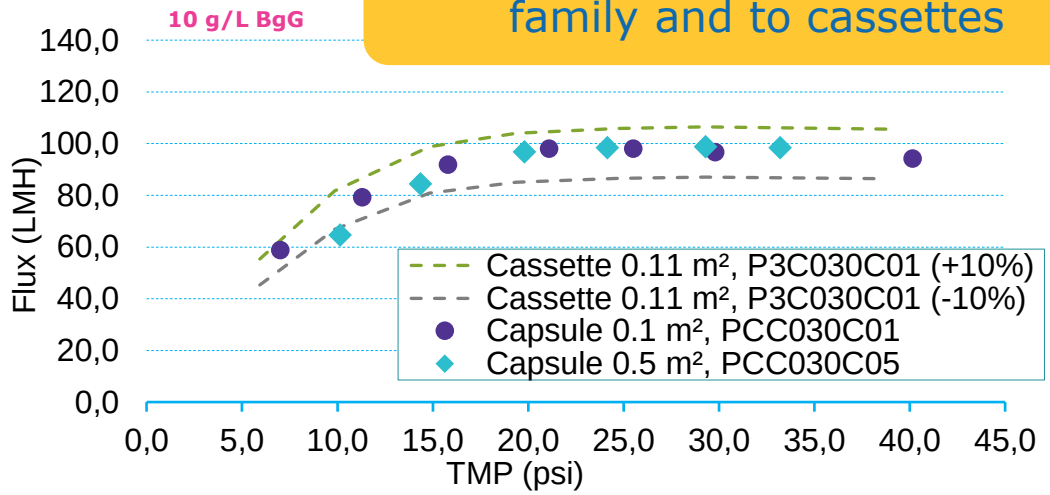
Pellicon capsule (single-use) vs cassette (multi-use)

Comparable flux and pressure drop

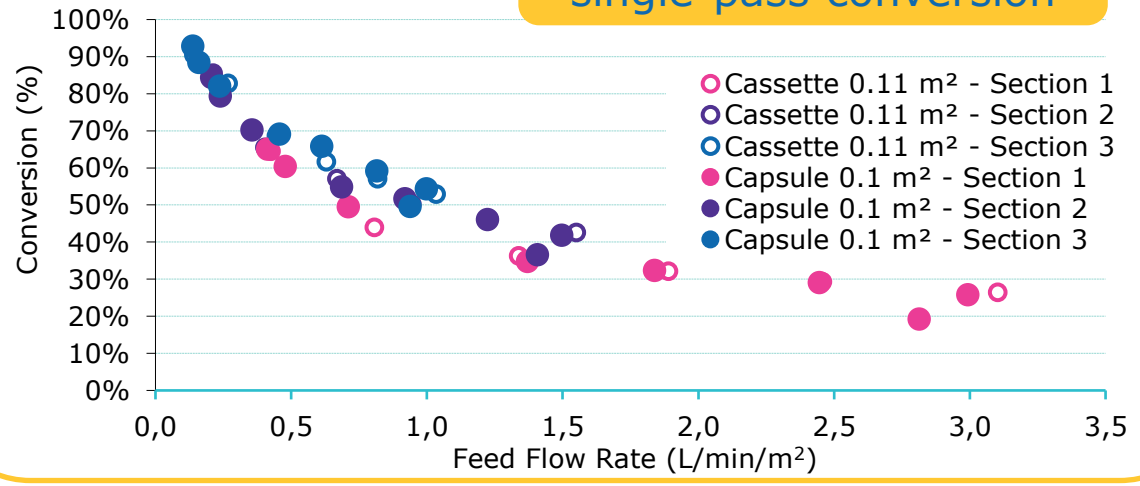




Linear scalability within capsule family and to cassettes



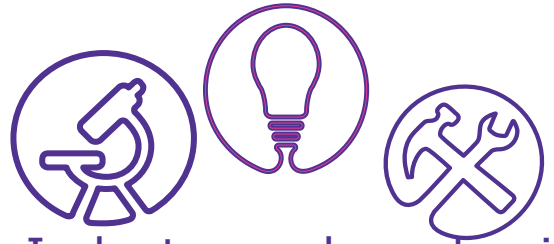
[SPTFF] Comparable single-pass conversion



How We Serve the Plasma Industry

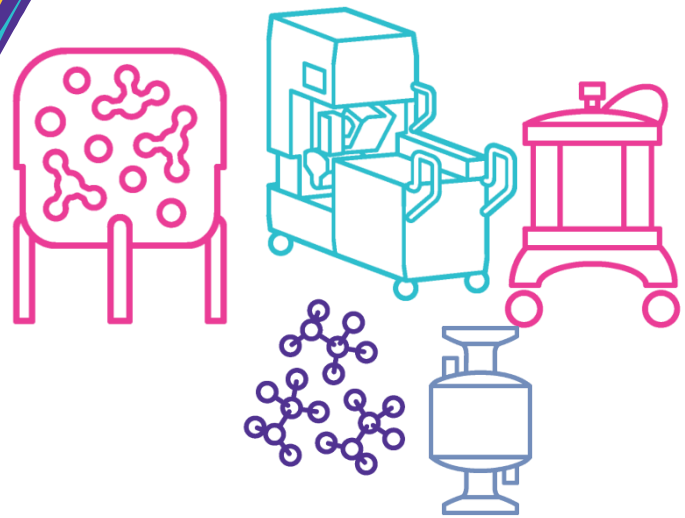
MERCK

Collaborations
Training



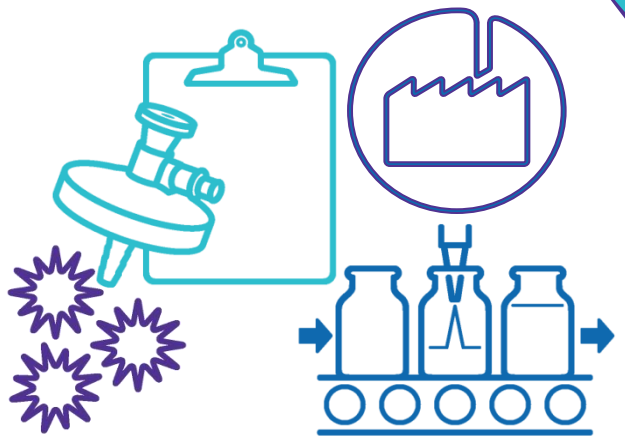
Industry and academic partnerships

Products
Quality dossiers



Process Intensification
& downstream efficiency

Services
Process
development



Speed to clinic and simplified compliance

Millipore

Take home messages

1. Increasing self-sufficiency for PDMPs to address the global demand is a must, domestic production is a key starting point.

2. Product development can start simple (e.g. S/D treated cryo) to more complex (e.g. IgG, Albumin, Factor VIII).

   Think scalable when developing a process to ensure smooth progression from small (e.g. 100L) to large scale (e.g.2000L); incorporate disposable technologies to accelerate fractionation competency establishment.

3. Collaboration accelerates development of plasma production even starting form small scale

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Thank You

Q&A

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