Process intensification in plasma Immunoglobulin G manufacturing using novel technologies providing enhanced productivity

Collaboration work with Taipei Medical University, Taipei, Taiwan
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Senior Consultant Strategy Operationalization – traditional modalities – APAC

IPFA/PEI 27th, May 4-6, 2021

Agenda

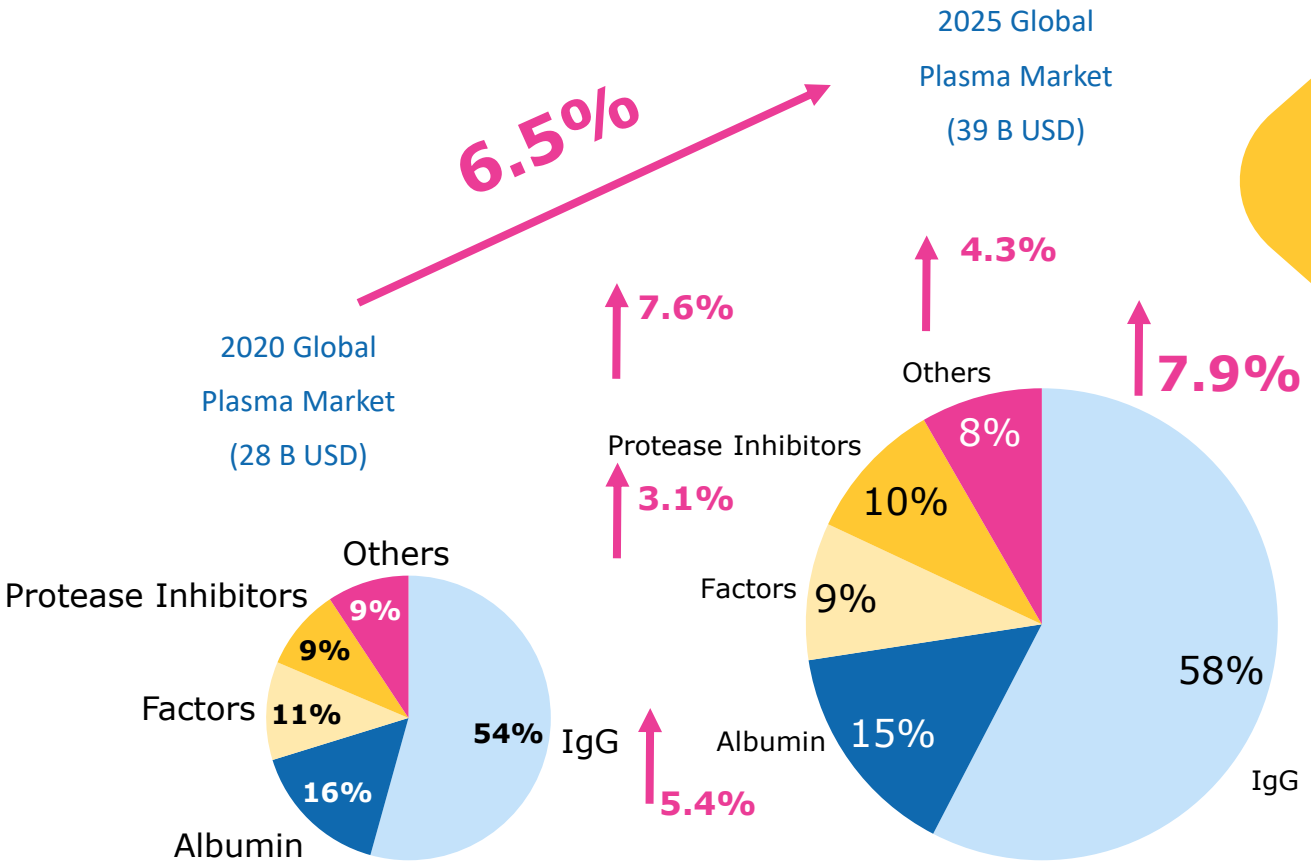
1. **Introduction – IGG purification**
2. **Study summary**
2. **Conclusions**

Millipore®

Preparation, Separation,
Filtration & Testing Products

Immunoglobulin G

High market demands drives exploration in process optimization



Population growth

SCIG Adoption

Shortage

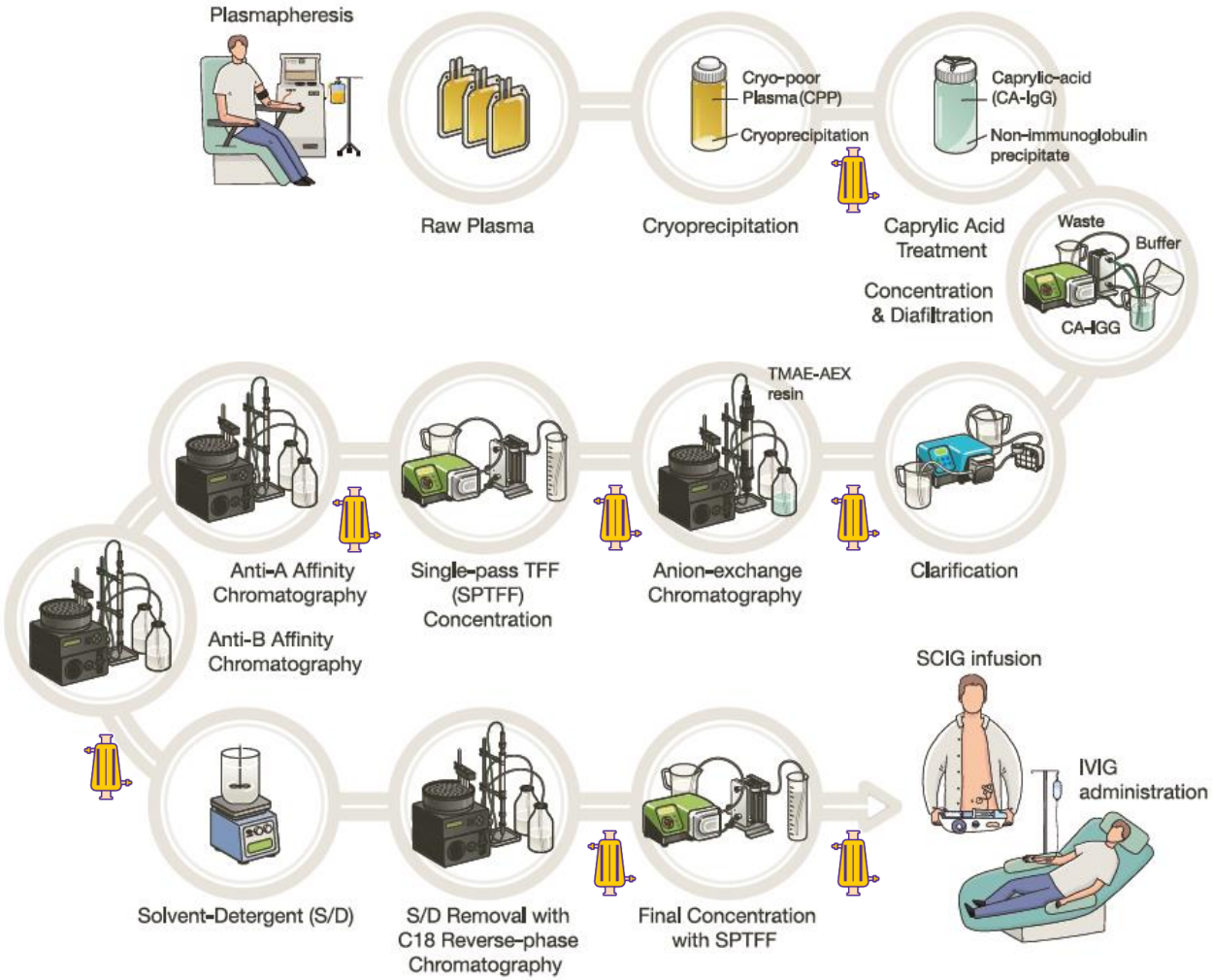
ON/Off label use

Improvement on Quality & Productivity

Source: Plasma fractionation markets report 2020, marketsandmarkets.

From Plasma donation to patient adminstration

Experimental flow with key steps used in purification



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

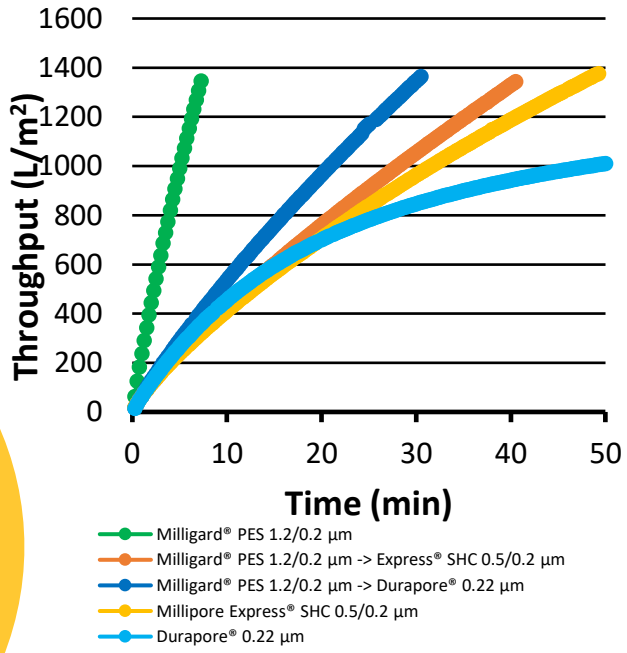
Confidential – Manuscript under review

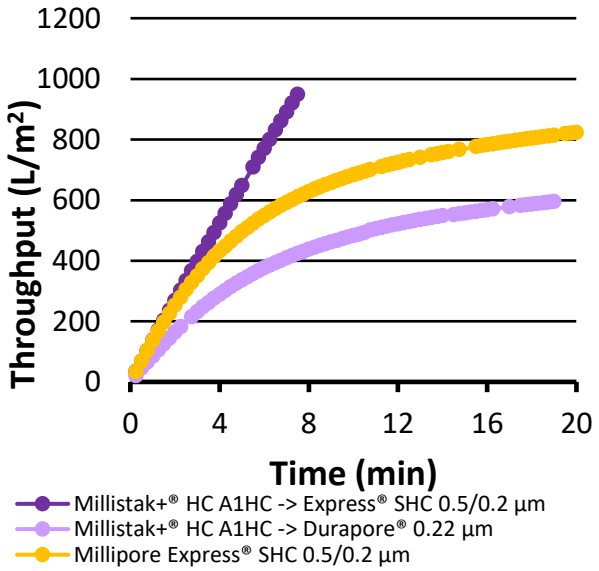
Various intermediate filtration steps reducing bioburden

Prefilters provide significant protection effect to sterile filters and can be used stand alone

Bioburden filters & clarification filters used in the study:

- Millstak+® A1HC
- Milligard® PES 1.2/0.2
- Millipore Express® SHC 0.5/0.2
- Durapore® filter0.22





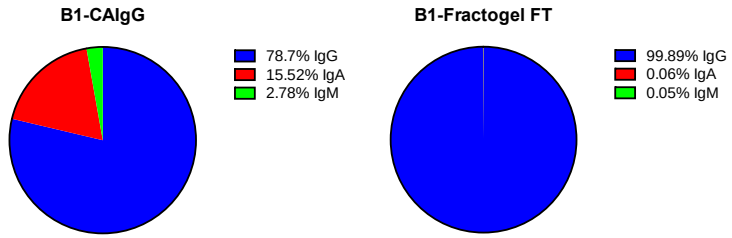
Steps	Selected Prefilters (Recovery %)	Selected Sterile Filter	Recovery (%)
Post CA	MPES (100%)	Durapore® filter	> 99
Post UF/DF	Millistak+® A1HC	Millipore Express® SHC	~ 100
Post AEX		Millipore Express® SHC	~ 100
Post 1st SPTFF	MPES (100%)	Durapore® filter	> 96
Post Anti A/B		Millipore Express® SHC	~ 100
Post C18 resin	MPES (100%)	Millipore Express® SHC	~ 98
Post final SPTFF		Millipore Express® SHC	~ 86

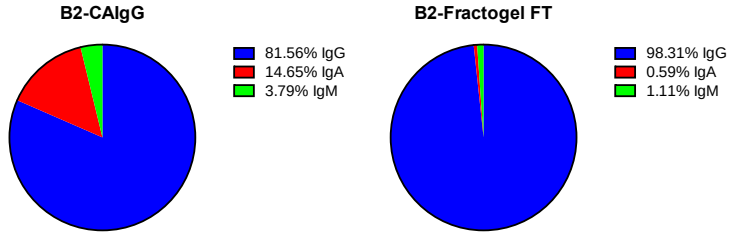
Observations:

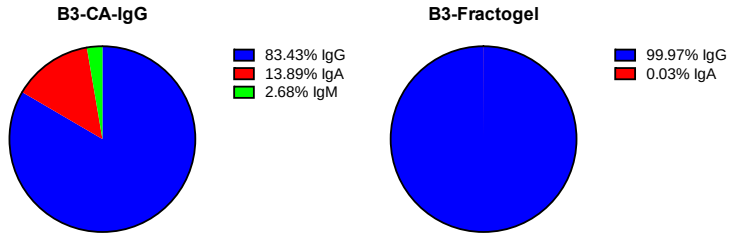
- w/o prefilter- Millipore Express ® SHC generally performs better
- w/ prefilter – sometimes Durapore® performs better
- Prefilter shows significant protecting effect / reducing membrane area needed for sterile filters.
- Millistak® A1HC clarification filter reduced significantly the particles/ precipitation.

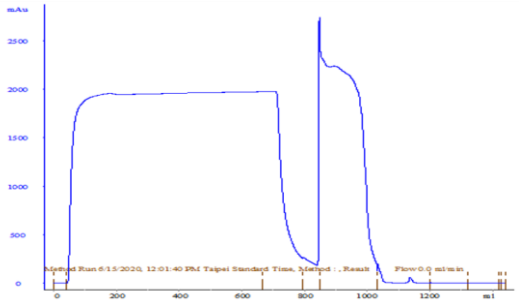
Anion Exchange chromatography (FT)
Major step to remove IgA & IgM

Pilot scale in 3 batches
IgG/IgA/IgM

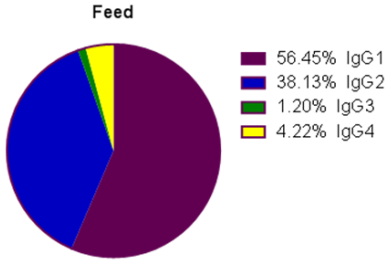


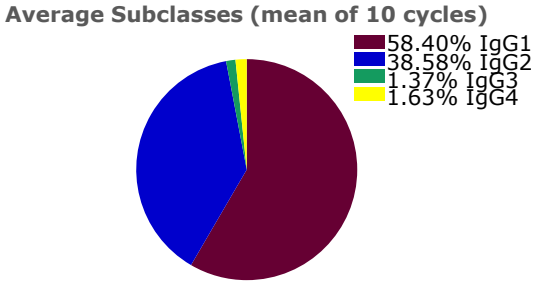






Small scale in 10 cycles
IgG1/IgG2/IgG3/IgG4





Summary:

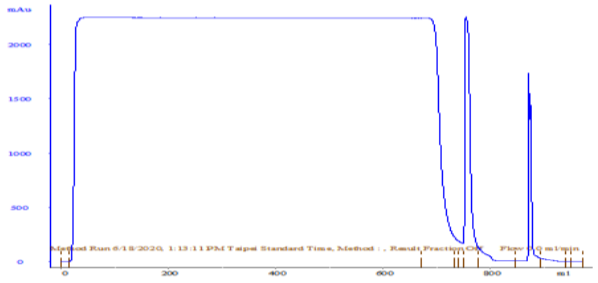
1. Optimal pH for loading identified @ pH 6.0
2. Purity IgG avg. 82% to 99% in small and pilot scale.
3. 200 cycles test with standard cleaning conditions confirms the robustness.
4. No changes in IgG subclasses.
5. No thrombogenicity activity detected

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

Millipore®

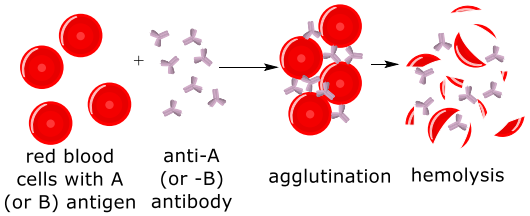
Immunoaffinity chromatography
Robustly reducing the blood-type specific isoagglutinins

Eshmuno® P Anti-A (FT)

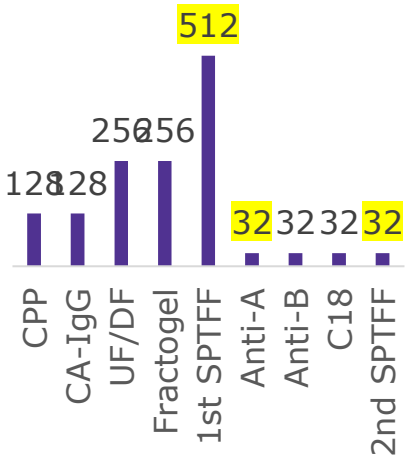


Eshmuno ® P Anti-B (FT)

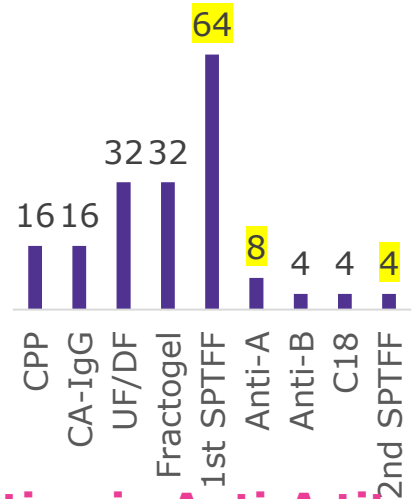
	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B



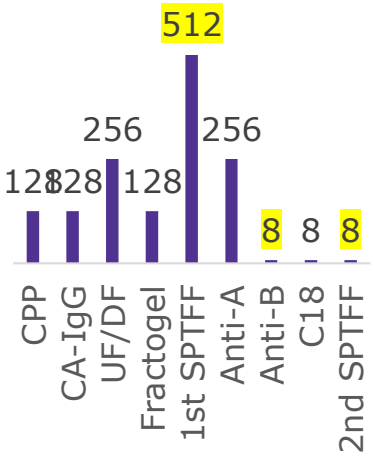
Batch 3 Anti-A titer



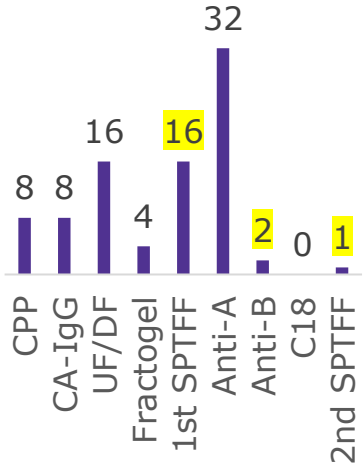
Batch 2 Anti-A titer



Batch 3 Anti-B titer



Batch 2 Anti-B titer



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.

Virus Inactivation and removal by C18 RP-chromatography

Efficient removal of S/D by Licroprep C18 (40 – 63um) to non-detactable level

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.

C.

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

Summary:

A. 0.3% TnBP + 1% 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.

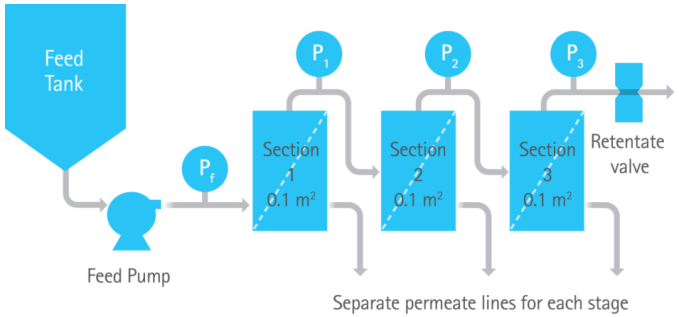
C. TnBP residual tested on GC-MS with results < 1ppm, Triton X-100 residual tested by HPLC with results < 2ppm, showing a robust removal with the loading quantity defined (6ml S/D-IGG/ml C18 packed resin), with capacity tested at 14 ml S/D-IGG/ml C18 resin)

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

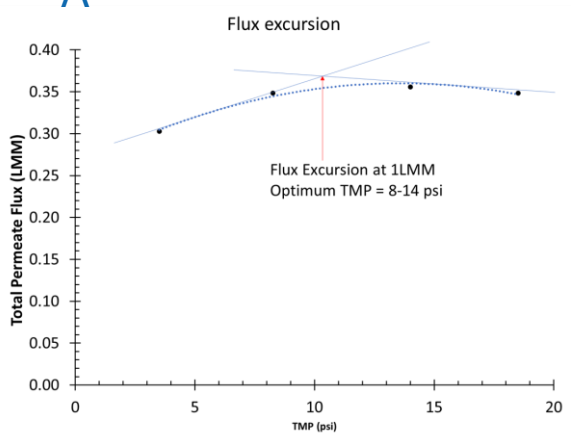
*Table A source: Hsieh YT, Mullin L, Greenhalgh P, Cunningham M, Goodrich E, et al.: Single-use technology for solvent/detergent virus inactivation of industrial plasma products. *Transfusion* 2016; 56: 1384-93.

Single-Pass Tangential Flow Filtration

A gentle method for inline concentration & high concentration ultrafiltration



A



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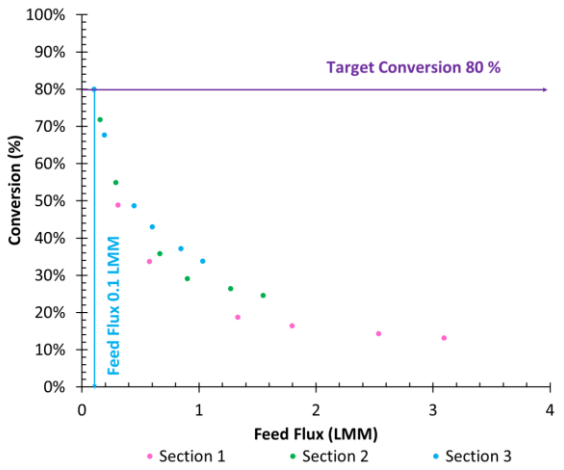
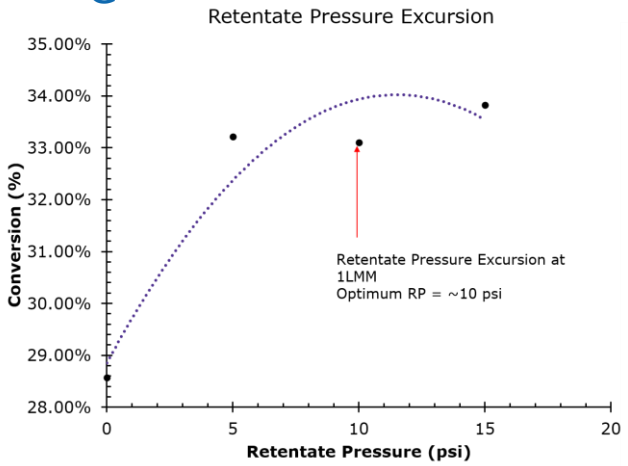
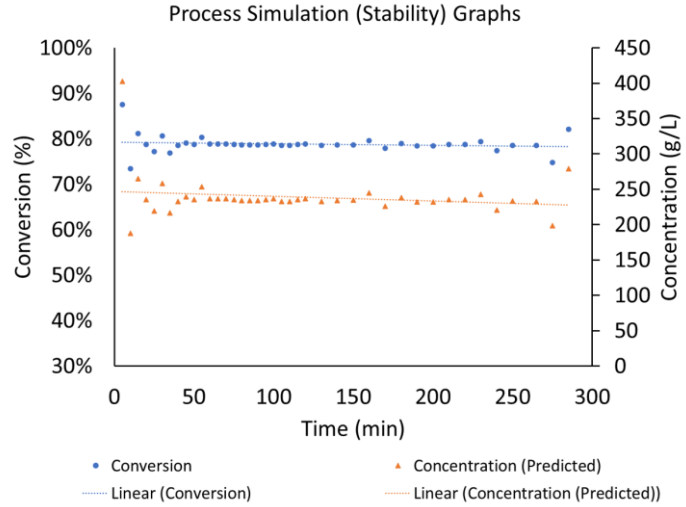


C



D

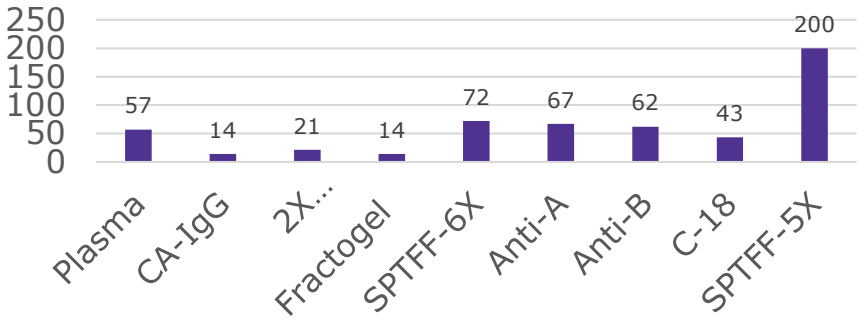


Summary:

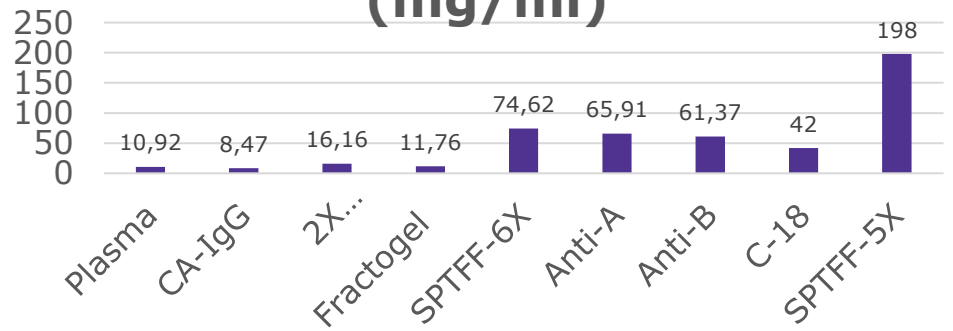
1. Sequential optimization to identify parameters to reach target concentration 20%. (200 mg/ml)
2. SPTFF before Eshmuno® P Anti-A/B step reduced loading time, buffer consumed, also improved the AC performance.
3. SPTFF for final concentration successfully provided a gentle (low shear force), scalable, high recovery, and easy-to-operate method for high concentration ultrafiltration.

Overall Quality test results
Satisfying concentration effect and IgA/IgG removal ability

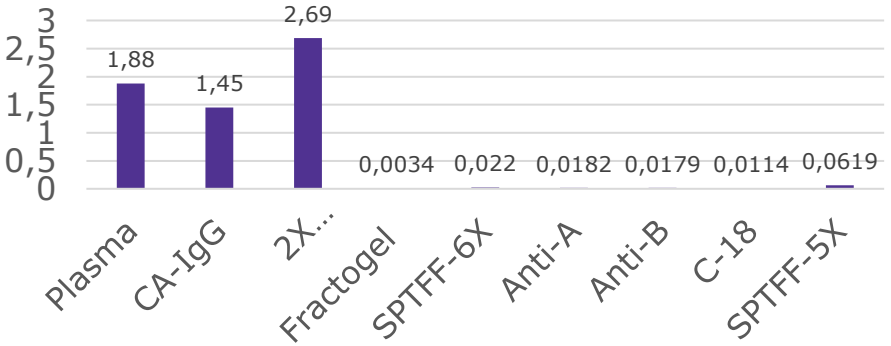
Total protein concentration (mg/ml)



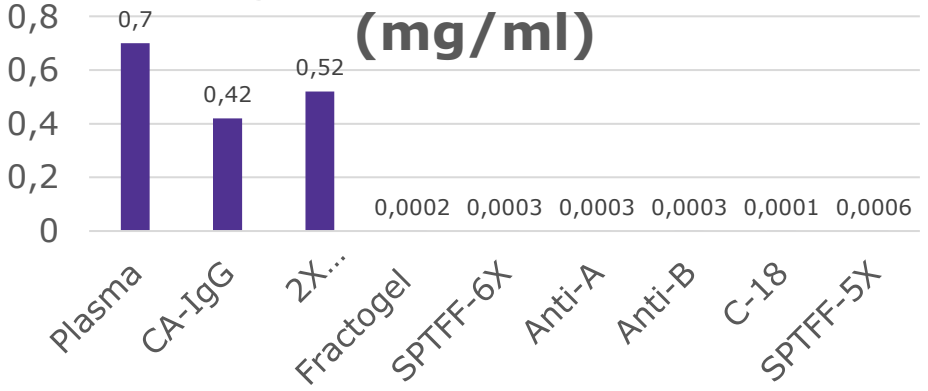
IgG concentration (mg/ml)



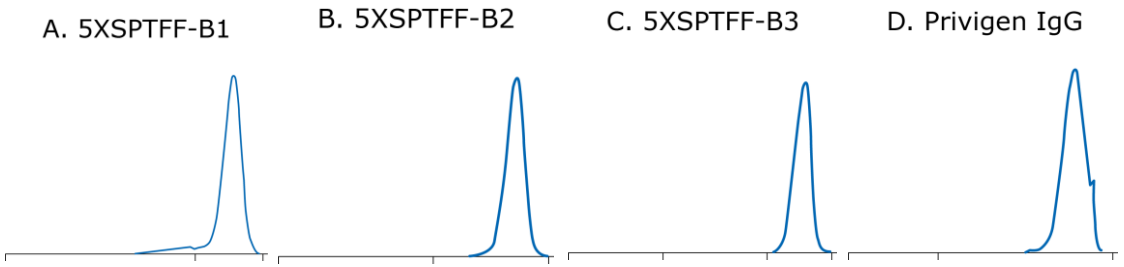
IgA concentration (mg/ml)



IgM Concentration (mg/ml)

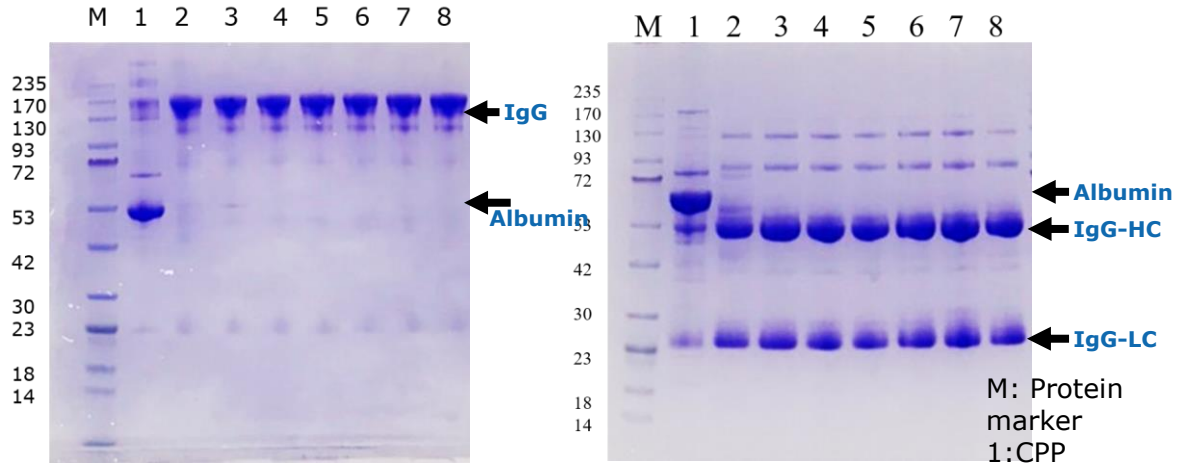


Quality check benchmarking market product
Comparable purity to market product



Fractions	Non-IgG	IgG
Unit	%	%
5XSPTFF-B1	4.8	95.2
5XSPTFF-B2	1.1	98.9
5XSPTFF-B3	0.6	99.4
Privigen® IgG	0.3	99.7

Zone electrophoresis confirmed the high purity of the final IgG. The purity of batch 3 (5X SPTFF) reaches almost 100%.



SDS-PAGE under non-reducing (left) and reducing (right) conditions showing the step wise purification process, purity was enhanced especially after AEX step.

M: Protein marker
1:CPP
2: CA-IgG
3: Fractogel ® FT
4: Anti-A-FT
5: Anti-B-FT
6: C18
7:SPTFF-5X
8:Privigen IgG

*5 µg of protein loaded on to 4-12% Bis-Tris SDS-PAGE

Conclusions

Proof of concept for a generic process with intensified processing:

1. Milligard® PES 1.2/0.2 prefilters effectively reduced the filtration area needed for sterile filters e.g. Millipore Express® SHC or Durapore® filters
2. Clarification using Millistak^{+}® HC A1HC to facilitate downstream purification.
3. Fractogel® TMAE (M) anion exchange chromatography for efficient removal of IgA and IgM, with well maintained IgG subclasses.
4. Eshmuno® P anti-A and Eshmuno® P anti-B chromatography for removal of anti-A and anti-B agglutinins.
5. S/D used in virus inactivation can be efficiently removed using Licroprep® C18 RP chromatography.
6. Use of SPTFF as a mild and robust approach to concentrate IgG to a target of 20% concentration.
7. Recovery from 92 – 100% for each step resulting in an overall process recovery of greater than 70% under a worst-case scenario, with opportunities to improve further with additional optimization.
8. Such flow through methods combined with single-pass TFF technology should be readily scalable, and easy to apply for various IgG products including polyvalent IgG, hyperimmune, or convalescent immunoglobulins.

Acknowledgements

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Thank You

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