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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.

Analytical methods performed

Quality control performed along the purification process to make sure the final product meet therapeutic IgG quality criteria:

- Total protein (Biuret)
- pH
- Conductivity
- Turbidity
- IgG (ELISA + Zone electrophoresis)
- IgA (ELISA)
- IgM (ELISA)
- IgG sub-classes (ELISA)
- Anti-A and Anti-B titer (Agglutination test)
- Gamma proteins by zone electrophoresis
- SDS-PAGE reducing / non-reducing conditions
- Thrombogenic and proteolytic activity potential
  - FXI Assay (FXIa Hyphen)
  - Thrombin Generation Assay (TGA, Technoclone)
- HPLC for Triton X-100 determination
- GC for TnBP determination

Figure 1 provides a summary of the intensifed process for IgG plasma purification. The process begins with plasma cryoprecipitation followed by caprylic acid treatment to yield the crude IgG solution, which serves as the starting material for the downstream purification. The yellow capsule icons in the figure indicate the seven bioburden reduction/sterile filtration steps that were incorporated. Downstream purification steps include clarification, followed by ion exchange chromatography in flow-through mode to remove IgM and IgA and single-pass tangential flow filtration (SPTFF) to concentrate the solution prior to anti-A and anti-B agglutinin removal. The final steps include solvent/ detergent treatment and its removal on C18 reverse phase chromatography, and finally, SPTFF concentration.

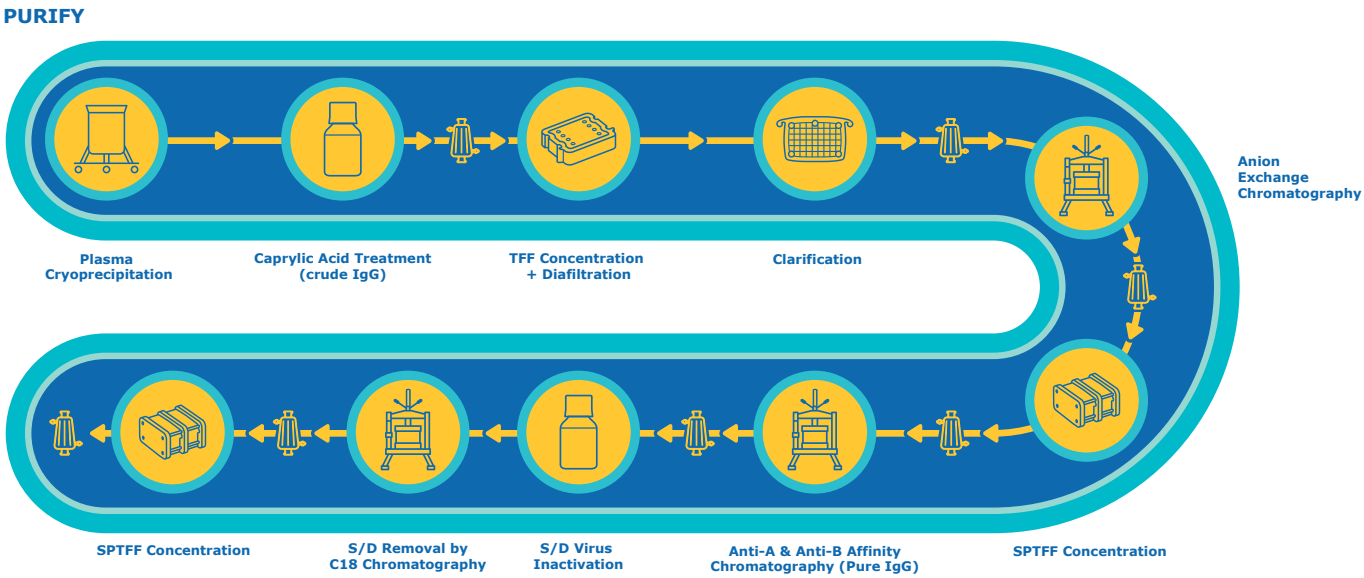


Figure 1. Summary of optimized process for Plasma IgG purification.

Cryoprecipitation and Caprylic Acid Treatment

To prepare the crude IgG for use in this study, frozen human plasma was obtained by apheresis or from whole blood; each batch size started with 10L. Plasma was subjected to cryoprecipitation by thawing at 1 – 3 °C overnight, then centrifugation to eliminate the cryoprecipitate. The supernatant (cryo-poor plasma, CPP) was subjected to a precipitation step using 5% caprylic acid at pH 5.5 to yield a 85 – 90% pure IgG, fraction which also contained major contaminants including IgA and IgM. The crude IgG feed stock was centrifuged at 6000xg for 20 minutes to remove any insoluble particles and then filtered using a Milligard® polyethersulfone (PES) prefilter and other sterile filters.

Evaluation of Prefilter Prior to Sterile Filtration

Membrane-based prefilters are used to limit the variability of process streams by removing plugging contaminants thereby protecting sterilizing-grade filters. For intermediate filtration, it can be stand alone, or coupled with a sterilizing-grade filter. In this study, Milligard® PES 1.2/0.2 µm filters were evaluated for their ability to improve the throughput of the Millipore Express® SHC and Durapore® sterilizing-grade filters. Milligard® PES filters contain polyethersulfone (PES) membranes of different pore sizes for efficient particle and bioburden removal from a broad range of process streams and are compatible with caustic sanitization, gamma irradiation, autoclave and steam-in-place (SIP) sterilization methods.

Figure 2 compares the throughput of Millipore Express® SHC 0.5/0.2 µm and Durapore® CVGL 0.22 µm with and without Milligard® PES 1.2/0.2 µm as prefilter. Minimum plugging was observed on the Durapore® CVGL 0.22µm filter following Milligard® PES 1.2/0.2 µm prefilter, which suggests that Milligard® PES filter has provided good protection by removing larger particles.

Table 1 summarizes the results and sizing recommendations for the post-caprylic acid treatment sterile filter evaluation. Based on results of the study, 1 × 20" Milligard® PES 1.2/0.2 µm cartridge and 1X Opticap® XLT30 Durapore® filter was recommended for sterile filtration of a 3000 L batch in 1 hour process time. This is a classical sizing example showing the protection effect of adding the Milligard® PES prefilter. Sterile filtration coupled with a Milligard® prefilter showed similar filtration profiles.

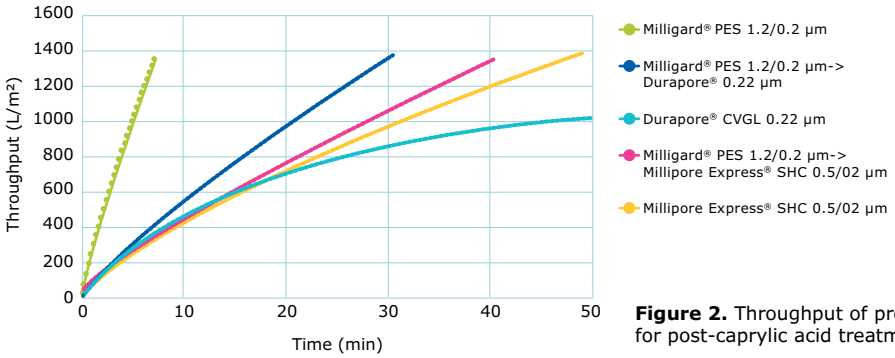
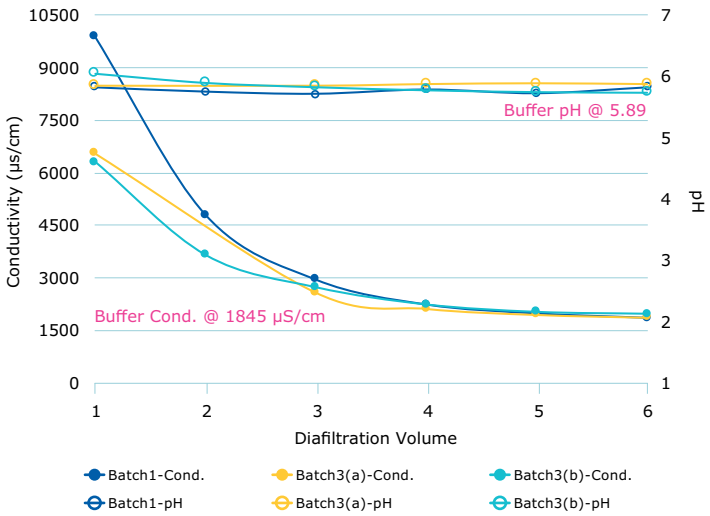


Figure 2. Throughput of pre-filters and sterile filter combinations for post-caprylic acid treatment (Vmax test).

Vmax Trial				Filter Sizing Recommendations					
Prefilter	Final Filter	Vmax	Trial Loading	Area Minimum	Area Suggested	Recommended Configuration	Process Loading	Estimated Process Time	Safety Factor
		(L/m²)	(L/m²)	(m²)	(m²)		(L/m²)	(hour)	
-	**Milligard® PES 1.2/0.2 µm**	**21106**	**1346**	**0.61**	**1.2**	**1 × 20" cartridge**	**2500**	**0.29**	**1.97**
Milligard® PES 1.2/0.2 µm	**Durapore® 0.22 µm**	**16002**	**1344**	**1.37**	**2.07**	**1 × Opticap® XLT30**	**1449**	**0.55**	**1.51**
NA	Durapore® CVGL 0.22 µm	1440	1106	2.82	6.21	3 × Opticap® XLT30	483	0.18	2.2
Milligard® PES 1.2/0.2 µm	Millipore Express® SHC 0.5/0.2 µm	12662	1365	1.73	2.94	2 × Opticap® XLT30	1020	0.47	1.7
NA	Millipore Express® SHC 0.5/0.2 µm	9961	1376	1.94	2.94	2 × Opticap® XLT30	1020	0.54	1.51

Table 1. Post-caprylic acid treatment sterile filter results and sizing summary.

The caprylic acid IgG (CA-IgG) was then concentrated and diafiltered against the chromatographic equilibration buffer using a batch TFF method prior to the subsequent chromatographic purification. After six diafiltration volumes, caprylic acid was successfully removed and the target pH and conductivity were achieved (**Figure 3**).



- 6x diafiltration was carried out under constant volume mode at ~2x VCF.
- pH and conductivity from permeate were taken at each diafiltration volume (DN). As shown in the diafiltration profiles, **target pH and conductivity were reached at 5-6 DN.** Typically 2 DN will be added as safety factor.
- Initial concentration : 8.74 mg/mL
- Final concentration: 16.16 mg/mL

Figure 3. Ultrafiltation/diafiltration successfully removed caprylic acid from the IgG fraction, data showed consistent removal from 3 pilot scale batches.

Clarification and Sterile Filtration

Post-diafiltration, a reduction in filtrate clarity was observed leading to lower throughput on the Millipore Express® SHC sterile filter of 823 L/m^2. A1HC was included to improve the filtrate clarity to protect the downstream filter. Filtration through A1HC was controlled at a constant flow of 170 LMH to allow sufficient residence time for adsorption between the contaminants and depth filter.

Figure 4B shows a flat resistance profile of Millistak+® A1HC at ~800 L/m^2, which indicates that there is the possibility to challenge the loading further. However, **Figure 4A** showed improved throughput with filtrate generated from Millistak+® A1HC, suggesting a protective effect with prefilter Millistak+® A1HC.

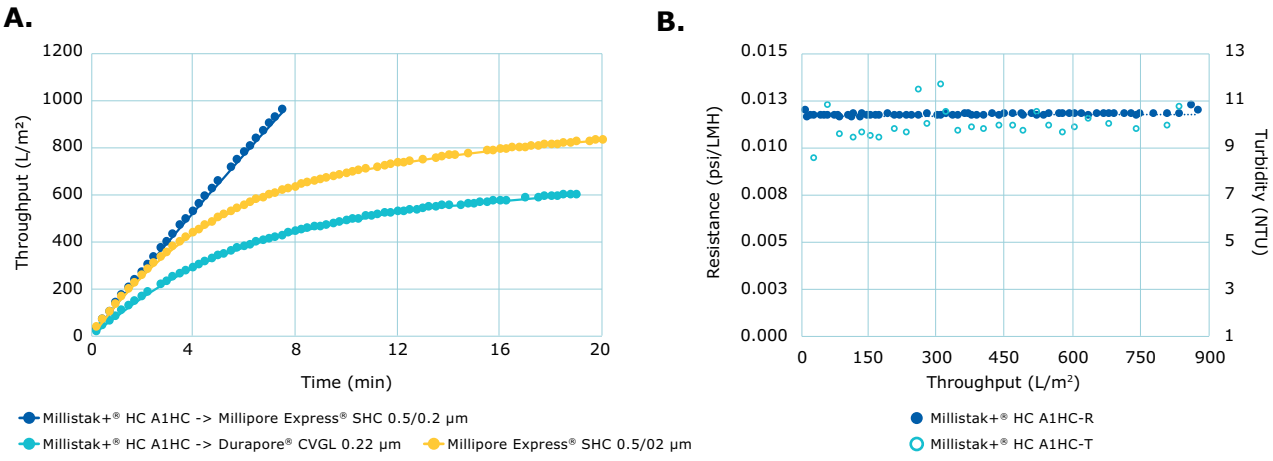


Figure 4. Throughput of filters evaluated for clarification and sterile filtration of the IgG fraction following ultrafiltration/diafiltration (A). Pmax study data for Millistak+® A1HC showing consistent resistance as throughput increases, indicating no plugging in the range of throughput tested (B).

Flow-Through Anion Exchange Chromatography – Optimization

The optimum loading conditions for Fractogel® resins were evaluated in small-scale at pH values of 5.7, 6.0 and 6.3 in flow-through mode. Up to 40 mL of the caprylic acid purified IgG was continuously loaded onto a 2.5 mL column packed with tri-methyl ammonium ethyl Fractogel® TMAE equilibrated with 25 mM sodium acetate at pH 5.7, 6.0, or 6.3 and run using a linear flow-rate of 180 cm/h. Samples of the flow-through were collected at different time points. Bound proteins were eluted with 500 mM sodium acetate wash buffer and IgG, IgA, and IgM were assessed in the flow-through samples and the wash. Three independent experiments were conducted at each equilibration buffer pH. **Figure 5** shows that there was no apparent impact of pH on IgG recovery (flow-through) and IgA and IgM removal (binding), demonstrating Fractogel® EMD TMAE (M) resin robustness in the pH 5.7 to 6.3 range. Based on this information, pH 6.0 was used for loading conditions.

Using the same conditions, 0.5M NaOH (for odd runs) and 1M NaOH (for even runs) were used to mimic standing CIP conditions. The IgG flow-through obtained every ten cycles was collected and analyzed to assess purity parameters and sub-class distribution. Robustness was tested over 200 cycles to ensure the consistency of performance on impurity removal (**Figure 6A**). The optimized chromatographic conditions also provided satisfactory preservation of the IgG sub-class distribution, although sub-class 4 is more easily bound to an anion exchanger (**Figure 6B**).

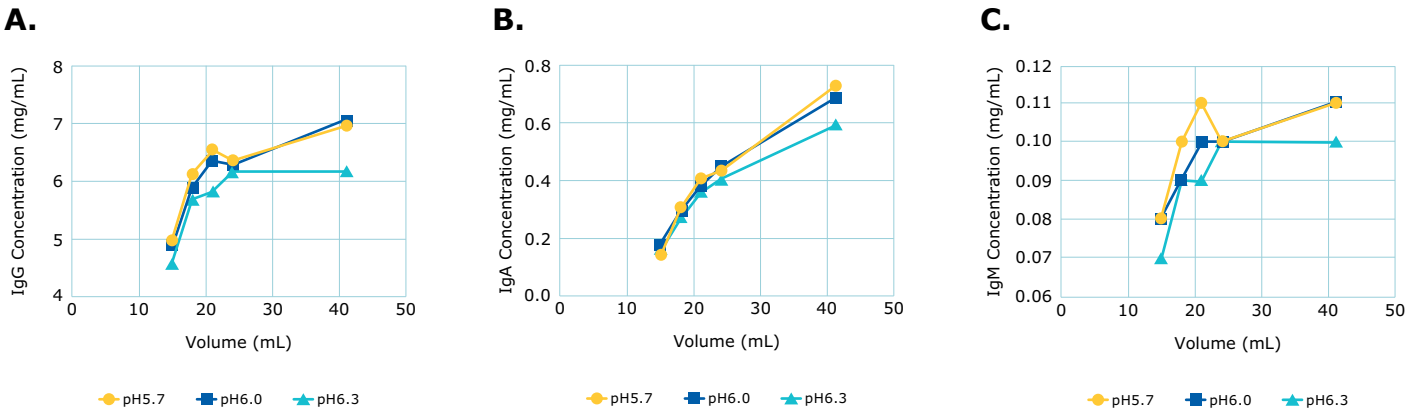
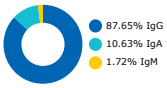


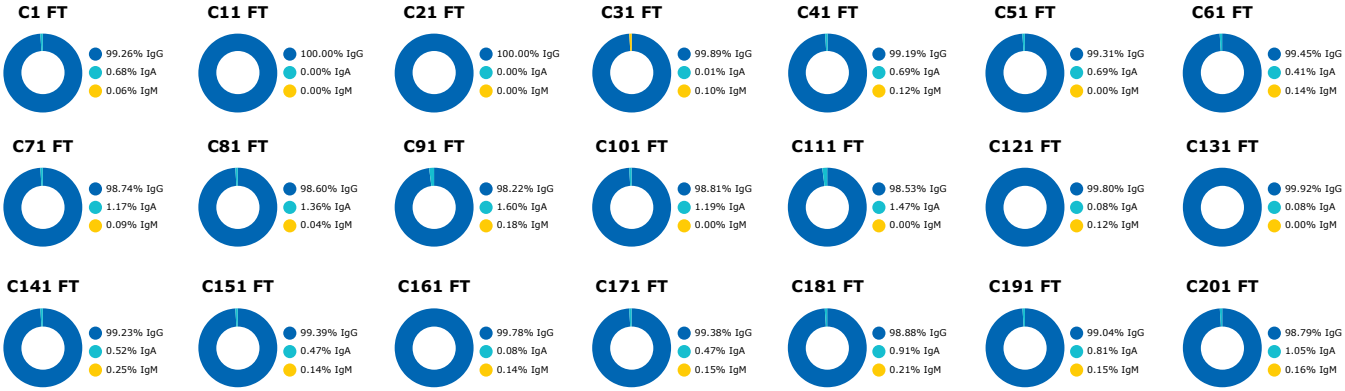
Figure 5. Evaluation of pH effect on Fractogel® TMAE(M) binding capacity.

A IgG Recovery

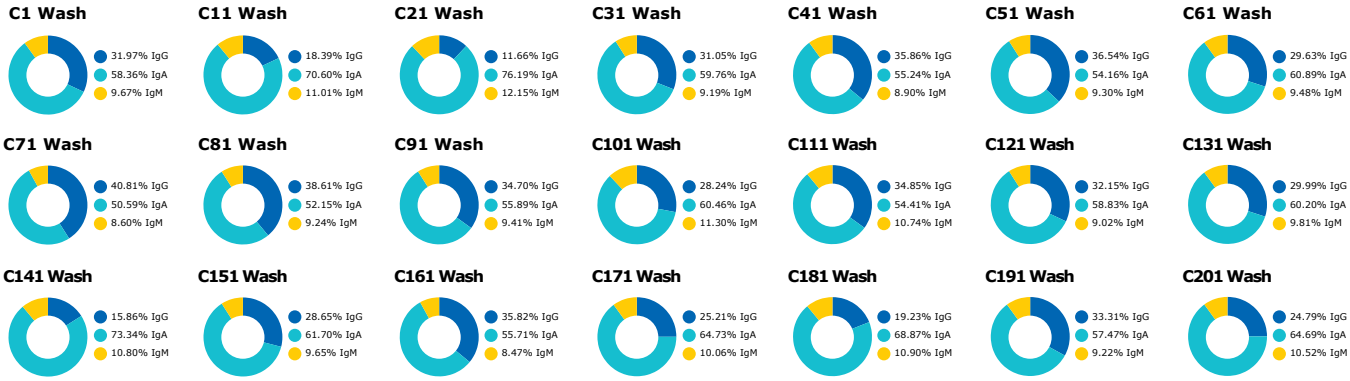
Feed



Flow Through

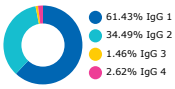


Wash



B IgG Subclass Recovery

Feed



Flow Through

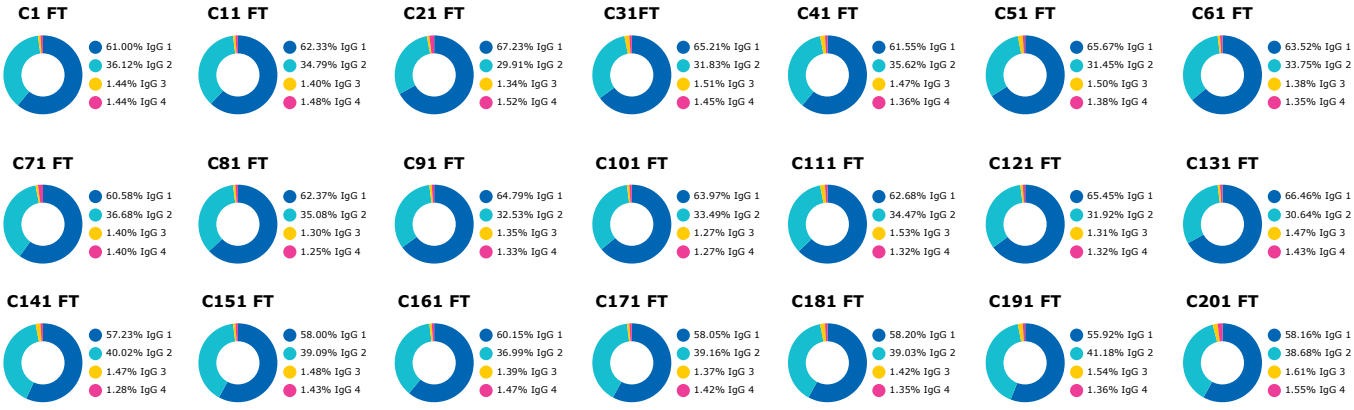


Figure 6. Robustness of the Fractogel® TMAE anion exchange chromatographic step. Consistent performance purity enhancement from 1 to 201 cycles (A). Successful preservation of IgG subclasses from 1 to 201 cycles (B).

IgA and IgM Removal by Anion-Exchange Chromatography – Pilot-Scale Process Purification

Using the optimized loading conditions defined above, Fractogel® EMD TMAE anion exchange chromatography was used following the clarification step for removal of IgA/IgM from three pilot-scale batches of plasma. An average IgG purity improvement from 81% to 99% was achieved; total protein loaded was approximately 137 mg/mL resin (**Figure 7**). As a result of purity, the average remaining IgA content was 0.23%, and the remaining IgM content was reduced to 0.39%; batch #3 showing nearly a complete removal of IgM.

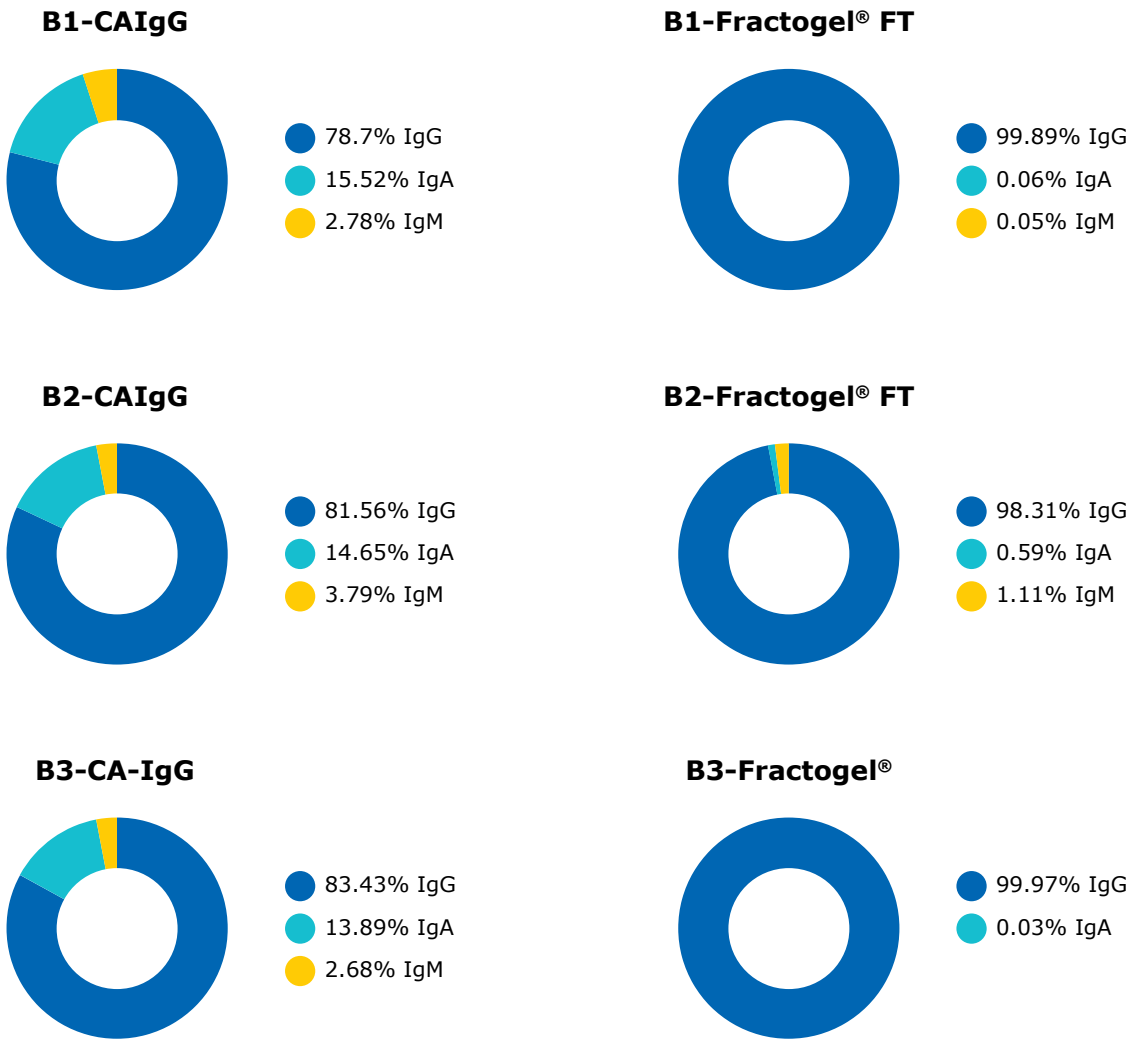


Figure 7. Removal of IgA and IgM using Fractogel® EMD TMAE anion exchange chromatography for three different pilot-scale batches of plasma.

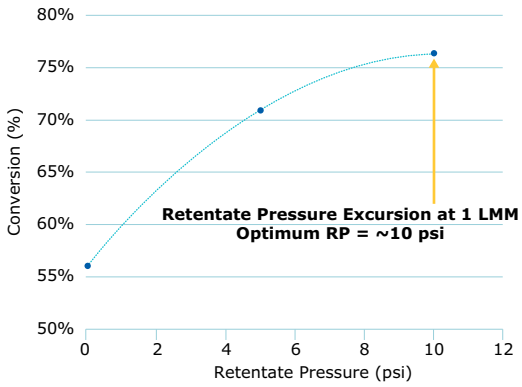
Learn More:

Webinar: Overcoming high concentration challenges in IgG purification
Webinar: Addressing Immunoglobulin (Ig) Purification Challenges with Chromatographic Technologies
Webinar: Chromatography: Chromatographic strategies for IVIG purification – Part 2

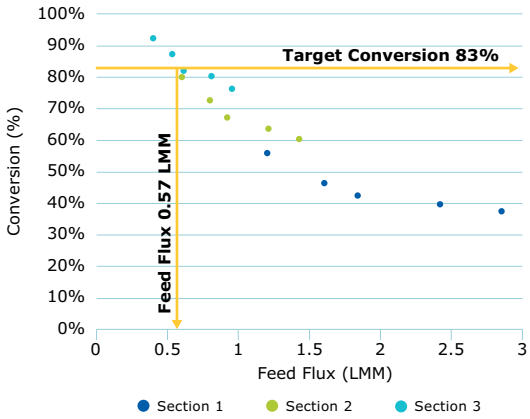
Concentration using Single-Pass Tangential Flow Filtration (SPTFF)

Following the Fractogel® TMAE anion exchange step, a 6X volume concentration was performed using a SPTFF system with Pellicon® 3 Biomax® membrane. This step achieved an optimal loading concentration (> 40 mg/mL) for the Eshmuno® P Anti-A/ Anti-B chromatography step and reduced the time and volume needed for subsequent steps to remove anti-A and anti-B isoagglutinins (**Figure 8**). In this study, 6X SPTFF concentration successfully increased the IgG sample concentration from 12 mg/mL to 74 mg/mL.

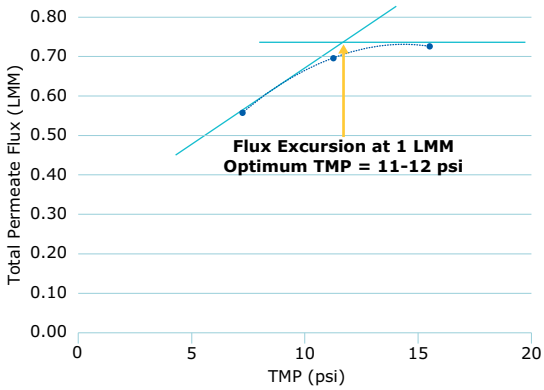
A. Retentate Pressure Excursion



B. Feed Flux Excursion



C. Permeate Flux TMP Excursion



SPTFF successfully concentrated the sample around 6 times concentration achieved optimal loading concentration as for binding capacity, reduced volume and time needed for subsequent anti-A and anti-B affinity chromatography steps.

D.

Process Parameters			
Initial concentration	12 mg/mL	**Initial volume**	4 L
Final concentration	75 mg/mL	**Final volume**	670 mL
Feed flux	0.57 LMM	**TMP**	11-12 psi
Conversion	83%	**Retentate Pressure**	10 psi

Figure 8. SPTFF concentration of the IgG sample (C). TMP excursion experiment defining the optimal TMP at 11-12 psi (B). Optimization excursion with three sections defining optimal feed flux for 83% conversion at 0.57L MM (A). Retentate pressure excursion defining optimal retentate pressure ~ 10 psi (D). Summary of conditions and overall concentration results.
*LMM= liters per minute per square meter.

Recommended Reading:

Pellicon® Single-pass TFF white paper

Anti-A and Anti-B Agglutinin Removal

Prior to use of plasma-derived IgG as therapeutics, manufacturers must ensure removal of anti-A and anti-B agglutinins as trace amounts have been associated with increased patient risk for hemolysis, a serious and sometimes fatal complication. In this study, Eshmuno® P anti-A and anti-B, two distinct affinity-based chromatography resins, were used to effectively remove anti-A and anti-B isoagglutinin antibodies from pools of TMAE-polished IgG. The loading quantity was approximately 2500 mg IgG/mL of anti-A or anti-B resin, corresponding to 66% dynamic binding capacity at the defined residence time, while capacity tested was approximately 3800 mg CA-IgG/mL Anti-A resin (data not shown). Samples were tested at 30 mg/mL concentration. Chromatographic conditions were 25 mM sodium acetate (pH 6.0) for the equilibration buffer, 1.5M NaCl in 250 mM sodium acetate (pH 4.5) for the elution buffer, and 0.5M/1.0M NaOH for CIP solutions. Results showed consistent 8 – 16x reduction in Anti-A titer and 16 – 32x reduction in Anti-B titer (**Figure 9**).

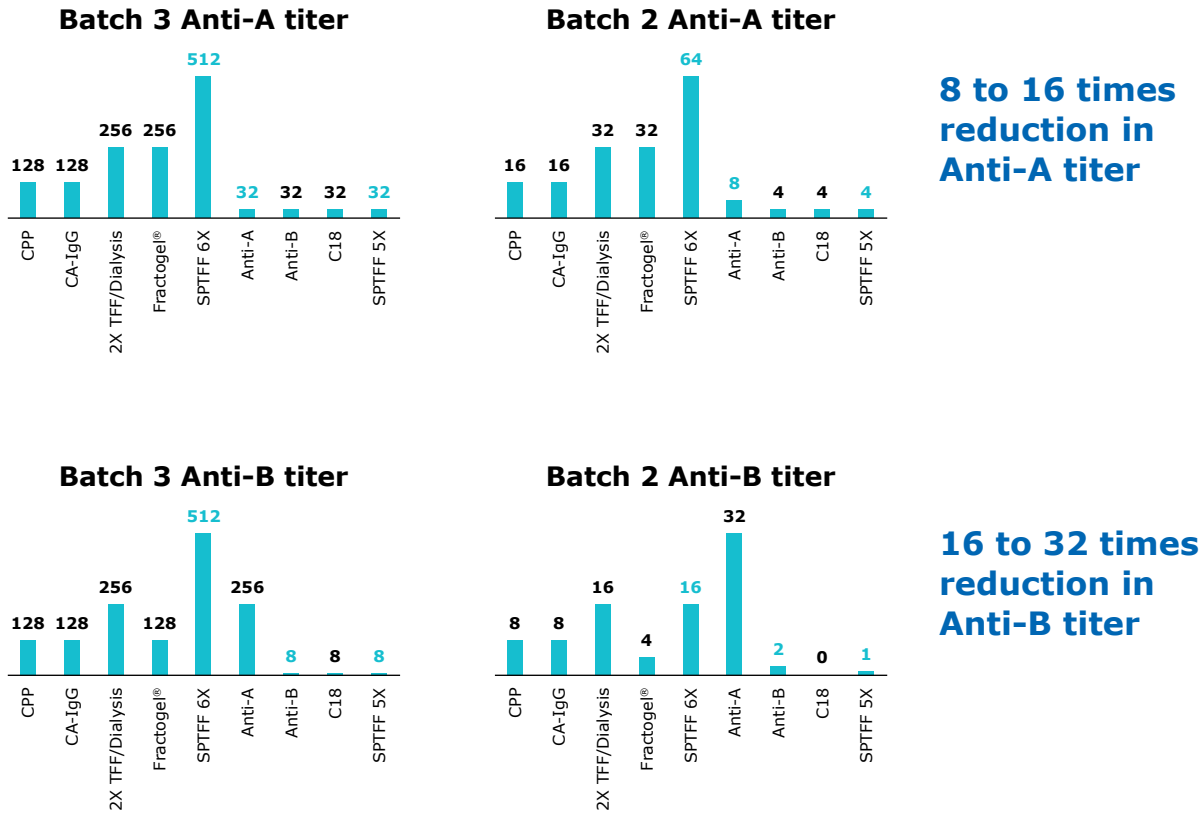


Figure 9. Eshmuno® P anti-A and anti-B enabled effective removal of anti-A and anti-B agglutinins.

Recommended Reading:

Reduction of Isoagglutinin in Intravenous Immunoglobulin (IVIG) Using Blood Group A- and B-Specific Immunoaffinity Chromatography: Industry-Scale Assessment

Solvent/Detergent Treatment and Removal by C18 Chromatography

Inactivation of enveloped viruses using solvent-detergent (SD) has been used in the plasma industry for many years to reduce the risk of blood borne pathogen transmission. Solvents such as tri-n-butyl phosphate (TnBP) and detergents such as Triton® X-100 and Tween® 80 are commonly used; Triton X-100/ TnBP requires the least amount of time to achieve > 4 Log reduction value for virus inactivation (**Table 2**; see recommended reading section below for additional information).

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

Table 2. XMuLV and BVDV inactivation using 0.3% (v/v) TnBP/1% (v/v) Triton® X-100 in human plasma.

In this study, S/D treatment was applied to the IgG batches and consisted of 0.3% tri(n-butyl) phosphate (TnBP) plus 1% Triton X-100. The mixture was incubated for 1 hour at 25 °C for experimental purposed and to mimic the conditions routinely used for the S/D virus inactivation of plasma products and other biologicals. A LiChroprep® RP-18 (40-63 µm) column was used in a flow-through mode to remove the S/D (**Figure 10**). The binding capacity for the S/D agents was up to 20 mL of S/D-IgG per 1mL packed C18 resin. In the pilot scale study, the loading ratio was reduced to 6 mL of S/D-IgG onto per 0.63g (1 mL) resin, in order to ensure a good safety factor. As a result, residual Triton X-100 in the S/D-IgG fractions after passing through the C18 resin was undectactable (< 2 ppm) as measured by HPLC. Residual TnBP in the S/D-IgG fractions was also undetactable (< 1 ppm) as measured by a GC-MS high sensitivity assay. It is worth noting that even after 5x SPTFF final concentration the residual S/D quantity remained low, proving the robustness of the process.

A.

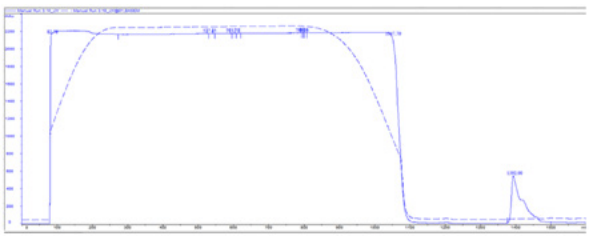


Figure 10. (A) Typical chromatography of flow-through Licroprep® C18 reverse phase chromatography.

B.

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

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

Figure 10. (B) Results of residual content measured by HPLC for Triton X-100, and GC-MS for TnBP.

Learn More:

Webinar: Solvent Detergent Viral Inactivation using Single-use Technology in Blood Fractionation Processes

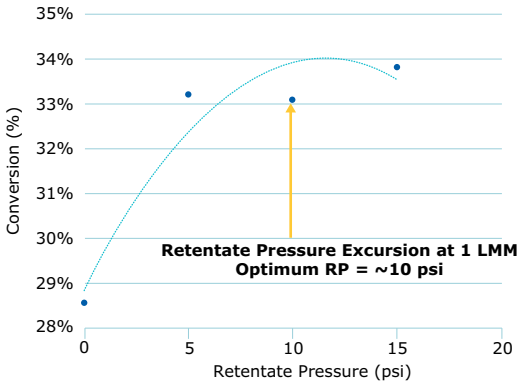
Recommended Reading:

Journal Article: Single-Use technology for solvent/detergent virus inactivation of industrial plasma products

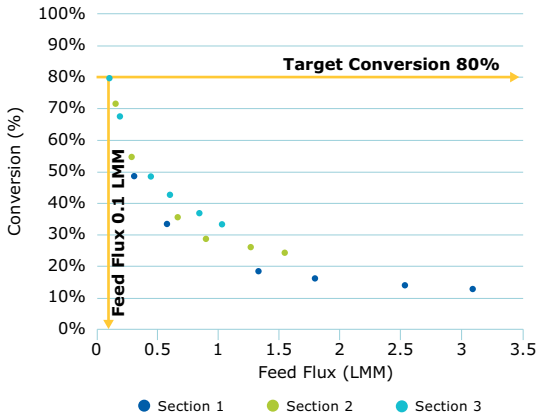
Final SPTFF Concentration

Following removal of the S/D agents, a series of optimization studies was performed to identify optimum parameters to be used to achieve the target concentration of 200 mg/mL. The studies included retentate pressure excursion and feed flux excursion to identify optimum TMP and feed flux. **Figure 11** summarizes the results required for a target of 80% conversion. A process simulation run was performed using the identified optimum parameter to demonstrate the ability to achieve a concentration of 200 mg/mL with process stability test over 300 minutes. The stable trend indicates the robustness of the conversion rates which represents stable target product concentration.

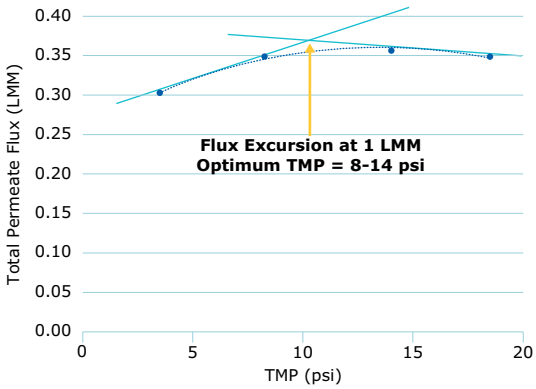
A. Retentate Pressure Conversion Excursion



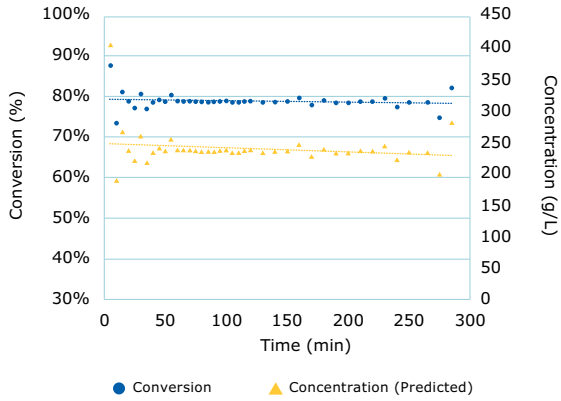
B. Feed Flux Excursion



C. Permeate Flux TMP Excursion



D. Process Simulation (Stability)



Process Parameters			
Initial concentration	51 mg/mL	**Initial volume**	726 L
Final concentration	221 mg/mL	**Final volume**	145 mL
Feed flux	0.1 LMM	**TMP**	11-12 psi
Conversion	80%	**Retentate Pressure**	10 psi

Figure 11. Process parameters obtained from retentate pressure and feed flux excursion used to achieve the final target concentration of 200 mg/mL of the IgG product.

Learn More:

Webinar: How to reach high plasma protein concentration with single-pass TFF

Webinar: High Viscosity Ultrafiltration Formulation for Plasma IgG and mAbs

Summary of the Filtration results in IgG Purification Process

Table 3 provides a summary of the pre-filters and sterile filters evaluated in the IgG purification process, along with the selected technologies and product recovery. Combinations of sterile filters Durapore® CVGL and Millipore Express® SHC, with or without Milligard® PES as prefilters delivered the desired performance. As observed in this study, when sterile filters were used alone, Millipore Express® SHC 0.5/0.2 µm offered better performance. However, when a Milligard® PES 1.2/0.2 µm prefilter was included, Durapore® CVGL 0.22 µm offered somewhat better performance, meaning that less membrane area was required for the same amount of throughput.

This full IgG purification study focusing on intermediate filtration steps is described in an application note entitled ***Filtration in Plasma IgG Purification.***

Steps	Pre-filter	Sterile Filter	Selected Pre-Filter (recovery %)	Selected Sterile Filter	Total Recovery (%)
Post Caprylic Acid Treatment)	Milligard® PES 1.2/0.2 µm	• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm	Milligard® PES 1.2/0.2 µm	Durapore® CVGL 0.22 µm	> 99%
Post UF/DF	Milligard® PES 1.2/0.2 µm Milligard® PES 1.2/0.45 µm Millistak+® A1HC	• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm	Millistak+® A1HC	Millipore Express® SHC 0.5/0.2 µm	~ 100%
Post CA-IgG passed through Fractogel resin		• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm		Millipore Express® SHC 0.5/0.2 µm	~ 100%
Post CA-IgG 6X SPTFF concentration	Milligard® PES 1.2/0.2 µm	• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm	Milligard® PES 1.2/0.2 µm (100%)	Durapore® CVGL 0.22 µm	> 96%
Post CA-IgG passing through Anti-B resin		• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm		Millipore Express® SHC 0.5/0.2 µm	~ 100%
Post S/D- pure IgG passing through C18 resin	Milligard® PES 1.2/0.2 µm	• Durapore® CVGL 0.22 µm • Millipore Express® SHC 0.5/0.2 µm	Milligard® PES 1.2/0.2 µm (100%)	Millipore Express® SHC 0.5/0.2 µm	~ 98%
Post Pure IgG 5X SPTFF		• Durapore® 0.22 µm • Millipore Express® SHC 0.5/0.2 µm		Millipore Express® SHC 0.5/0.2 µm	~ 86%

Table 3. Sterile filtration steps in the IgG process and selected Pre-filter/sterile filter combinations.

Figure 12 summarizes the total protein, IgG, IgA and IgM concentrations resulting from the final batch process. The recovered IgG was at a concentration of 200 mg/mL while the IgA and IgM concentrations were extremely low, confirming the purification effect using only one step Fractogel® TMAE (M). In other cases, two different anion chromatography steps can be used in sequence; Fractogel® TMAE (M) followed by Fractogel® DEAE (M) would achieve even better IgA/IgM removal. The flow-through mode provided ease of operation and higher recovery.

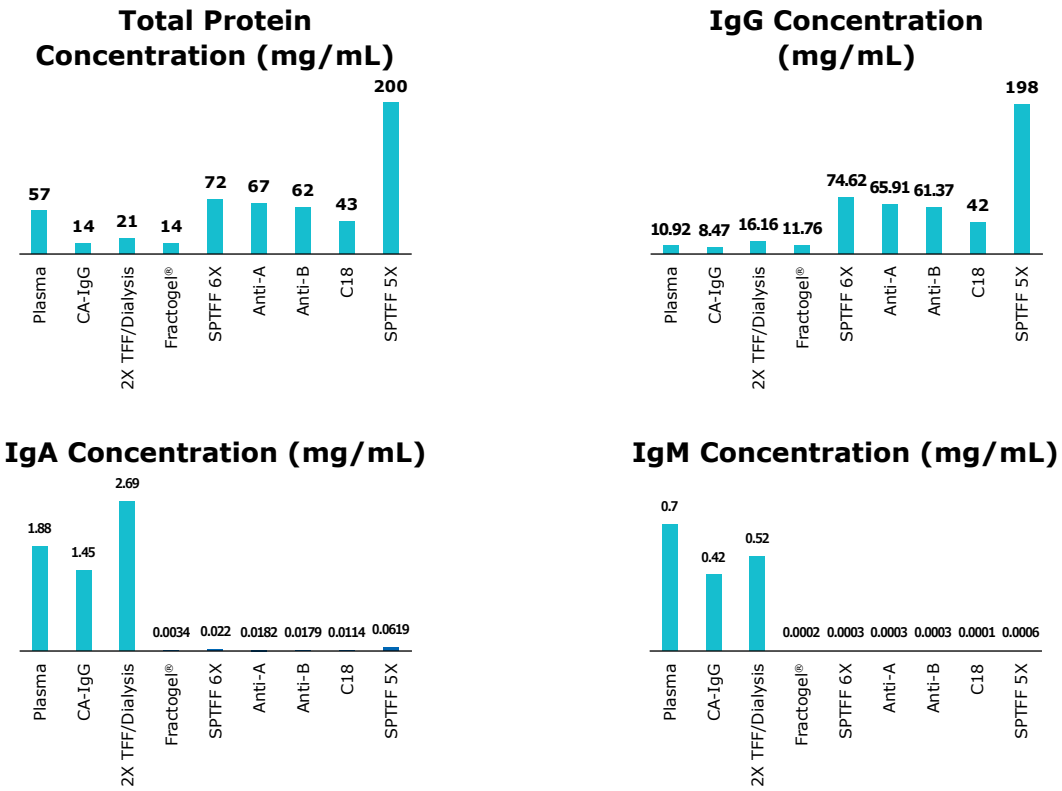


Figure 12. Total protein, IgG, IgA and IgM concentrations achieved by the process.

Figure 13 shows the typical step recovery results obtained from the pilot-scale purification run, with overall theoretical process recovery reaching more than 70%. The flow-through chromatography step and SPTFF technology provided ease of operation and reduced loss for each step; one should keep in mind, however, that small-scale experiments generally show lower recovery data as comparison to large-scale, due to the volume used.

The flow-through process provides ease of operation and also a satisfying overall theoretical process recovery > 70%.

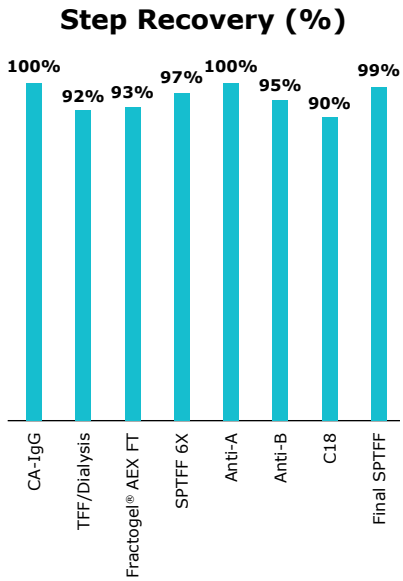


Figure 13. Typical recoveries obtained in the pilot-scale purification.

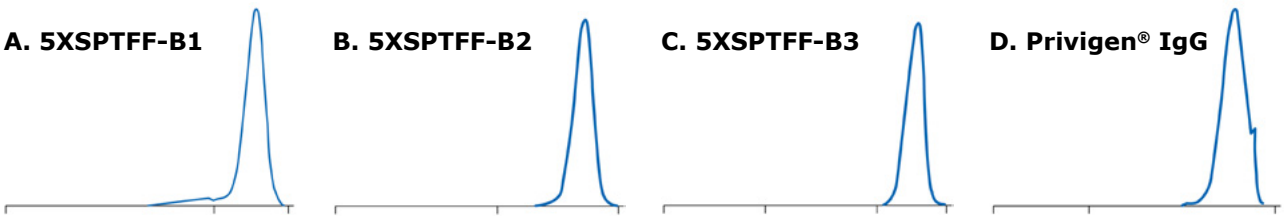
Table 4 shows the level of FXI/FXIa in various stages of purification; FXI/FXIa is considered to be a contributor to the thromboembolic effects of IVIG preparations. Our data show that the final IgG had undetectable FXI/FXIa, confirming the capacity of the caprylic acid treatment to remove or inactivate FXIa [1]. In addition, the purified IgG did not exhibit thrombin generation activity using the Thrombin Generation Assay (TGA) as the Fractogel® TMAE was consistently found to remove procoagulant factors.

	Lag Phase (min)	Thrombin (nM)	Time to Peak (min)	Velocity Index	AUC
CPP	4.75 ± 0.50	52.16 ± 14.73	27.25 ± 19.92	6.98 ± 6.93	954.20 ± 325.20
CA-IgG	4.00 ± 0.00	27.20 ±5.92	44.50 ± 0.58	0.67 ± 0.15	291.24 ± 155.68
Fractogel®	BDL	BDL	BDL	BDL	BDL
SPTFF	BDL	BDL	BDL	BDL	BDL

Table 4. Thrombin generation assay (TGA) conducted on IgG fractions over the various stages of purification.
BDL = below detection limit.

To examine the product purity, zone electrophoresis (**Figure 13**) and SDS-PAGE under non-reducing and reducing conditions (**Figure 14**) confirmed the high purity of the final IgG. The zone electrophoresis study included Privigen®, a marketed plasma IgG product, as a benchmark.

The SDS-PAGE protein profile during each purification process step revealed an increase in IgG purity and removal of protein contaminants (e.g. albumin). The profile of the final IgG was similar to that of Privigen®, reaching more than 99% purity in batch #3.



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

Figure 14. Zone electrophoresis of 5x SPTFF IgG batches and standard sample Privigen®.

Zone electrophoresis confirmed the high purity of the final IgG after process adjustments (Batches 2&3). The purity of batch 3 (5X SPTFF) reaches almost 100%.

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

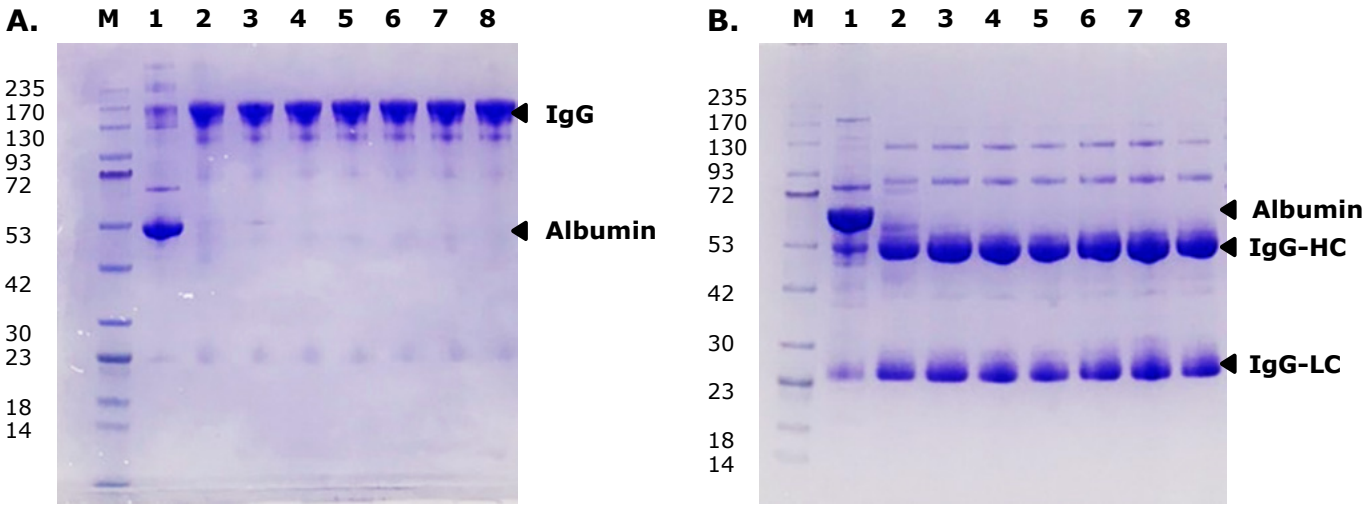


Figure 15. SDS-PAGE profile under (A) non-reducing and (B) reducing conditions of IgG samples and Privigen®. Loading quantity: 5 µg of protein loaded on to 4 – 12% Bis-Tris SDS-PAGE.

Conclusion

Purification of IgG from plasma is a time-consuming process with many steps. With a growing number of therapeutic applications for plasma IgG, and persistent shortage around the globe, optimization of the purification process to increase yield and productivity, while maintaining a cost-efficient process, is essential.

This whitepaper described a collaboration project which developed a “generic” process to accelerate and streamline the purification of IgG from human plasma from various starting materials. The process has since been modernized and optimized using a series of innovative technologies, capable of ensuring contaminant removal and virus safety. In this study, S/D treatment was used for virus inactivation and optimal conditions for removal of the S/D agents was developed. It should be noted, however, that to meet regulatory requirements, industrial applications require additional orthogonal virus reduction steps such as nanofiltration and/or low pH incubation, which both should be readily integrated.

The flow-through mode process based on Fractogel® TMAE(M), Eshmuno® P anti-A and anti-B chromatography robustly resulted in an isoagglutinin-depleted IgG fraction with well-maintained IgG sub-class distribution and depletion of IgA and thrombogenic activity. This fraction can be S/D-treated with efficient S/D removal by C18 reverse-phase chromatography and concentrated to 20% with SPTFF, which helps address the growing demand of subcutaneous home administration by primary immunodeficient patients. As an overall result, an IgG purity of 99% was achieved as demonstrated by zone electrophoresis and SDS-PAGE; this purity is comparable to a marketed IgG protein. This flow-through process can be monitored reliably by controlling key process parameters, is readily scalable and can be applied for various IgG products including polyvalent IgG, hyperimmune or convalescent immunoglobulins.

Highlights of the intensified process include:

- Use of SPTFF technology with Pellicon® 3 membrane cassettes as a mild and robust approach to concentrate IgG, realizing in-line concentration for intensified chromatography, and to reach final target concentration of 20%.
- Protection of sterile filters by Milligard® PES 1.2/0.2 µm prefilters, effectively reducing the filtration area needed for Millipore Express® SHC or Durapore® CVGL filters.
- Clarification using Millistak+® HC A1HC can facilitate downstream purification.
- Fractogel® TMAE(M) anion exchange chromatography for efficient removal of IgA and IgM.
- Eshmuno® P anti-A and Eshmuno® P anti-B chromatography for removal of anti-A and anti-B agglutinins.
- Recovery from 92 – 100% resulting in an overall process recovery greater than 70%, with the likelihood of opportunities to improve further with additional optimization.

Citation list:

1. Wu YW, Champagne J, Toueille M, Gantier R, Burnouf T: Dedicated removal of immunoglobulin (Ig)A, IgM, and Factor (F)XI/activated FXI from human plasma IgG. ***Transfusion*** 2014; 54: 169-78
2. Article "Process steps for the fractionation of immunoglobulin (Ig) G depleted of IgA, isoagglutinins, and devoid of *in vitro* thrombogenicity" from **Blood Transfusion** 021; DOI 10.2450/2021.0159-21

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