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Application Note

Integration of Bioburden Reduction and Sterile Filtration in Human Plasma IgG Purification

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Human plasma contains coagulation factors, protease inhibitors, anticoagulants, albumin, polyvalent and hyperimmune immunoglobulins (IgGs) that can be used for therapeutic purposes. The fractionation process used in extracting proteins from plasma must ensure sufficient bioburden reduction which is typically achieved by several sterile filtration steps. This application note presents an intensified IgG purification process of human plasma from optimized sterile filtration steps.

Figure 1 provides an overview of an optimized process for IgG plasma purification. The yellow capsule icons in the figure indicate the seven bioburden reduction/sterile filtration steps that were incorporated; these steps are described in **Table 1**.

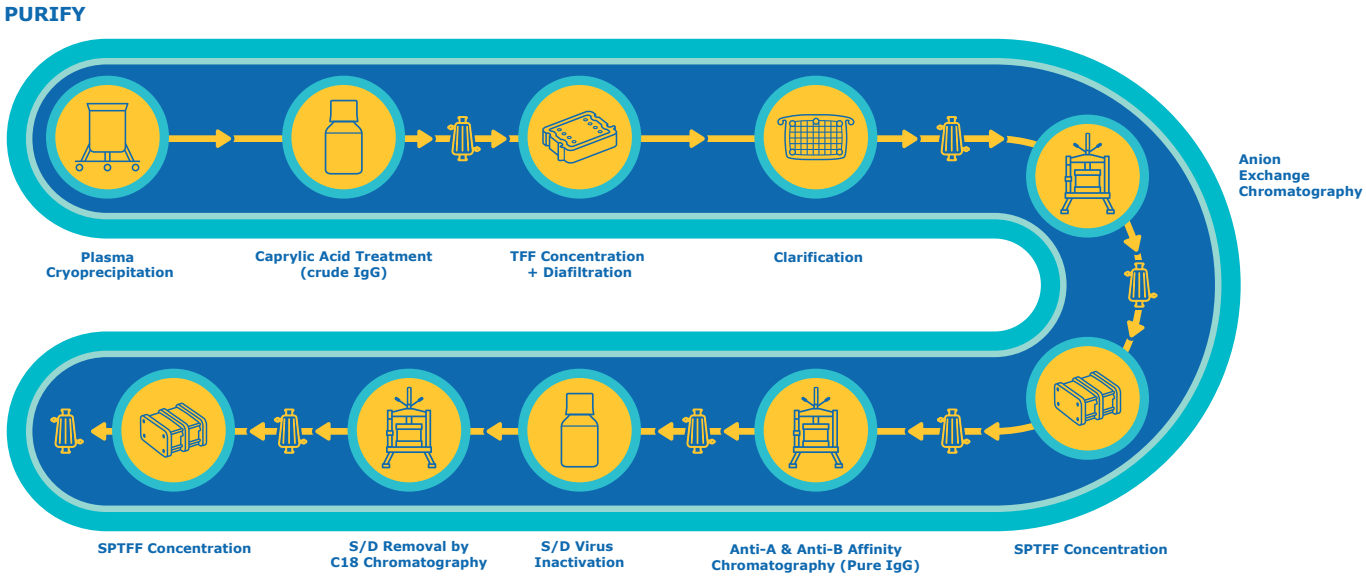


Figure 1. Summary of optimized process for IgG plasma fraction purification.

No.	Process step	Description
1	Caprylic Acid Treatment/centrifugation	Added 5% caprylic acid to precipitate non-IgG protein and centrifuged to remove precipitates to generate starting materials representing worse case of Fraction I+II+III in plasma fractionation process.
2	Batch TFF UF/DF	Concentrated and diafiltrated against the chromatographic equilibration buffer using Pellicon® 3 Biomax® (30 kDa, A screen) via tangential flow filtration (TFF) method prior to the subsequent chromatography purification steps.
3	Anion exchange (AEX) chromatography	Fractogel® EMD TMAE (M) anion exchange chromatography for primarily purification. Major IgA and IgM was removed.
4	Pre-affinity chromatography SPTFF concentration	Concentration was performed using Pellicon® 3 Biomax® (30 kDa, A screen) via Single-Pass TFF technology, to achieve an optimal loading concentration of >40 mg/mL for following affinity chromatography purification.
5	Affinity chromatography	Eshmuno® P anti-A and anti-B, two distinct, affinity-based chromatography resins, were used to remove blood type anti-A and anti-B isoagglutinin.
6	S/D treatment and S/D removal by C18 reverse phase chromatography	Solvent/detergent (S/D) treatment was applied to the IgG batches for enveloped virus inactivation. The LiChroprep® RP-18 (40-63µm) column was used in a flow-through mode to remove the S/D.
7	SPTFF final concentration	Single-Pass TFF technology using Pellicon® 3 Biomax® (30 kDa, D screen) to achieve final target concentration of 200 mg/mL.

Table 1. Steps in the IgG purification process that incorporated sterile filtration.

Filter Sizing Methods

Constant pressure (Vmax™) and constant flow (Pmax™) methods were used in the filter sizing studies (**Figure 2**).

The Vmax™ method predicts the capacity of filters using constant pressure testing, based on the gradual pore plugging model. Unlike traditional flow decay methods, this method allows prediction of filter lifetimes (volumetric loading capacities) without having to actually run the filter until it is completely plugged.

Gradual pore plugging occurs when colloids or suspended matter collect on the sides of filter pores to gradually block them off, until a state of total occlusion is eventually reached. This gradual blocking of the pores occurs in a distinct geometric pattern. In a Vmax™ test, the time and volume collected up to that time are recorded at regular intervals. Data are then plotted as time/volume versus time. If the data plot as a straight line, this indicates the filter is plugging by the gradual pore plugging model and the formulas of the Vmax™ method can be applied to predict filter life.

Pmax™ sizing method involves determining the filter resistance to flow as a function of throughput. The advantages to this method is that it provides a basis for filter train selection and is independent of the plugging model. This method is commonly used to size depth filters and other charged filters that exhibit complex fouling models where particle retention occurs via size exclusion and adsorption. During the experiment, the operator measures and records upstream and downstream pressures across the depth filter and the filtrate turbidity at regular time intervals. Typical flux values range from 100-200 LMH (for a µPod® device at 23 cm^2, the flowrate is 4-8 mL/min). The test concludes when either the pressure reaches a maximum of 20 psi or the turbidity breakthrough point is acheived.

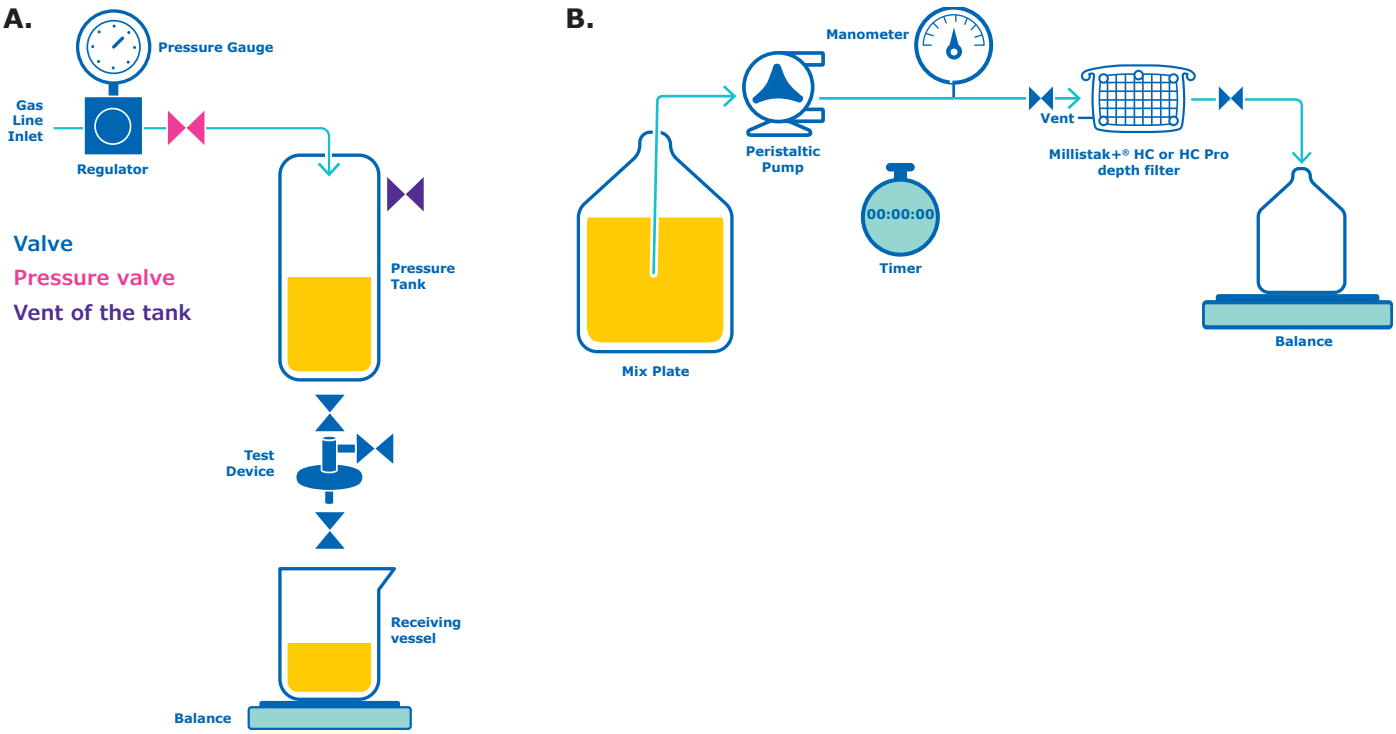


Figure 2. Schematics summarizing the Vmax™ (A) and Pmax™ (B) methods filtration.

Evaluation of Filters

The filters evaluated in the capacity studies are shown in **Table 2**. Based on the quality of the feed and the goal of the filtration step (particulate control, bioburden reduction and/or sterility assurance), different configurations of filters were evaluated.

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 Millpore 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.

Filter Name	Filter Area	Membrane Pore Size	Composition & Symmetry	Type
Millipore Express® SHC Optiscale® 25 mm	3.5 cm^2	0.5 / 0.2 µm	PES*, asymmetric	Membrane filter
Durapore® 0.22 µm Optiscale® 25 mm	3.5 cm^2	0.22 µm	PVDF**, symmetric	Membrane filter
Milligard® PES Optiscale® 25 mm	3.5 cm^2	1.2 / 0.2 µm	PES, asymmetric	Membrane filter
Millistak+® HC µPod A1HC	23 cm^2	<0.5 µm (Nominal pore size)	Cellulose fibers with inorganic filter aid (DE65 + ED70) Mixed esters of cellulose (RW01)	Depth filter

Table 2. Details of filters evaluated in the study.

** Polyethersulfone (PES) membranes*
*** Polyvinylidene fluoride (PVDF) membranes*

Figure 3 summarizes the results of the Vmax™ and Pmax™ studies evaluating filters for the seven plasma IgG purification steps.

Post-Caprylic acid treatment and centrifugation: In a single step filtration, the Millpore Express® SHC filter showed better capacity and less flux decay than the Durapore® filter. In a two step filtration train with Milligard® PES 1.2/0.2 serving as a prefilter, the performance of both sterile filters was improvedby 10% (Millpore Express® SHC) and 60% (Durapore®), taking the throughput values at 30 minutes time Therefore for this step a filtration train using Milligard® PES 1.2/0.2 following by Durapore® 0.22 μm was selected.

Post-batch TFF UF/DF: Due to the lower clarity of the feed, the sterile filter SHC has lower efficiency in terms of throughput. A depth filter (A1HC) was recommended as prefilter to remove the impurities. The resistance remained stable at 0.01 psi/LMH, and instantaneous turbidity did not increase during the trial. The final pool turbidity was 9.8 ntu (data can refer to white paper). Following clarification, the Millpore Express® SHC and Durapore® sterile filters were evaluated. The capacity on Millpore Express® SHC with A1HC prefilter increased 48 times higher in capacity with minimum plugging observed as compared to direct SHC (without prefilter), suggesting that the A1HC filter successfully protected the sterile filter.

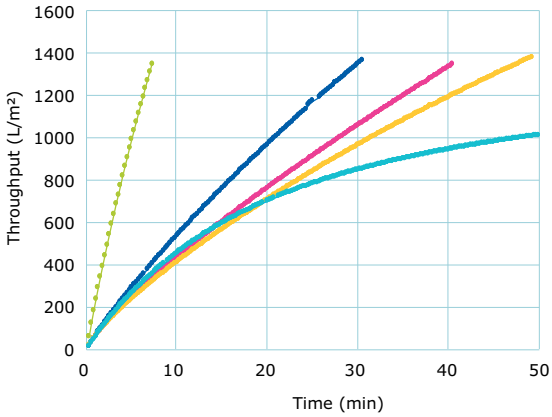
Post-AEX and Post-affinity chromatography: In theory, the feed coming from chromatography steps will have relatively low turbidity and be a clean solution. As such, the Millpore Express® SHC and Durapore® filters were evaluated directly in these steps. Based on the results, the SHC filter delivered better performance in these two steps because of the multi layer design and asymmetric membrane structure.

Post-SFTFF concentration: Due to the sample turbidity is higher than 50 ntu, the prefilteration step was added to protect the following sterile filter and resulted in process improvement. Milligard® PES 1.2/0.2 served as a prefilter and immediate flux decay (more than 90%) was observed with Vmax of 650 L/m^2. The SHC filter delivered higher intitial flux because of the multi layer design and asymmetric membrane structure. However, both SHC and Durapore were observed to have high Vmax values of 2197 L/m^2 and 1789 L/m^2, respecatively.

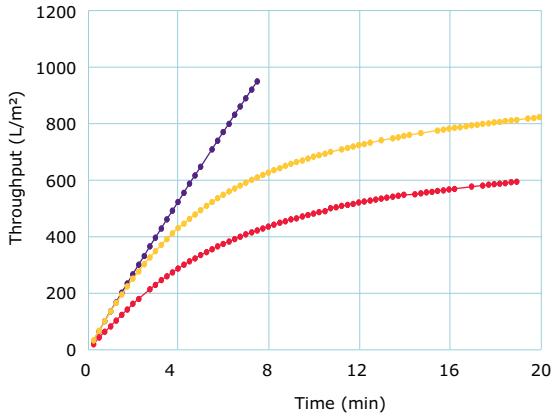
Post-C18 reversed phase chromatography: Since the Millipore Express® SHC and Durapore® filters alone were immediately plugged with low throughput, a Milligard® PES 1.2/0.2 filter was used as a prefilter. As a result, Vmax™ capacity of the sterile filters improved 7.6 times and 23 times, respectively.

Post-SPTFF final concentration: Due to high concentration and viscosity of the feed solution, the double layer design and asymmetric membrane structure of the Millipore Express® SHC filter was expected to be helpful in enhancing filtration performance; in contrast, Durapore® filters have a single layer design and a symmetric membrane structure. Future optimization consideration includes the addition of prefilters such as Milligard® PES, Milligard® and Polysep® II and is expected to futher enhance the overall efficiency.

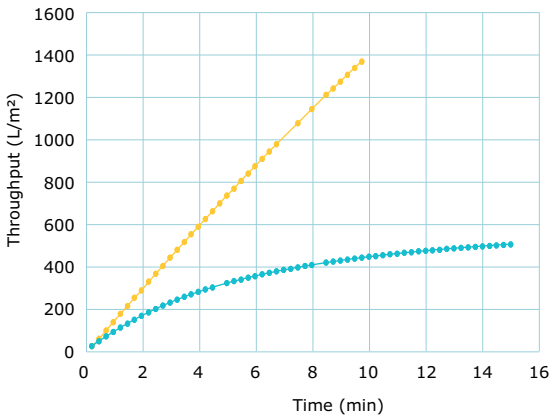
Step 1: Post-Caprylic Acid Treatment/centrifugation



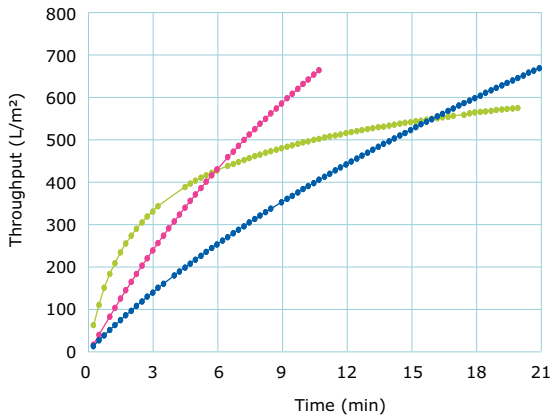
Step 2: Post-Batch TFF UF/DF



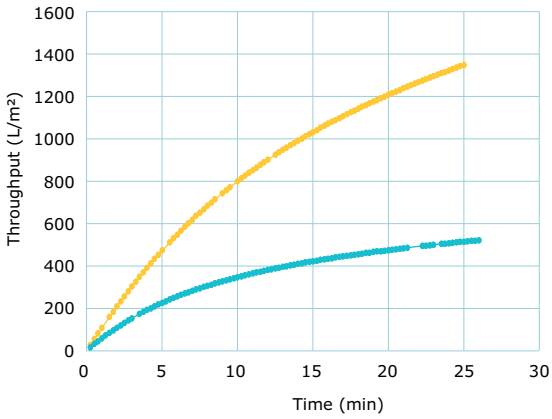
Step 3: Post-AEX Chromatography



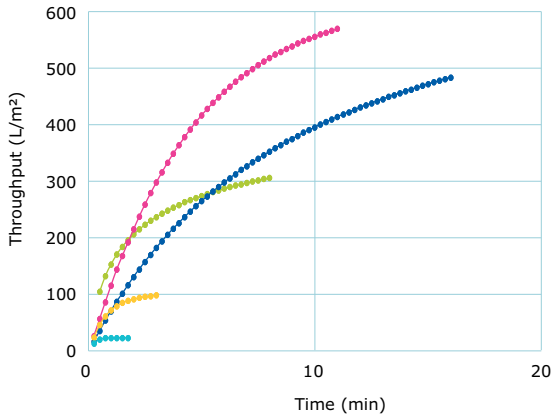
Step 4: Post-SPTFF Concentration



Step 5: Post-Affinity Chromatography



Step 6: Post-C18 Reverse Phase Concentration



Step 7: Post-SPTFF Final Concentration

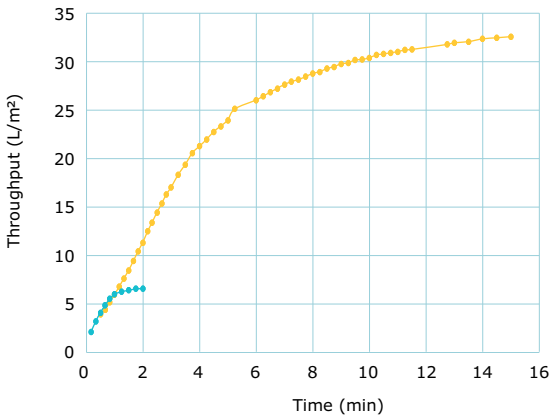


Figure 3. Summary of Vmax™ and Pmax™ results.

Discussion and Conclusion

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 ensuring patient safety is essential. Intermediate filtration including bioburden reduction filtration and sterile filtration can contribute to process efficiency while ensuring product safety.

This study evaluated the use of a variety of prefilters and filters for bioburden reduction and sterile filatration during an intensified process for purification of IgG from a human plasma feedstream. **Table 3** summarizes the recommended filters based on Vmax™ and Pmax™ sizing results. For relatively clean intermediate filtration steps, Millipore Express® SHC can deliver higher intitial flux compared with Durapore® filters because of the multi layer design and asymmetric membrane structure of SHC; both can provide robust sterile assurance throughout the process. In the high high concentration, high viscosity and low turbidity product, the Milligard® PES 1.2/0.2 µm filter improved the capacity of sterile filters in the plasma IgG purification process. However, for the higher turbidity stream, the Millistak+® HC A1HC depth filter would be the optimal choice as it can offer a protective effect to downstream sterile filters. All the configuration can achieve >90% product recovery except for the final filtration. Due to a high product concentration, it is difficult to achieve recovery when the filter is completely plugged in the small scale trial. This recovery issue could be optimized in large scale because of the safety factor and product recovery method.

Filtration configuration can vary depending on the process need. For example, if bioburden reduction filters are sufficient, Milligard® PES can be used in a standalone manner; Milligard® PES 1.2/0.2 µm nominal and 1.2/0.45 µm filters can remove greater than 6 logs of Brevundimonas diminuta and Serratia marcescens respectively. For non-critical process steps, these filters are an attractive alternative to sterilizing filters for reducing bioburden and improving processing efficiency.

In summary, this study provided a full concept and trial design for intermediate and final filtration filter selection.

Steps	IgG Concentration (mg/mL)	Clarification	Prefiltration	Sterile filtration	Recovery (%)
Post-Caprylic Acid Treatment/centrifugation	8.4	NA	Milligard® PES 1.2/0.2 µm*	Durapore® 0.22 µm	>99
Post-Batch TFF UF/DF	16.2	Millistak+® HC A1HC**	NA	Millipore Express® SHC 0.5/0.2 µm	>99
Post-AEX chromatography	11.8	NA	NA	Millipore Express® SHC 0.5/0.2 µm	>99
Post-SPTFF concentration	75	NA	Milligard® PES 1.2/0.2 µm	Millipore Express® SHC 0.5/0.2 µm	>96
Post-Affinity chromatography	45.8	NA	NA	Millipore Express® SHC 0.5/0.2 µm	>99
Post-C18 reverse phase chromatography	42	NA	Milligard® PES 1.2/0.2 µm	Millipore Express® SHC 0.5/0.2 µm	>98
Post-SPTFF final concentration	198	NA	NA	Millipore Express® SHC 0.5/0.2 µm	>86

Table 3. Filter recommendations for bioburden reduction and sterile filtration during plasma IgG purification.

**The sample was collected from post centrifugation suspension, so the sample is relatively clear.*
***Due to a small amount of precipitation that appeared in the product solution, Millistak+® HC A1HC was used in this step as a prefilter instead of Milligard® PES.*

References:

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 2021; DOI 10.2450/2021.0159-21

3. White Paper: "Intensification of Human Plasma IgG Purification for Intravenous and Subcutaneous Administration"; Lit. No. MS_WP7867EN 07/2021 Ver 1.0

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