An Abbreviated Affinity Chromatography Cascade Process for Factor VIII/von Willebrand Factor Complex and Immunoglobulin G.

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ABSTRACT

A novel affinity adsorbent has been designed with a fully synthetic (NCE) ligand that selectively binds the Factor VIII/ von Willebrand Factor Complex while allowing essentially quantitative recovery of the immunoglobulin G fraction of recovered and source plasma. IgG is recovered from the Cascade sequence using MAbsorbent A2P®, with or without prior removal of the albumin fraction. The Cascade intermediate can be further processed to remove remaining protein impurities such as IgM and IgA as well as traces of HSA and transferrin. The downstream process is also applicable to other IgG intermediates of the Cohn process.

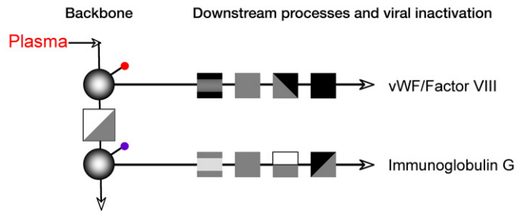
PRIMARY OBJECTIVES

- Direct capture of target proteins from Human Plasma using affinity adsorbents.
- The flow through can be loaded directly onto the next adsorbent in the cascade for capture of the next target protein and the order of the columns can be somewhat modified.
- Scale-up from 10mL columns to 2.5 L for vWF/FVIII and 18 L for IgG.
- Higher recovery of the target proteins over conventional plasma purification techniques.
- Minimal loss of other plasma proteins on the specific adsorbents.

CASCADE PROCESS

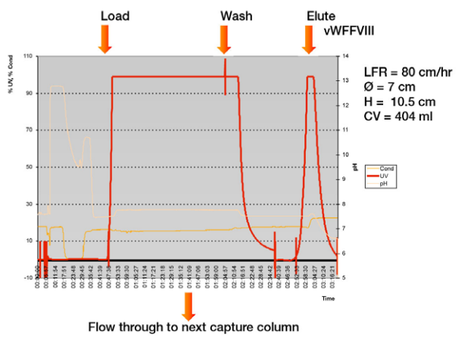
The use of affinity adsorbents to selectively capture target proteins from plasma with high recoveries and the ability to be scaled for manufacturing has not previously been successful. Affinity adsorbents were developed that allowed for capture of the target proteins from plasma at relatively high recoveries and purities. The flow through from the first column can be directly applied to the second affinity adsorbent with minimal to no further adjustment. The subsequent flow through can further be applied to additional affinity adsorbents to capture the desired proteins from the plasma. Once the target protein has been captured from the plasma feed stream, conventional down stream processing is performed.

ABBREVIATED CASCADE PROCESS



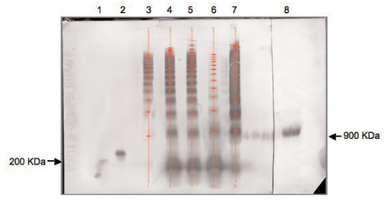
vWF/FACTOR VIII COLUMN

Human Source plasma is adjusted to pH 7.5 and loaded directly onto the vWF/FVIII affinity adsorbent. The flow through from this step is applied directly to the next affinity column in the cascade. The vWF/FVIII is eluted from the affinity resin and is used for downstream purification of the vWF/FVIII complex.



vWF MULTIMERS

Agarose gel for vWF multimers from affinity adsorbent.



Lane No.	Sample
1	Molecular Weight Markers
2	Reduced vWF Std
3	Non-Reduced vWF Std (13 Multimers)
4	S.A.R.P. Plasma Control (17 Multimers)
5	LCS vWF Load Plasma (17 Multimers)
6	LCS vWF Flow Through (12 Multimers)
7	LCS vWF Eluate (17 Multimers)
8	IgM (Ponceau S Stained)

RECOVERY OF vWF AND FVIII

Recovery of the vWF and FVIII from the affinity adsorbent is determined by ELISA. The activity of the recovered proteins is measured by their respective activity assays. The purification factor from plasma is calculated from the ratios of specific protein to total protein content in plasma and in the eluate.

	Recovery (n=20)	Purification Factor from plasma	Specific Activity
vWF ELISA	43.8%	37.9	
vWF Ristocetin Binding	49.1%		79.5 U/mg
FVIII ELISA	67.9%	59.3	
FVIII Chromogenic Activity	41.0%		0.232 IU/mg

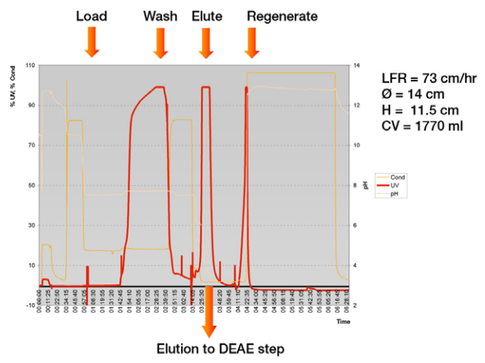
RECOVERY OF OTHER PLASMA PROTEINS

The recovery of other plasma proteins in the flow through from the vWF/FVIII affinity column was calculated by Nephelometry (n=20). This flow through was then used to further purify IgG.

IgG	HSA	AAT	Transferrin	IgA	IgM
98.1%	100.1%	99.5%	97.4%	96.8%	85.7%

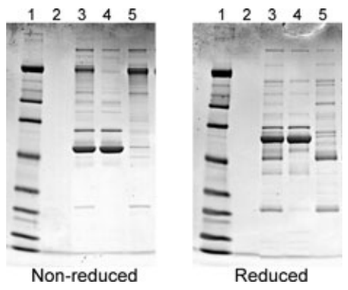
IMMUNOGLOBULIN G COLUMN (MAbsorbent A2P)

The flow through from the previous cascade column was used as load for the MAbsorbent A2P column. In some cases, the HSA was removed first by affinity chromatography. Otherwise, the HSA remained in the load for this adsorbent. No significant differences were detected with or without the prior removal of HSA.



RECOVERY OF IgG FROM MAbsorbent A2P

Non-reducing and reducing SDS-PAGE (8-16% Tris-Glycine) for the IgG A2P column samples; G250 Coomassie Stain



Gel Lane	Description
1	MW Broad Range Standard
2	Blank
3	IgG A2P Load
4	IgG A2P Flow through
5	IgG A2P Elution

Mean Step Recovery of IgG (n=17)	94.7%
Mean recovery from plasma (n=17)	84.1%
Purification Factor from plasma	3.7

There are 3 columns run in the cascade prior to loading on the A2P adsorbent. The calculation for the recovery from plasma takes into account the cumulative losses from all the columns.

COMPOSITION OF THE IgG SUBTYPES

The composition of the subtypes after elution from the A2P capture adsorbent remains constant from the starting plasma, through 3 other capture columns (for three additional proteins) to the A2P column.

Plasma Load vs. IgG Eluate from MAbsorbent A2P Mass of Protein (mg)

Sample	IgG Total (Sum of IgG_1–IgG_4)	IgG_1	IgG_2	IgG_1	IgG_4
Plasma load	26,254	16,960 (64.6%)	7,400 (28.2%)	762 (2.9%)	1,132 (4.3%)
IgG load	25,579	16,206 (64.0%)	7,295 (28.8%)	770 (3.0%)	1033 (4.1%)
IgG elution	24,579	16,160 (65.7%)	6,787 (27.6%)	719 (2.9%)	913 (3.7%)

RECOVERY OF OTHER PLASMA PROTEINS

The step recovery of other plasma proteins in the flow through of the MAbsorbent A2P column is relatively high. The majority of the IgM is in the flow through while only about 50% of the IgA is in the FT. The recovery of IgG, IgA and IgM in the eluate is also summarized below. There is very little IgM in the eluate and about 50% of the IgA from this step.

Recovery in the Flow through

HSA	AAT*	Transferrin	IgA	IgM
95.0%	128.7%	100.3%	49.1%	85.2%

* Recovery of alpha-1 anti-trypsin at this step is >100% due to interference in the assay in the presence of caprylate

Recovery in the Eluate

IgA	IgM	IgG
51.4%	14.3%	94.7%

The recovery of IgG with the HSA removed prior to the A2P column in the cascade was 88.3%. (average of 6 runs)

DOWNSTREAM PROCESS – IMMUNOGLOBULIN G

MAbsorbent® A2P elution
Adjusted to pH 5.0

Purabead® DEAE HF chromatography
Flow through mode

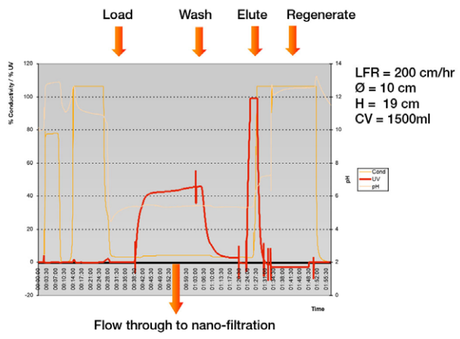
Nanofiltration

Final formulation - 5%(w/v) IgG solution
Low pH hold

IVIG

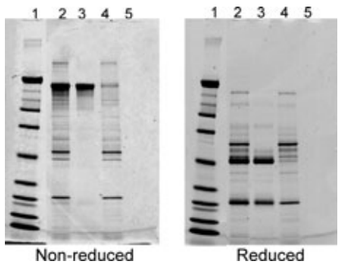
CHROMATOGRAPHY ON PURABEAD DEAE HF

The elution from the A2P column is adjusted to pH 5.0 in Sodium Acetate buffer and loaded directly onto the DEAE HF column. This column is run in the negative capture mode so that all the other proteins stick to this resin and the IgG is in the flow through.



RECOVERY OF IgG FROM DEAE

Non-reducing and reducing SDS-PAGE (8-16% Tris-Glycine) for the IgG A2P column samples; G250 Coomassie Stain



Gel Lane	Description
1	MW Broad Range Standard
2	IgG DEAE Load
3	IgG DEAE Flow through
4	IgG DEAE Elution
5	Blank

Mean Step Recovery of IgG (n=11)	92.2%
Mean recovery from plasma (n=11)	70.9%
Purification Factor from plasma	4.6

There are 4 columns run in the cascade prior to loading the A2P eluate on the DEAE resin. The calculation for the recovery from plasma takes into account the cumulative losses from all the columns.

COMPOSITION OF THE IgG SUBTYPES

The composition of the subtypes after the DEAE column does not significantly change from plasma through the DEAE FT.

Plasma Load vs. IgG DEAE FT Mass of Protein (mg)

Sample	IgG Total (Sum of IgG_1–IgG_4)	IgG_1	IgG_2	IgG_1	IgG_4
Plasma load	26,254	16,960 (64.6%)	7,400 (28.2%)	762 (2.9%)	1,132 (4.3%)
IgG DEAE load	22,284	14,899 (66.9%)	5,586 (25.1%)	791 (3.5%)	1,008 (4.5%)
IgG DEAE Flow through	21,915	14,699 (67.1%)	5,467 (24.9%)	753 (3.4%)	996 (4.5%)

SUMMARY

- Direct capture of the vWF/FVIII complex from plasma with high recoveries has been accomplished using an affinity adsorbent.
- The flow through can be loaded directly onto the next adsorbent in the cascade for capture of the next target protein with minimal loss of other proteins of interest.
- The IgG present in the “protein-depleted” plasma can be captured on the A2P adsorbent with high recoveries. The IgG can be further purified with high recovery at each step and minimal modification of the IgG subtype distribution.
- The affinity adsorbents offer higher recovery of proteins of interest over conventional plasma purification techniques with minimal loss of other plasma proteins on the specific adsorbents.

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