

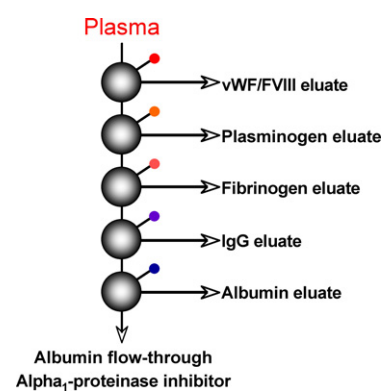
A Comparison of Cascade Plasma Fractionation Intermediates from Site to Site and Scale-up to 30 L Pools

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ABSTRACT

The initial version of Cascade Process was successfully transferred from the American Red Cross to Hemosol Corporation (4 L scale) followed by scale up to 30 L. The initial criteria for transfer were based on the yield of target protein products (vWF-FVIII; Plasminogen; Fibrinogen; IgG; Albumin; A1PI). Additional process optimization at the American Red Cross has included considerations for potency, recovery and intermediate impurity profiles. Yields of target proteins were comparable between sites and scales. FVIII and vWF potency were improved by scale-up, improved elution conditions and by addition of classic stabilizers to the eluted vWF-FVIII intermediate. Examination of impurity profiles have demonstrated substantial comparability between sites and scale. Subtle differences were noted in the initial transfer, for example, impurities can be removed by the Fibrinogen Capture and prevented from entering the IgG intermediate. Thus, important factors necessary to support the economics of the Cascade Process have been transferred to Hemosol. Additional optimization of critical process parameters related to impurity profiles continues at the American Red Cross.

CASCADE PLASMA FRACTIONATION BACKBONE AS TRANSFERRED TO HEMOSOL CORPORATION



THE CASCADE BACKBONE is a process that utilizes affinity adsorbents to selectively capture target proteins from plasma with high recoveries. The flow-through from the first column is directly applied to the second affinity adsorbent with minimal or no further adjustment. The subsequent flow-through is further applied to additional affinity adsorbents to capture other desired proteins from plasma. Captured target proteins are eluted for additional downstream processing to meet product target specifications. The adsorbents are stable to caustic cleaning and sanitation.

COMPARISON OF ANTIGEN YIELDS OF ARC 4L, HEMOSOL 4L & HEMOSOL 30L PPPS PREPARATIONS

Protein	ARC 4L (Std Dev)	Hemosol 4L (Std Dev)	Hemosol 30L (Std Dev)
vWF*	42.1% (10.0) ⁷	53.8% (17.6) ⁷	61.6% (22.5) ⁶
FVIII*	63.8% (9.2) ⁷	71.1% (11.3) ⁷	82.3% (33.5) ⁶
Pg	70.9% (4.6) ⁷	76.5% (15.3) ⁷	76.6% (15.0) ⁷
Fg	77.1% (4.2) ⁷	72.7% (4.3) ⁷	76.7% (9.2) ⁷
IgG	79.5% (10.4) ⁷	71.3% (4.1) ⁷	72.6% (17.7) ⁷
HSA	82.3% (9.8) ⁷	75.9% (6.1) ⁷	77.7% (14.6) ⁷
A1PI	84.2% (6.4) ⁷	75.1% (7.3) ⁷	94.6% (44.0) ⁷

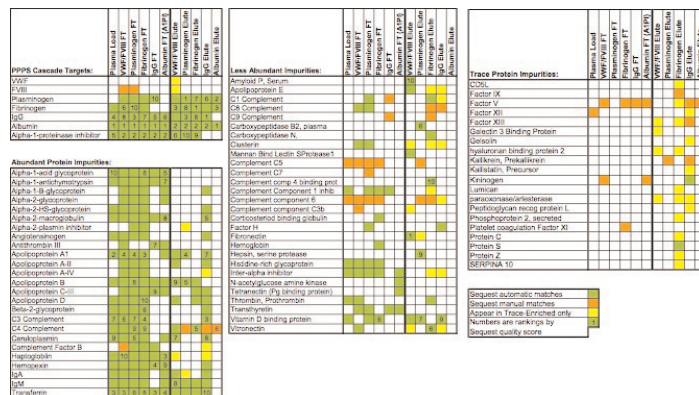
Numerical Superscript = N; *Determined by ELISA; All others determined by nephelometry

COMPARISON OF ACTIVITY YIELDS OF ARC 4L, HEMOSOL 4L & HEMOSOL 30L PPPS PREPARATIONS

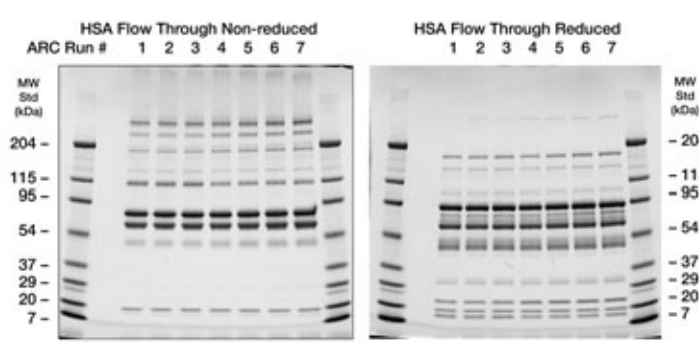
Protein	ARC 4L (Std Dev)	Hemosol 4L (Std Dev)	Hemosol 30L (Std Dev)
vWF	36.3% (10.3) ⁷ *47.5% (14.0) ²⁴	58.1% (11.9) ⁷	80.1% (32.3) ⁷
FVIII _{cg}	10.0% (4.1) ⁷ *27.8% (7.4) ²⁴	17.7% (10.7) ⁷	22.3% (9.6) ⁷
FVIII _{ch}	9.5% (3.2) ⁷ *39.9% (12.6) ²⁴	22% (11.8) ⁷	30.7% (14.9) ⁷
Pg	58% (9.2) ³	73.6% (5.4) ⁷	89.6% (5.7) ³
Fg	44.9% (7.3) ³	47.2% (3.3) ⁷	53.2% (7.1) ³
A1PI	89.4% (19.2) ³	90.4% (17.2) ⁷	*86.6% (5.2) ³

Superscript = N, cg =Coagulation, ch = Chromogenic, * Post transfer process improvements

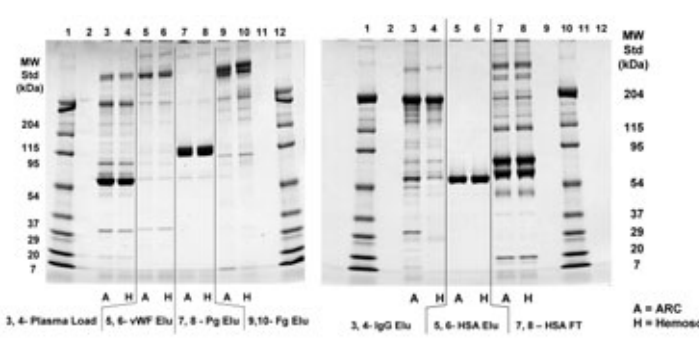
IDENTIFICATION OF PLASMA PROTEINS OR FRAGMENTS IN CASCADE INTERMEDIATES BY LC/LC/MS/MS



4L RUN CONSISTENCY ANALYSIS BY SDS-PAGE

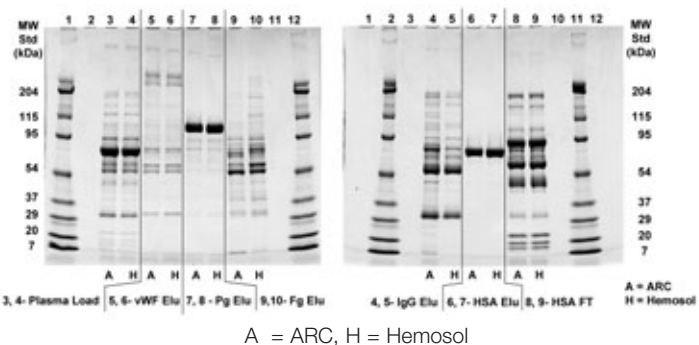


NON-REDUCED SDS-PAGE COMPARISON OF ARC AND HEMOSOL 4 L CASCADE ELUATES AND HSA FLOW-THROUGH INTERMEDIATES



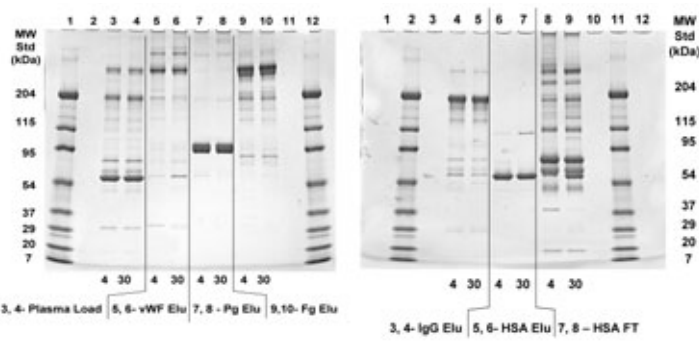
A = ARC, H = Hemosol
See attached booklet for band identification by in-gel digest and MALDI MS Peptide Mass Fingerprinting

REDUCED SDS-PAGE COMPARISON OF ARC AND HEMOSOL 4 L CASCADE ELUATES AND HSA FLOW-THROUGH INTERMEDIATES



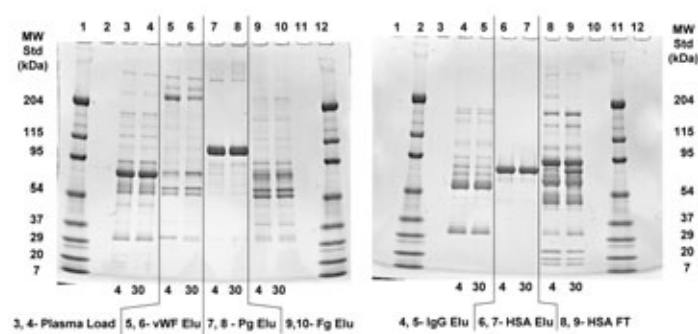
A = ARC, H = Hemosol
See attached booklet for band identification by in-gel digest and MALDI MS Peptide Mass Fingerprinting

NON-REDUCED SDS-PAGE COMPARISON OF HEMOSOL 4 L AND 30 L CASCADE ELUATES AND HSA FLOW-THROUGH INTERMEDIATES



4 = Hemosol 4L Run, 30 = Hemosol 30L Run
See attached booklet for band identification by in-gel digest and MALDI MS Peptide Mass Fingerprinting

REDUCED SDS-PAGE COMPARISON OF HEMOSOL 4 L AND 30 L CASCADE ELUATES AND HSA FLOW-THROUGH INTERMEDIATES



4 = Hemosol 4L Run, 30 = Hemosol 30L Run
See attached booklet for band identification by in-gel digest and MALDI MS Peptide Mass Fingerprinting

PLASMA LOAD ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
HSA	60.65%	60.57%	58.97%
IgG	12.62%	12.90%	15.50%
Fibrinogen	5.14%	4.99%	5.67%
Transferrin	3.95%	3.73%	3.39%
IgA	2.78%	2.89%	2.96%
A1PI	2.28%	2.37%	2.17%
Apolipoprotein A	2.18%	2.19%	0.02%
Alpha-2 Macroglobulin	1.85%	1.81%	2.30%
Complement C3c	1.83%	2.02%	1.68%
Haptoglobin	1.81%	1.72%	1.94%
Apolipoprotein B	1.41%	1.45%	1.35%
IgM	1.23%	1.20%	1.58%
Fibrinectin	0.69%	0.58%	0.79%
Antithrombin III	0.52%	0.54%	0.58%
Ceruloplasmin	0.40%	0.43%	0.43%
Complement C4	0.40%	0.41%	0.39%
Plasminogen	0.23%	0.23%	0.26%
vWF	0.02%	0.01%	0.02%
FVIII	0.002%	0.002%	0.200%
IgE	0.001%	0.001%	0.100%

*Determined by ELISA; All others determined by nephelometry

VWF-FVIII ELUTION ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
Fibrinectin	48.26%	46.51%	31.62%
Fibrinogen	21.57%	25.80%	39.20%
IgA	6.93%	4.75%	2.61%
Apolipoprotein B	5.01%	6.70%	7.35%
IgM	4.03%	3.87%	2.98%
HSA	3.30%	3.75%	7.29%
Alpha-2 Macroglobulin	2.99%	1.53%	2.42%
Complement C3c	2.56%	1.68%	1.32%
Ceruloplasmin	2.15%	1.65%	0.58%
Transferrin	1.12%	0.92%	0.89%
Apolipoprotein A	0.72%	0.54%	2.24%
vWF	0.61%	0.54%	0.40%
Complement C4	0.43%	1.42%	0.65%
Antithrombin III	0.22%	0.23%	0.18%
FVIII	0.10%	0.13%	0.10%

*Determined by ELISA; All others determined by nephelometry

PLASMINOGEN ELUTION ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
Plasminogen	75.75%	67.17%	63.45%
IgM	7.41%	8.16%	6.07%
IgA	2.20%	3.12%	4.03%
Apolipoprotein B	2.16%	2.82%	3.60%
Alpha-2 Macroglobulin	2.08%	2.52%	2.08%
IgG	1.97%	3.63%	2.89%
HSA	1.74%	1.81%	3.32%
Complement C4	1.55%	1.01%	1.98%
Fibrinogen	1.31%	2.82%	1.27%
Transferrin	0.94%	2.01%	2.54%
Ceruloplasmin	0.84%	1.21%	1.98%
Complement C3c	0.84%	1.01%	1.48%
A1PI	0.47%	1.11%	1.34%
Apolipoprotein A	0.47%	1.01%	0.42%
Antithrombin III	0.28%	0.60%	0.71%

FIBRINOGEN ELUTION ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
Fibrinogen	81.77%	65.81%	72.70%
IgG	6.79%	16.16%	9.98%
IgA	2.73%	3.02%	3.39%
IgM	2.08%	2.94%	3.63%
Complement C4	1.71%	3.88%	3.33%
HSA	1.10%	0.48%	0.91%
Apolipoprotein B	0.87%	1.10%	0.76%
Alpha-2 Macroglobulin	0.84%	1.08%	1.12%
Complement C3c	0.84%	3.93%	2.57%
Transferrin	0.43%	0.54%	0.21%
Ceruloplasmin	0.24%	0.30%	0.06%
A1PI	0.22%	0.27%	0.30%
Apolipoprotein A	0.22%	0.27%	0.57%
Antithrombin III	0.13%	0.16%	0.18%
Plasminogen	0.04%	0.05%	0.30%

IGG ELUTION ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
IgG	61.55%	79.09%	83.67%
HSA	13.19%	3.33%	2.01%
IgA	8.61%	11.42%	8.97%
Complement C3c	5.83%	0.38%	0.68%
Alpha-2 Macroglobulin	4.29%	1.80%	2.01%
Apolipoprotein B	1.40%	0.33%	0.44%
IgM	1.38%	0.58%	0.88%
Transferrin	1.23%	1.78%	0.24%
Ceruloplasmin	0.78%	0.73%	0.58%
Haptoglobin	0.70%	0.33%	0.26%
Complement C4	0.66%	0.07%	0.06%
Plasminogen	0.21%	0.04%	0.16%
Antithrombin III	0.16%	0.16%	0.16%
IgE	0.00002%	0.0004%	0.0401%

ALBUMIN ELUTION ANTIGEN PROFILE

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
HSA	97.33%	97.75%	95.54%
Apolipoprotein B	0.60%	0.45%	0.60%
IgM	0.44%	0.24%	0.54%
Complement C3c	0.21%	0.15%	0.12%
IgA	0.21%	0.16%	0.60%
Alpha-2 Macroglobulin	0.20%	0.15%	0.25%
Antithrombin III	0.16%	0.06%	0.09%
Fibrinogen	0.15%	0.30%	0.07%
IgG	0.14%	0.08%	0.21%
Haptoglobin	0.13%	0.26%	0.30%
Transferrin	0.13%	0.35%	0.16%
Ceruloplasmin	0.10%	0.07%	0.15%
A1PI	0.09%	0.17%	0.04%
Complement C4	0.09%	0.03%	1.26%
Plasminogen	0.01%	0.09%	0.06%

ALBUMIN FLOW-THROUGH ANTIGEN PROFILE (STARTING MATERIAL FOR DOWNSTREAM PROCESSING OF A1PI)

Protein	% of Assayed Antigens for Conformance Runs		
	ARC 4L	Hemosol 4L	Hemosol 30L
Transferrin	31.74%	34.92%	25.68%
A1PI	21.42%	23.03%	13.72%
Haptoglobin	16.23%	15.16%	12.93%
Alpha-2 Macroglobulin	8.25%	8.10%	8.28%
IgA	7.65%	6.02%	7.01%
IgM	4.39%	4.46%	4.71%
HSA	3.46%	3.12%	17.28%
Antithrombin III	2.06%	0.74%	2.90%
Apolipoprotein B	1.73%	1.11%	0.73%
Ceruloplasmin	1.00%	1.11%	1.15%
Apolipoprotein A	0.67%	0.74%	4.35%
IgG	0.60%	0.59%	0.60%
Complement C3c	0.60%	0.67%	0.48%
Complement C4	0.20%	0.22%	0.18%

SUMMARY

The initial version of Cascade Process was successfully transferred from the American Red Cross to Hemosol Corporation (4 L scale) followed by scale up to 30 L. The initial criteria for transfer were based on the yield of target protein products (vWF-FVIII; Plasminogen; Fibrinogen; IgG; Albumin; A1PI). Additional process optimization at the American Red Cross has included considerations for potency, recovery and intermediate impurity profiles. Yields of target proteins were comparable between sites and scales. FVIII and vWF potency were improved by scale-up, improved elution conditions and by addition of classic stabilizers to the eluted vWF-FVIII intermediate. Examination of impurity profiles have demonstrated substantial comparability between sites and scale. Subtle differences were noted in the initial transfer, for example, impurities can be removed by the Fibrinogen Capture and prevented from entering the IgG intermediate. Thus, important factors necessary to support the economics of the Cascade Process have been transferred to Hemosol. Additional optimization of critical process parameters related to impurity profiles continues at the American Red Cross.

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