

ABSTRACT

At Aragen, our typical steps to purify and formulate human IgG from transient system cultures up to 2 L involve clarification of harvest, affinity purification using Protein A and a combination of dialysis and concentration using centrifugal filter units for formulation. This platform can take from 5 to 7 days to complete, including assessment of the formulated antibody for purity. Recently introduced technologies such as Protein A membrane devices and TFF systems capable of handling ultrafiltration and diafiltration of samples as low as 1 mL have tremendously decreased the turnaround time down to 3 days. The typical and improved platforms will be compared.

Typical Client's Expression, Purification and Formulation

- Transiently expressed an antibody either in CHO-S or HEK293 cells.
- Affinity purification only.
- Formulate antibody at a specified concentration and buffer, i.e. 5 to 8 mg/mL in PBS, pH 7.4.
- Final product purity >95% by SDS-PAGE and <5% aggregates by SEC-HPLC.
- Endotoxin levels in final product should be <1 EU/mg.

Key Equipment and Consumables

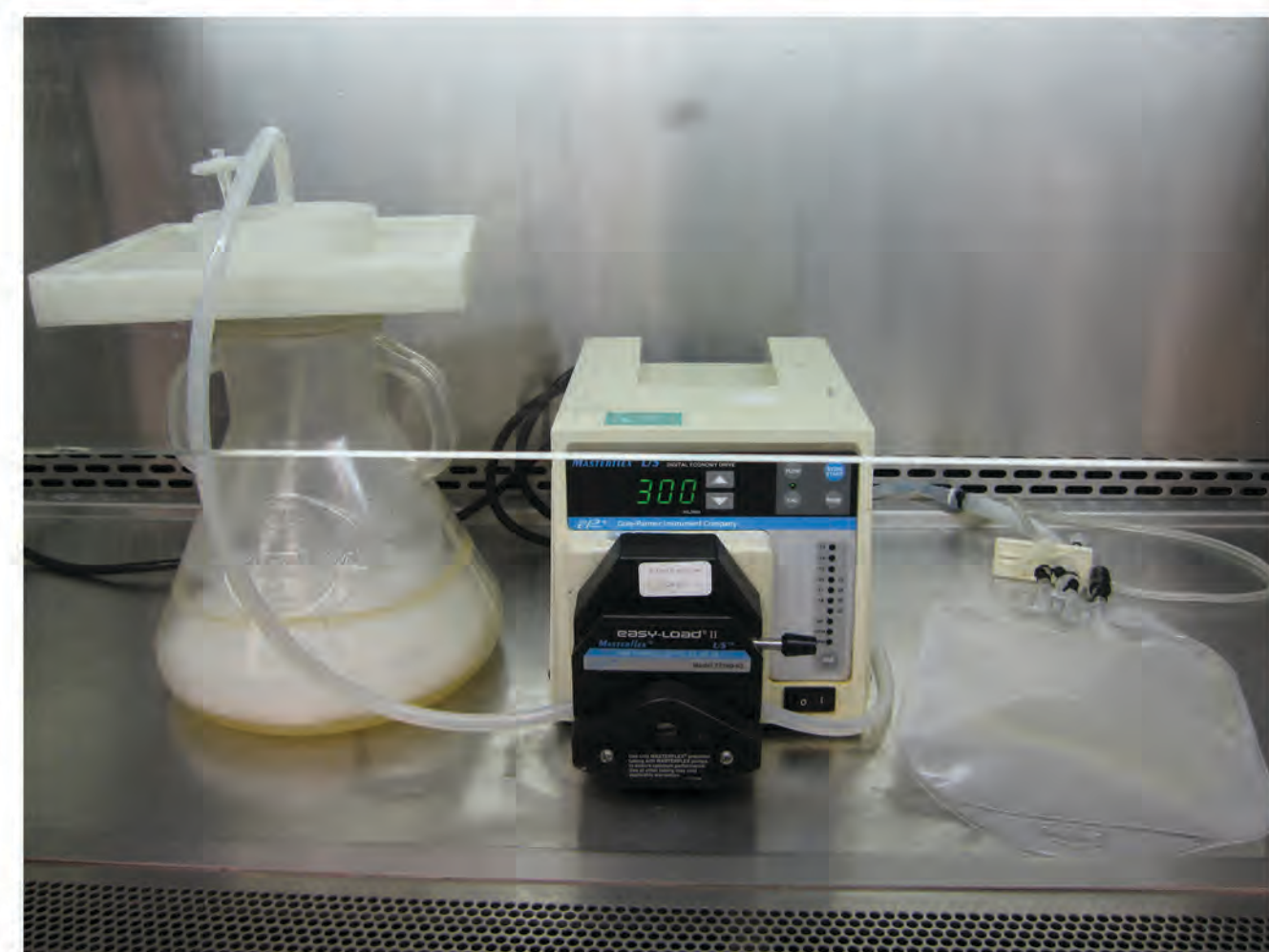
Equipment

1. GE Healthcare ÄKTA pure 150
2. Spectrum Labs KR2i TFF System®
3. Masterflex Pump
4. Trinean DropSense™ with cDrop
5. Agilent 1100 HPLC System
6. Charles River Endosafe®-MCST™
7. ThermoFisher Pierce™ Power Stainer

Consumables

1. GORE™ Protein Capture Device
2. Spectrum Labs MidiKros® Filter Module
3. Thomson Instrument Company Rapid Clear Cap
4. Trinean Slide-40
5. TOSOH Bioscience TSKgel Protein A-5PW
6. Charles River Endosafe®-MCST™ Cassettes
7. ThermoFisher NuPAGE™ 4-12% Bis-Tris Protein Gels

Cell Culture Harvest Clarification Step



- A Rapid™ Clear Cap provides 0.2 µm cell harvest clarification and is installed on top of a Thomson Optimum Growth™ Flask.
- A pump draws the cell culture through the cap and into a sterile disposable bag.
- Recovery after clarification ranges from 90% to 96%.

Processing time includes set-up, clean-up and documentation.

Protein A Purification Step Using the Gore™ Protein Capture Device

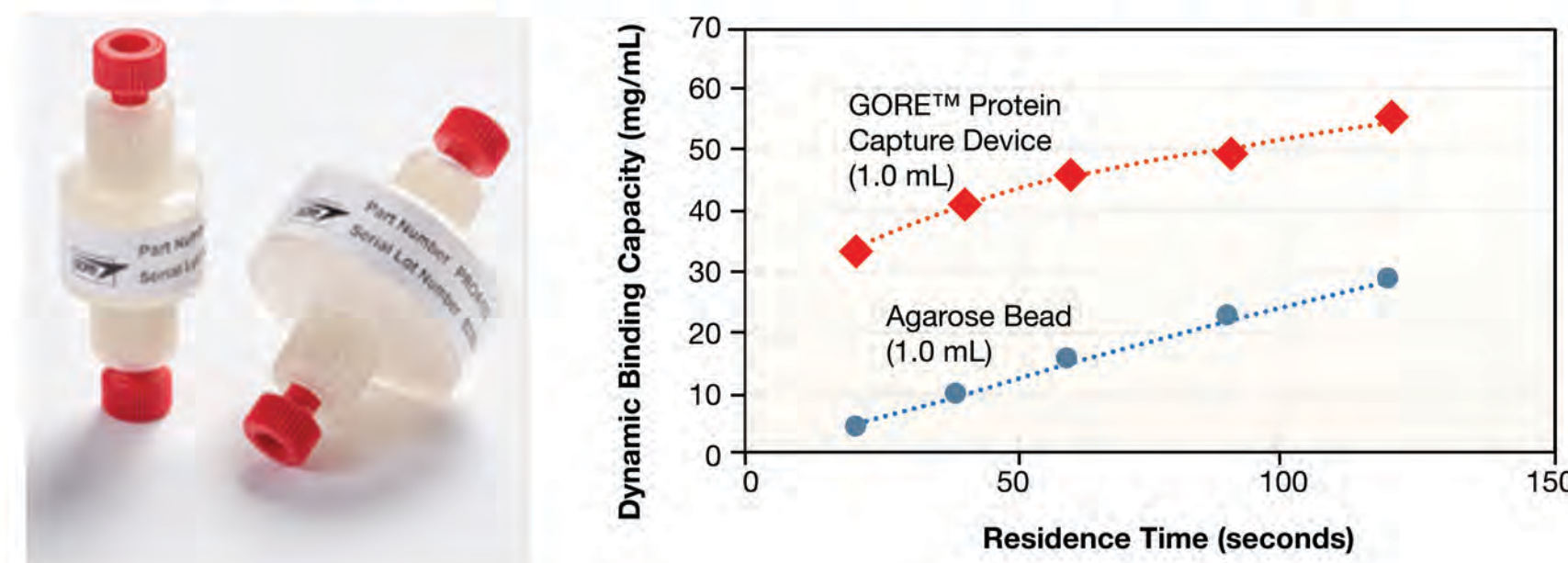
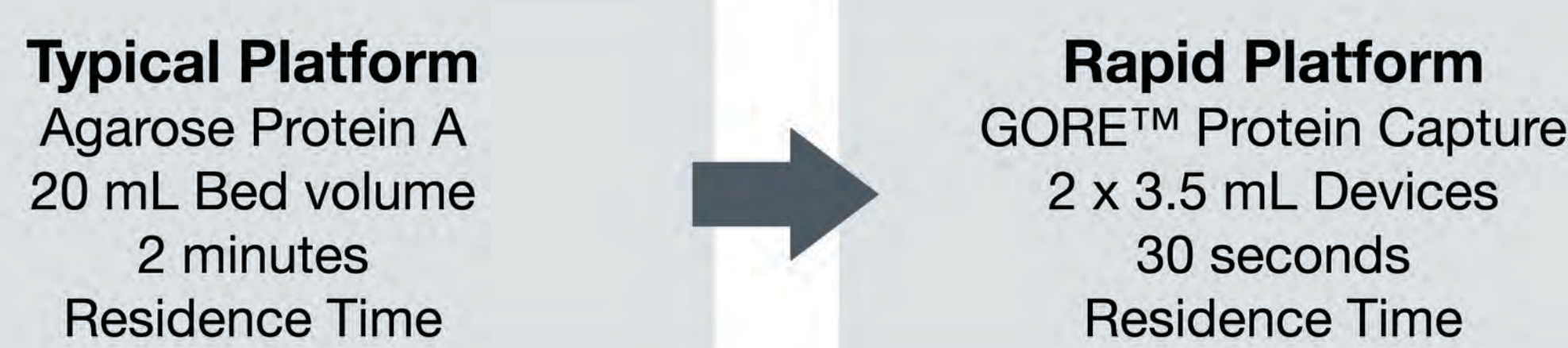
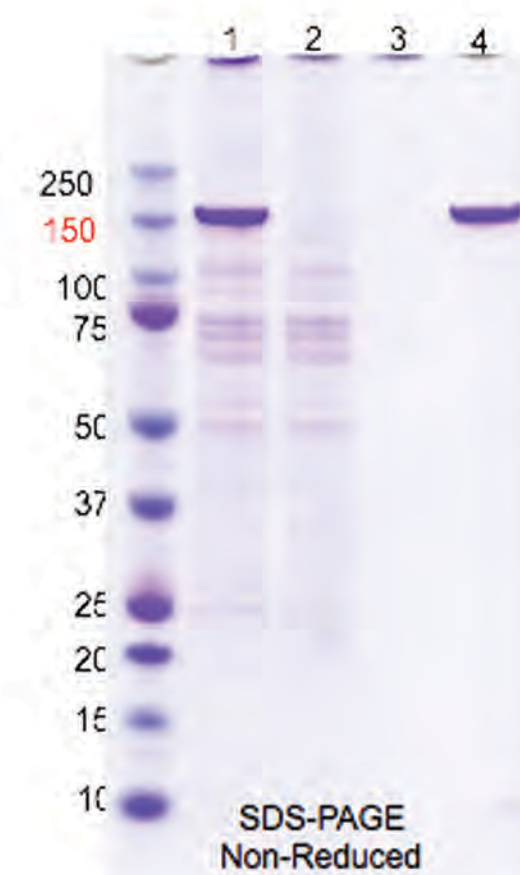


Image and graph provided by W. L. Gore and Associates



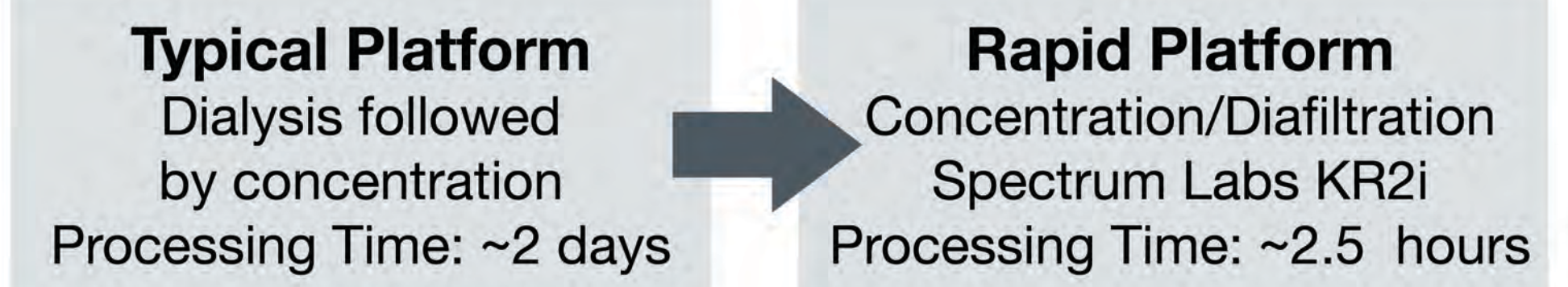
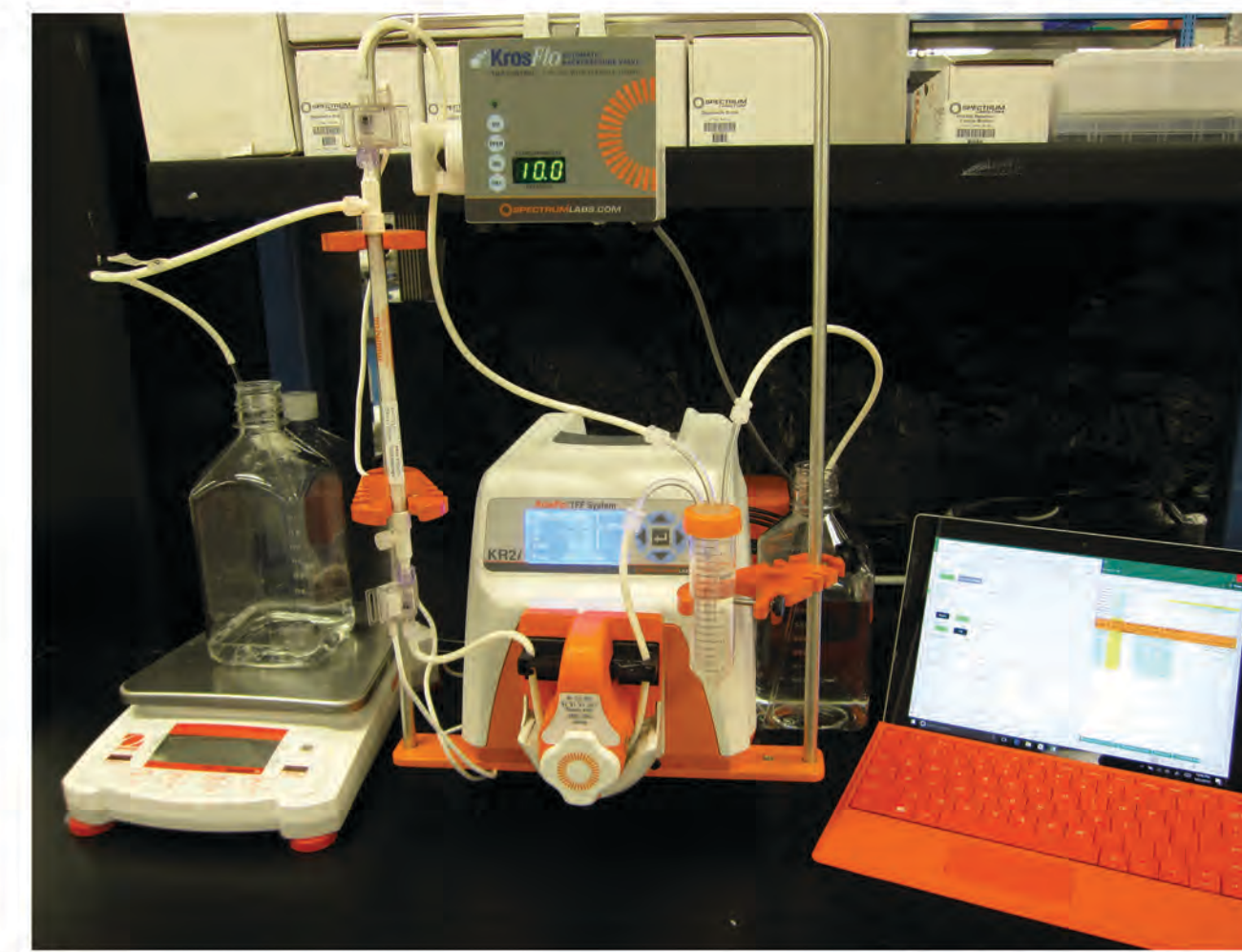
- 93% of antibody (lane 4) was recovered from 1 L of clarified harvest (lane 1) using two GORE™ Protein Capture devices connected in series.
- No antibody was observed in the flow through and wash (lanes 3 & 4).



	Processing Time	Titer	Elution Concentration	Concentration Factor
GORE™ Protein Capture	~2.5 hours	174 mg/L	6.4 mg/mL	37.6
Agarose Protein A	~5 hours		3.8 mg/mL	22.4

Processing time includes set-up, clean-up and documentation.

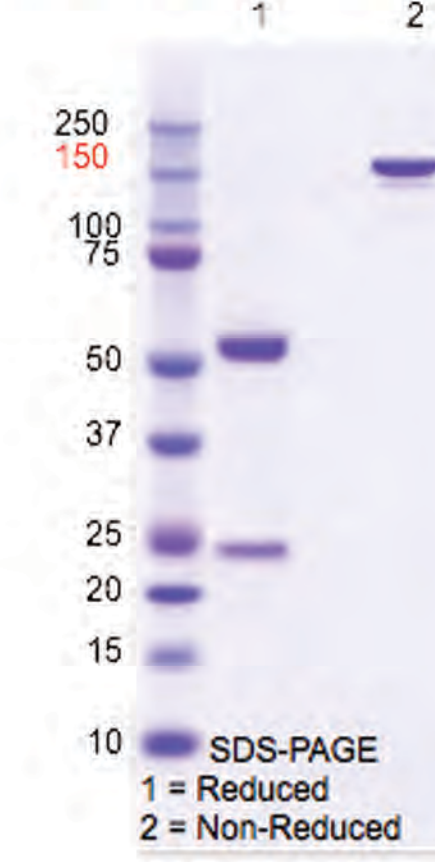
Formulation Step



- Since the concentration of the elution from the GORE™ Protein Capture Device was within 5 to 8 mg/mL, a concentration step was eliminated.
- A diafiltration against 10 diavolumes of PBS, pH 7.4 was performed.

Processing time includes buffer preparation, set-up, CIP, clean-up and documentation.

Final Product Assessment – SDS-PAGE Staining



- Traditional staining of SDS-PAGE gel requires an hour to overnight staining followed by destaining steps to achieve desired staining results.
- Use of Pierce™ Power Stainer achieves similar or better staining results in 10 minutes.

Processing time includes buffer preparation, set-up, clean-up and documentation.

Summary

Step Name	Step Recovery (%)	Endotoxin (EU/mg)	Purity SDS-PAGE (%)	Aggregates SEC-HPLC (%)
Clarified Harvest	96	0.429	Not determined	
Protein A Elution	93	0.003	>95	<1
Final Product	90	0.009	97	<1

- The overall recovery and purity of the antibody achieved from the new rapid platform are similar to our existing typical platform.
- The GORE™ Protein Capture device and the Spectrum Labs KR2i TFF System® were key technologies in reducing turnaround time.
- A similar rapid approach has been implemented on transient or stable cell cultures from 5 L to 25 L with a turnaround time of 4 days.

Step Name	Typical Platform	Rapid Platform
Harvest Clarification	Day 1 AM	Day 1 AM
Protein A Purification	Day 1 PM	Day 1 PM
Formulation	Day 2 & 3	Day 2 AM
Final Product Assessment	Day 4 & 5	Day 2 PM
Final Product Shipping	Day 6 or 7	Day 3 or 4

- The table above compares the schedule of the two platforms.
- In the rapid platform, the harvest clarification, Protein A purification and formulation steps can be completed on the same day and the final product shipped the following day.

Additional Protein Characterization Services

- Aragen offers the following high resolution analytical services to further characterize proteins such as molecular weight determination, post-translation modifications, pI determination, N-glycan profiling and thermal stability:
 - Capillary SDS, capillary isoelectric focusing (cIEF), capillary zone electrophoresis (CZE) and capillary-based N-Glycan profiling using SCIEX PA-800 Plus Pharmaceutical Analysis System
 - Thermal stability using Unchained Labs UNcle
 - Mass Spectrometry – COMING SOON
- Aragen also offers functional characterization assays to complement analytical services above.
 - Cell-based assays
 - Protein-protein interaction and antibody-antigen affinity measurements using FortéBio Octet Red