One Resin, Multiple Products: A Potential Solution for Supply Constraints

Ekta Mahajan
Pharma Technical Regulatory
Genentech, South San Francisco

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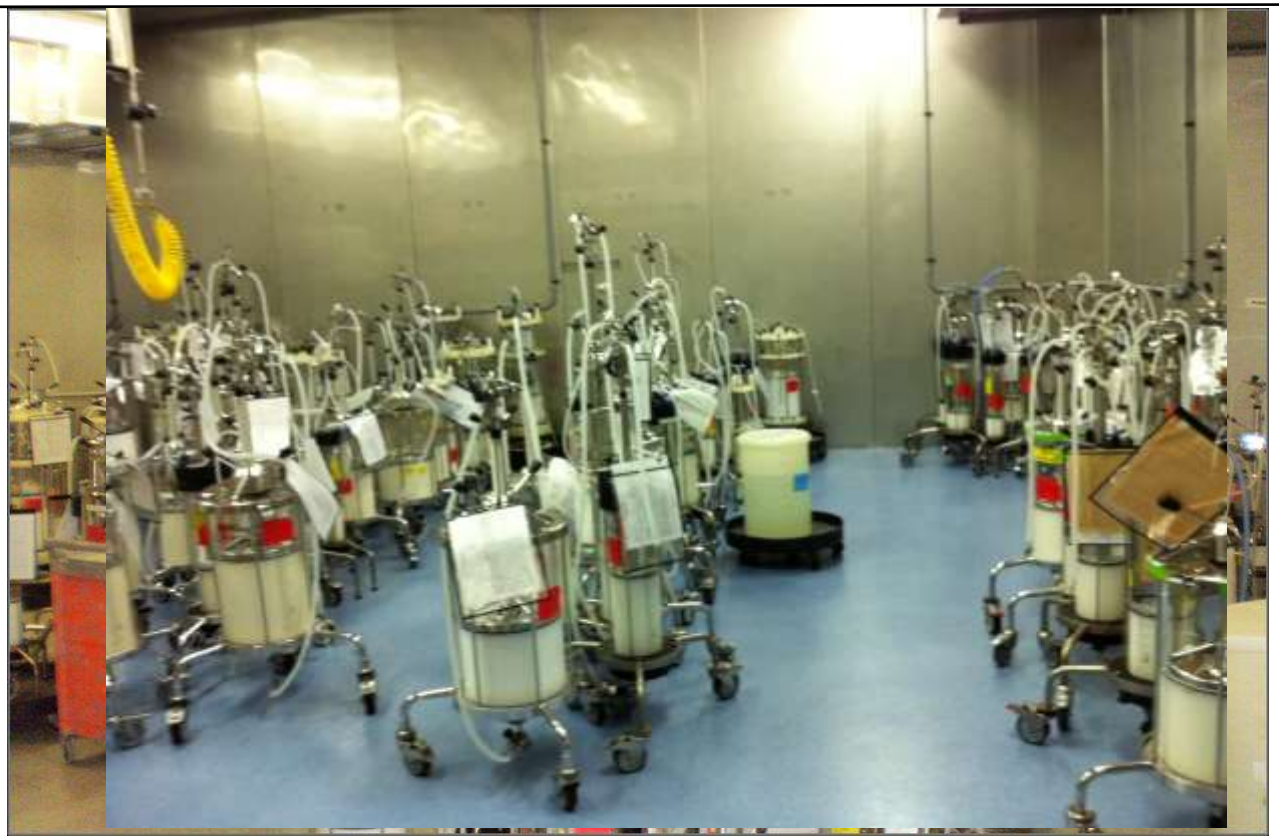
Pharma Technical Regulatory
Passion. Trust. Relationships.

Motivation



- Cost Savings
- Increase flexibility for process optimization
- Sustainability
- Improved Ergonomics
- *Manage Raw Material Supply during catastrophic events, e.g. pandemic*

Project Goal

Enable Mab Select SuRe™ columns to be used for multiple CHO products in pilot plant for tox supply (2009)

- Demonstrate low product carryover below a safe acceptable carryover level with improved column cleaning between two MAbs

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Safety Assessment by Toxicology group

Risk Assessment of IgG in 5 commercial Genentech MAbs

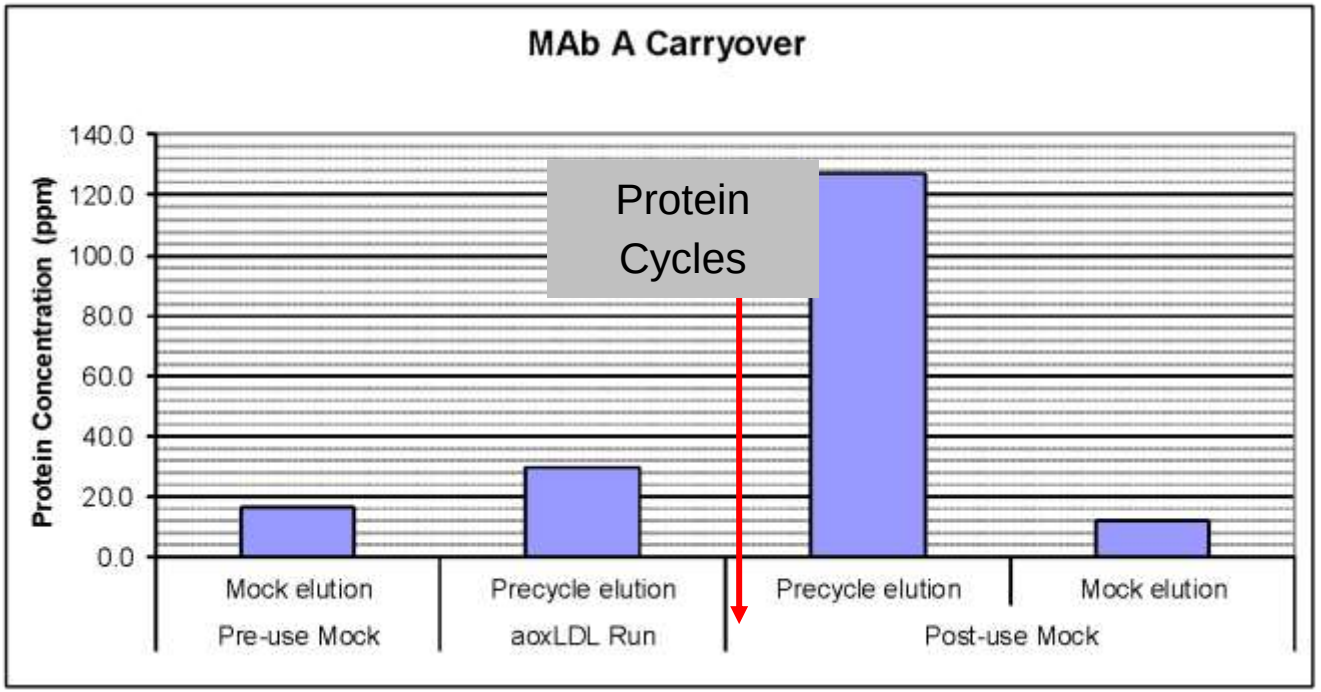
- Determination of Acceptable daily exposure (ADE) of IgG
- Evaluation of amount of IgG administered per dose (Estimated Daily Intake- EDI) of commercial Mabs
- Calculation of safety margin based on ratio of ADE to EDI

Safety margins are very high even for worst case scenario

Highest value of MAb carryover allowed = 100 ppm

Baseline Process: Protein Carryover

- MAb A
- Additional Cleaning -No



Mock Elution results indicate the need for additional cleaning

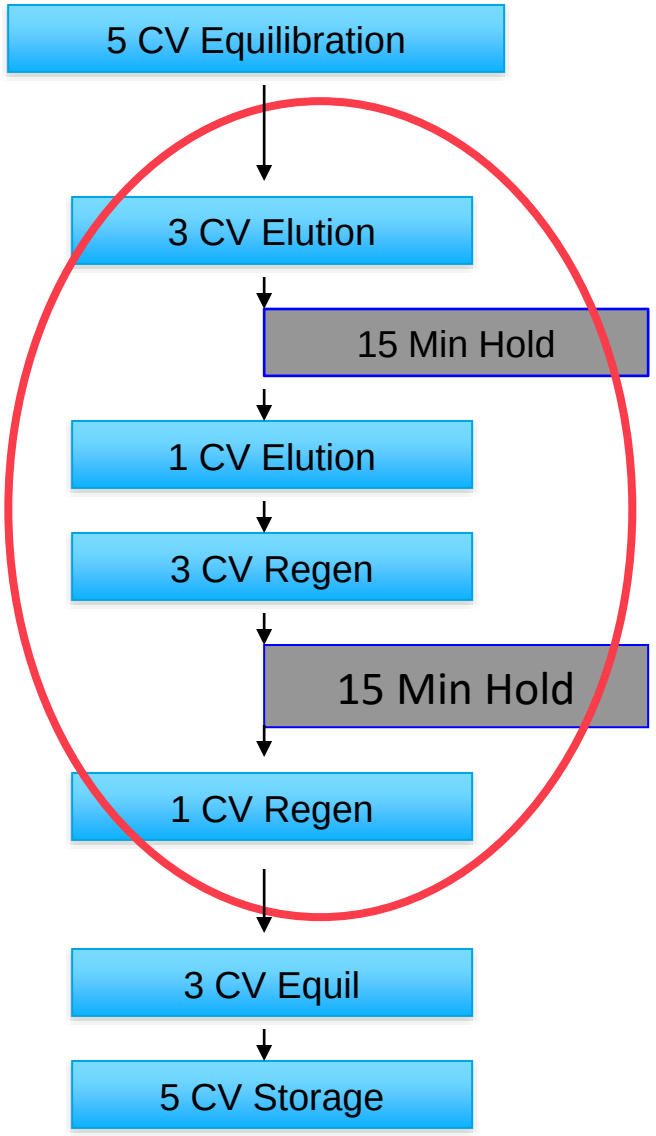
Lab Scale 5mL CV. 30g/L Load limit, Elution protein concentration: 10.51g/L
All samples are pooled.

Cleaning Approach

- **Static Holds: Provide extra residence time in a particular buffer without using extra buffer**
- **pH Cycling: Pulse back and forth between a basic buffer and an acidic buffer**

Buffer	Composition	pH	Function
Elution	0.15 M acetic Acid	2.9	Elutes bound IgG from protein A complex
Regeneration	0.1 N Sodium Hydroxide	12	Solubilizes lipids, proteins, nucleic acids; Denatures and cleaves the protein into small fragments; destroys endotoxin

Resin CIP protocol (time reqd~4.5 hours @ 20CV/hr)



Elution 0.15M Acetic Acid

Regen 0.1 N Sodium Hydroxide

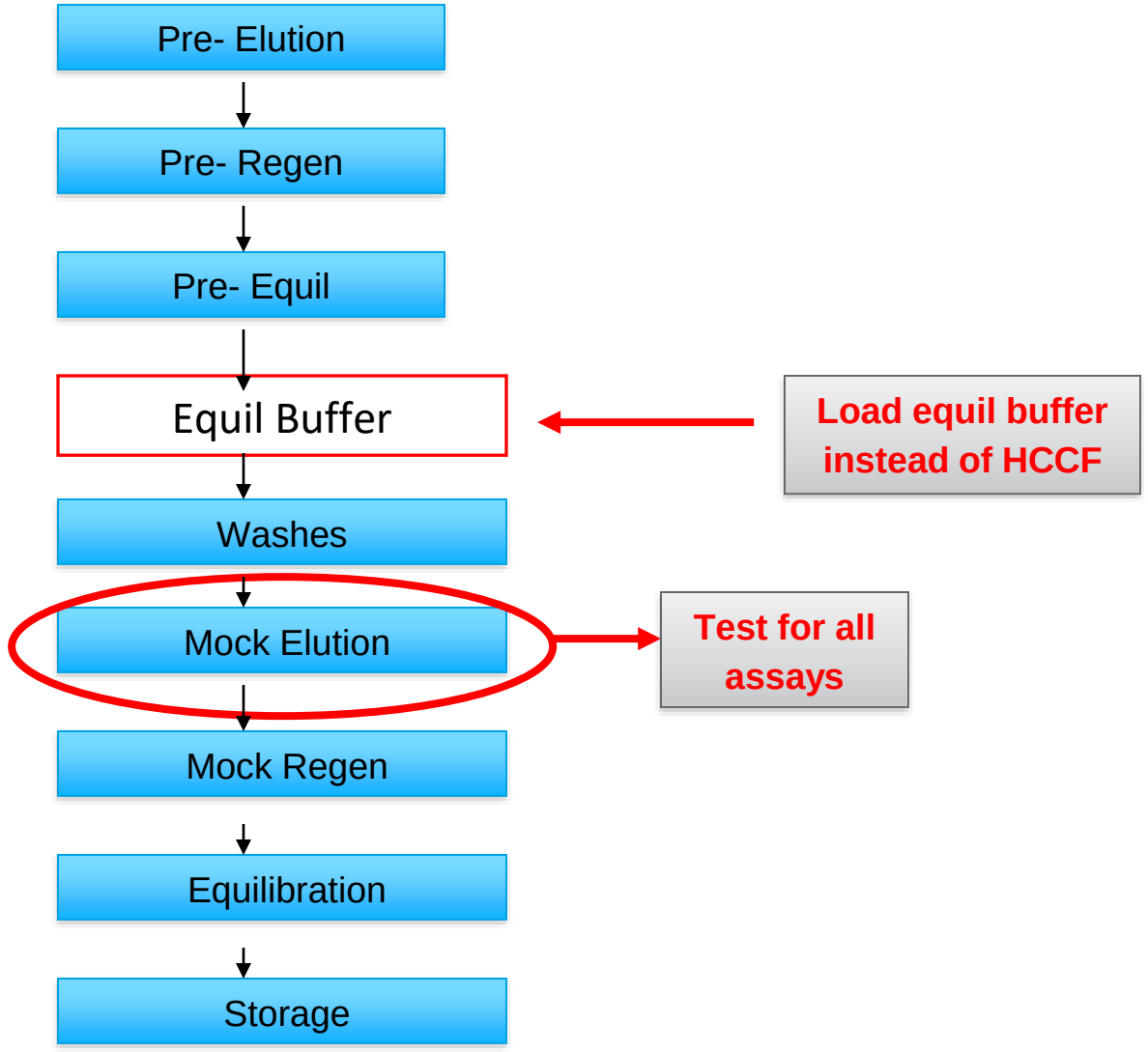
6x

Collect samples after 15 min hold

Analyze for carryover

CV = Column Volume

Mock Run Protocol (to verify protein carryover)

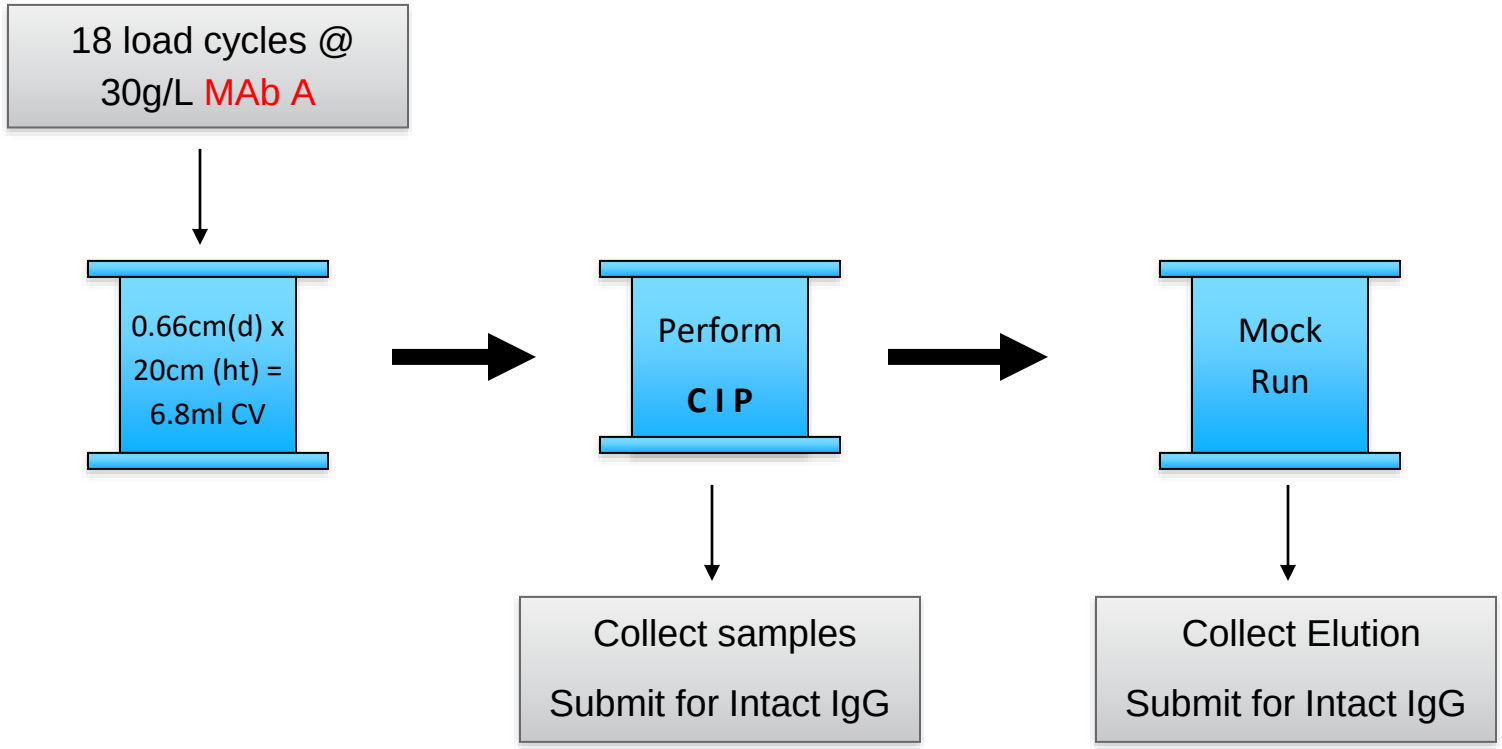


Assays

Assay Name	Comment
Intact Human Iggy ELISA	Binds to both Fab and Fc regions
Human Fc ELISA	Binds to only Fc region
CZE LIF- Total protein*	Any protein
CHOP	Chinese Hamster Ovary Proteins
Leached protein A	High leaching can cause loss in binding capacity

* Assay used for routine monitoring

Experimental Protocol for assessing MAb carryover



- Samples conditioned with low conc. of detergent to prevent protein sample sticking to the wall of the container
- Samples are adjusted to neutral pH

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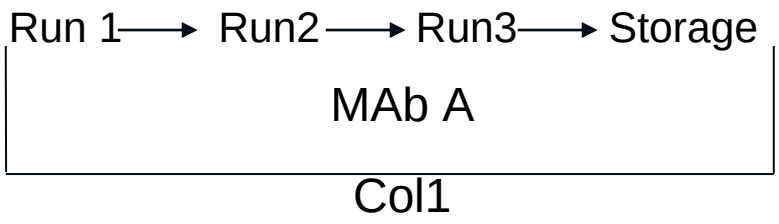
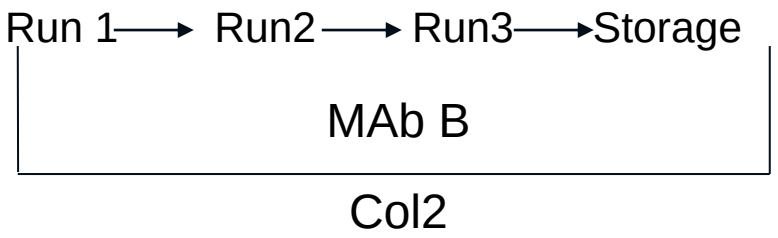
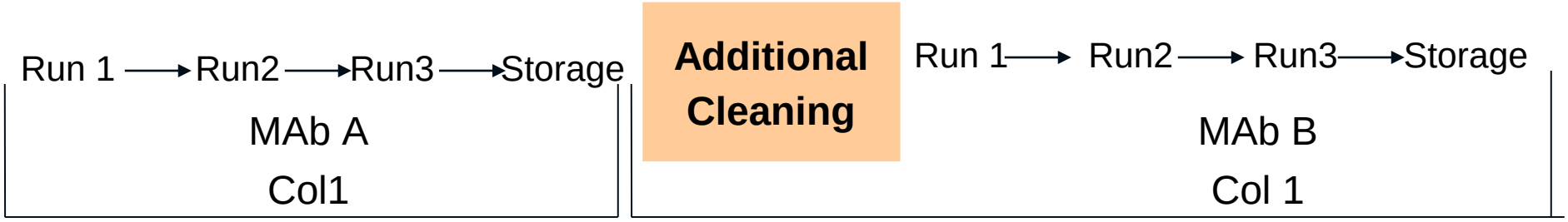
Process

Baseline process



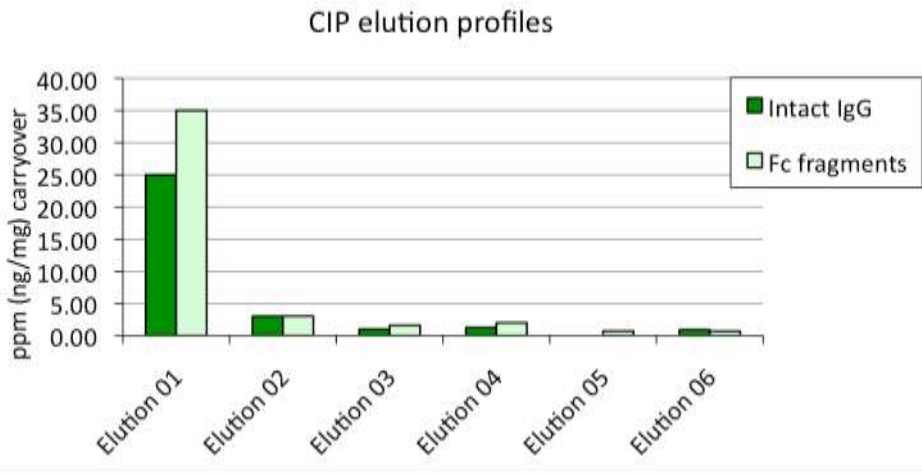


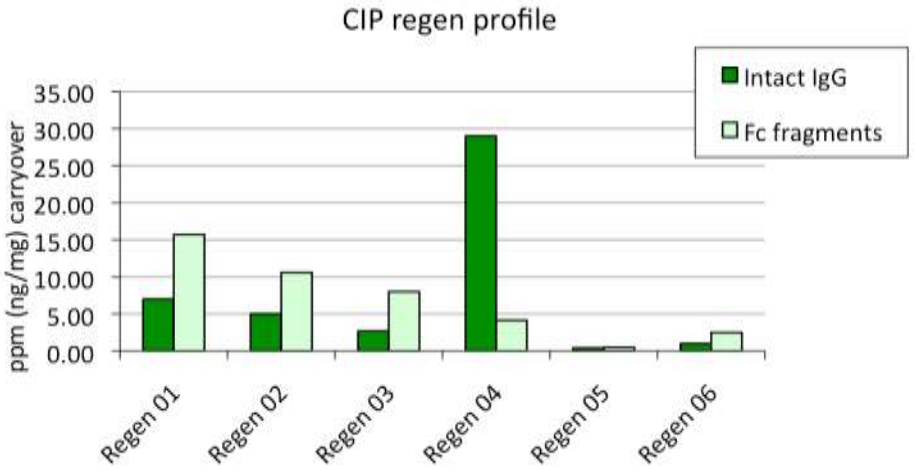
Proposed Process

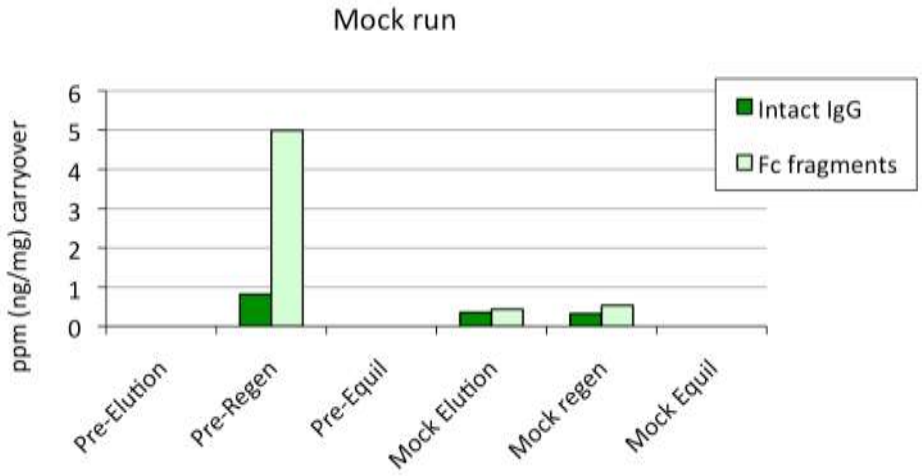


MAb C at lab scale

- Scale = 6.8 ml
- System- AKTA explorer 100
- Load – 18 cycles @ 30 g/L
- Process- purification platform process



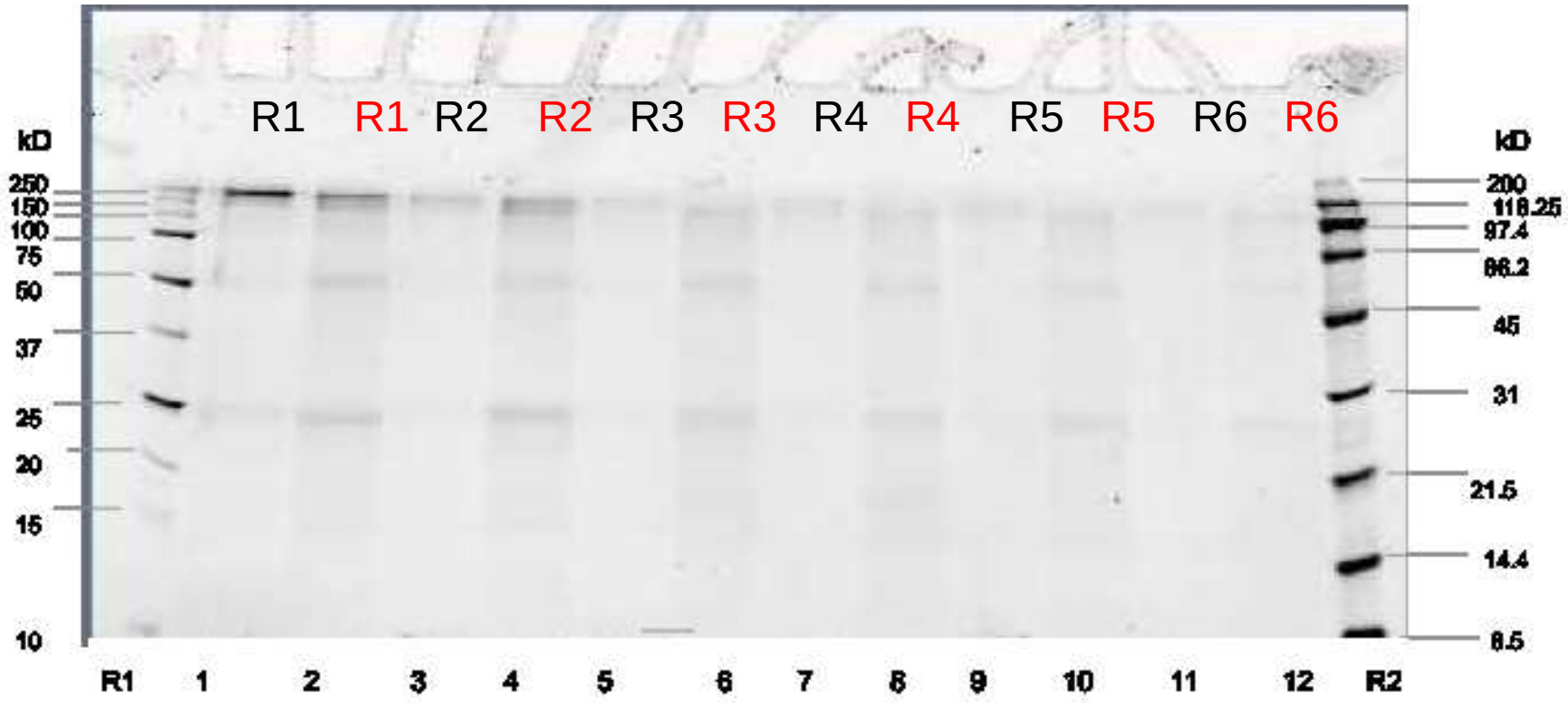




6 cycles of pH cycling and 15 min hold time shows < 1 ppm of contaminant antibody carryover

18% Tris-HCl Gel (MAb C) to see fragments

- Regen Samples -concentrated 25x
- Lanes marked in Red contain samples after the 15 min static hold



- Band Intensity decreases from Cleaning cycle 1 to cycle 6
- Fragments are cleaned out with each cleaning cycle

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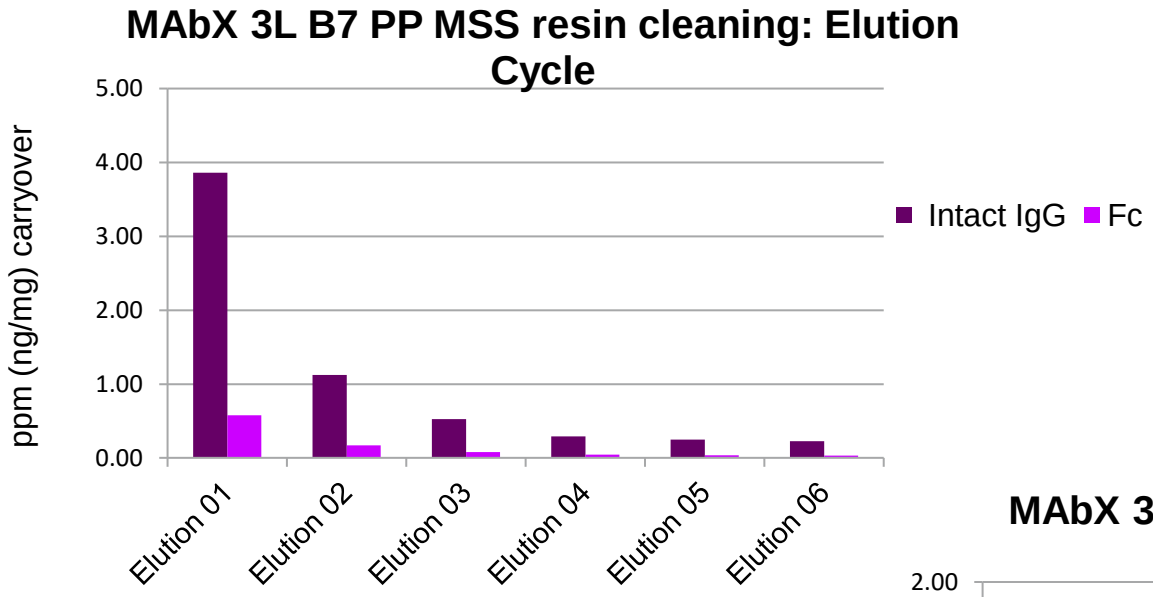
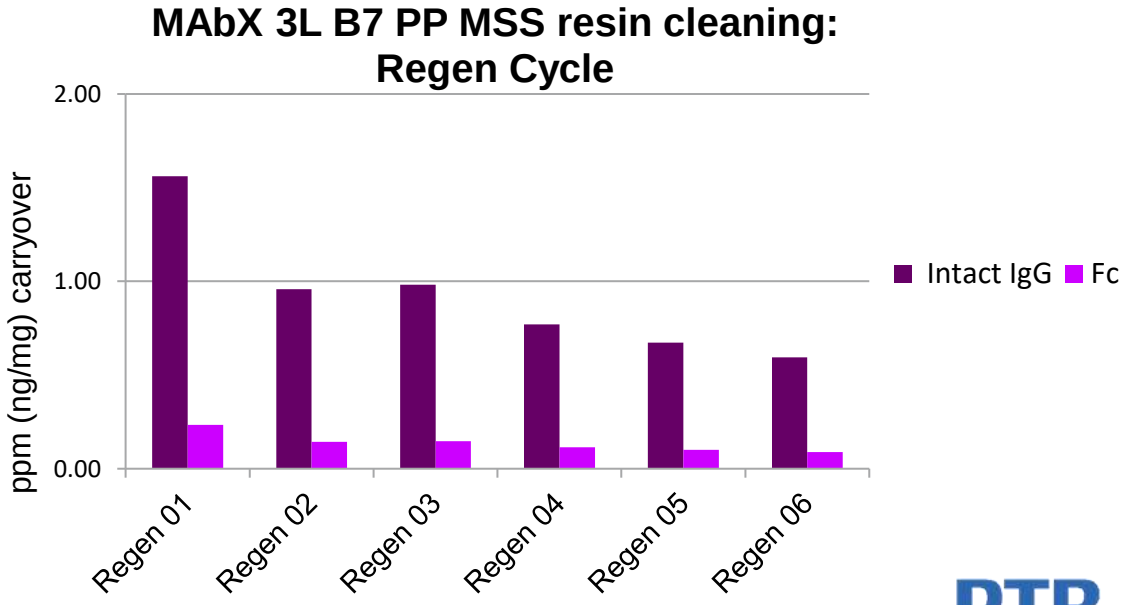
MAb X CIP resin cleaning at pilot scale





MAb Y 6.28L CIP Resin Cleaning Data

Sample Name	LpA	CZE-LIF	CHOP	CHO DNA
Units	ppm	ug/ml	ppm	pg/ml
Elution 02	6.24	0.35	0.74	<1.00
Elution03	2.72	0.28	0.63	<1.00
Elution04	3.08	0.35	<0.5	<1.00
Elution05	3.01	0.34	<0.5	<1.00
Elution 06	3.03	<0.25	<0.5	<1.00
Regen 06	23.7204	>2.5	1.1	27.05
Pre-Elution	1.4	<0.25	<0.5	1.12
Mock Elution	<1	<0.25	<0.5	<1.0

After 153 multi product load cycles on 6.28L MSS column with CIP resin cleaning in between products

PP Runs	Step Operation	% Yield	CHOP (ppm)	% aggregate	LProA ng/mg	CHO DNA pg/mg
400-L PP R1 (IND Tox)	HCCF		1,161,000			59,700
	Protein A	92	12,100	3.17	8	62
	Poros 50HS[1]	97	370	1.27	4	<0.5
	QSFF	99	< 3	1.27	<3	<0.5
	UFDF	**98**	**1**	**1.43**	**2**	**<0.5**
100-L PP R2	HCCF		2,591,520			
	Protein A	101	4,290	2.6	12	80
	Poros 50HS[2]	99	43	0.6	<10	<0.5
	QSFF	91	<1	0.6	<4	<0.5
	UFDF	**97**	**<1**	**0.6**	**2**	**<0.5**
400-L PP R3	HCCF		1,115,320	2.2		
	Protein A	101	10,870	1.6	10	
	Poros 50HS[1]	99	290	1.6	2	<0.5

Process Step	CHOP (ppm)		Lch pA (ng/mg)		DNA (pg/mg)	
	Lab	PP	Lab	PP	Lab	PP
MabSelect SuRe	12,000	4000 - 12000	15	8 - 12	960	62 - 80

Data by Marc Wong

Carryover Mock Elution measure by CZE-LIF

Molecule Name	Scale	CV	Total Protein (ug/ml)
MAb 1	Lab	6.8ml	<0.25
MAb 2	Lab	6.8ml	0.46
MAb 3	Pilot	3.0L	<0.25
MAb 4	Pilot	6.28L	<0.25
MAb 5	Pilot	6.28L	<0.25
MAb 6	Pilot	3.23L	<0.25
MAb 7	Pilot	6.28L	<0.25
MAb 8	Pilot	1.73L	0.25

- *Number of Molecules that have used MPUR since implementation : 22*

Summary

- Successfully implemented multi-product ProA resin (Mab Select SuRe™) use in pilot plants
- Data from lab as well as pilot scale experiments suggest that the CIP resin cleaning protocol with 6 cycles of 0.15M Acetic Acid and 0.1 N Sodium Hydroxide with 15 min hold time cleans the Mob Select Sure resin to <=5 ppm
- Actual Savings achieved since Implementation: ~10MM over 10 years

What is Next?

Can this approach be applied to Other resins?

Yes: Similar Approach as ProA resin

Multi-product resin reuse has been implemented successfully for 8 resins (including MSS)

No variations in product quality for MAb purified using MPUR or naïve resin

What about cGMP Manufacturing?

Regulatory Considerations

- **Patient Safety**
  - No impact to product quality
  - Product carryover negligible or within acceptable limits
  - No impact to product safety profile
- **Supply to Patients**
  - Uninterrupted supply to patients
- **Challenges**
  - Potential product cross-contamination
    - Strong binding of protein to resin
  - Global acceptance of the approach

Potential Regulatory Strategy Considerations

- Stage of molecule for first implementation
- Manufacture using both MPUR and naive resin to mitigate filing/approval delays
- Definition of worst case molecule
- Same or different strategies for Early Stage Clinical vs. Late Stage Clinical vs. Commercial

Acknowledgements

Resin Reuse Team

Yolanda Ng
Greg Embrose
Kapil Kothari
Jay Webber
Bijee George
Jimmy Sughara
Ron Massicotte
Aurelia Santa
Deborah O'Connor
Michelle Butler
Amy Lim
Marc Wong
Aileen Sanchez

AAT group
B7 pilot plant
Julie Nishihara
Rahul Parikh

Management

Terry Hudson
Paul Bezy
Stephen Gomez
Paul Motchnik
Phil Lester
Greg Blank
David Michaels
Debra Dunlap
Kevin Ford
Susan Cornelius
Julie Nishihara
Rahul Parikh

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Kavita Mistry
Sarah Kennett