The quest for a generic IgG purification process

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Conclusions

- ***A generic IgG purification process is not a practical goal for two reasons:***
- Diversity
- Dosage

- Possible exceptions

An adaptable platform

Conc./microfiltr.

Protein A

Virus inactivation

Cation exchange or

Hydrophobic interaction or

Ceramic hydroxyapatite

Virus filtration

Anion exchange

Conc./diafiltr.

Strengths, anion exchange

- Good removal of host cell proteins
- Good removal of leached protein A
- Excellent removal of DNA, endotoxin, retrovirus, even in flow-through mode
- IgG fully soluble under loading conditions

Limitations, anion exchange

- Capacity compromised by high product pI
- Seldom better than 10mg/mL with conventional exchangers, often half that or less
- Compensate with:
  - higher pH (enhances deamidation)
  - lower conductivity (may reduce pH control)
  - UNOsphere™ Q (capacity 3-5x conventional)
- Irreversible binding of DNA on all Q media

Strengths, cation exchange

- Very good removal of host cell proteins
- Very good removal of leached protein A
- Fair-good removal of DNA & endotoxin
- Good capacity, even on conventional exchangers.
- 3-5x greater capacity on UNOsphere™ S

Limitations, cation exchange

- Most IgGs partially insoluble under conditions required to support good binding capacity
- Compensate with on-line dilution
- Antibodies form stable ionic complexes with DNA, endotoxin, and other contaminants, and carry them through the method.
- Compensate by raising pH and/or conductivity on high capacity exchangers. This reduces capacity but gives better performance and reproducibility.
- Corrosive buffers

Strengths, ceramic hydroxyapatite

- Second only to protein A for overall purification performance
- Very good removal of host cell proteins
- Very good removal of leached protein A
- Very good removal of DNA, endotoxin
- Excellent removal of aggregates
- Excellent removal of metal contaminants
  - Improves product homogeneity
  - Improves product stability
- IgG fully soluble under loading conditions

Limitations, ceramic hydroxyapatite

- Media unstable below pH 6.25, citrate, EDTA
- Non-phosphate buffers cause slow degradation
- Media scavenges metal contaminants which displace calcium, cause discoloration, and may alter performance.
- Ceramic composition requires care during packing and unpacking

Strengths, hydrophobic interaction

- Good removal of host cell proteins
- Fair removal of leached protein A
- Fair-good removal of aggregates
- Excellent removal of DNA, endotoxin

Limitations, hydrophobic interaction

- Compromise: stronger ligands give better capacity and do so at lower salt concentration but with lower recovery and higher risk of aggregation.
- Weaker ligands require very high salt concentrations to achieve good capacity. Give good recovery, little or no aggregation, but elute in high salt and require on-line dilution to load the sample.
- Concentrated salts are corrosive and “encrustive”
- Ammonium and phosphates a disposal issue
- Citrate viscous, sodium sulfate limited solubility

A template approach

- The key to identifying the most effective combination is to evaluate each of the options in the context of a complete fully integrated purification process.
- Preliminary evaluation with 1mL columns
- **Special qualification:** a protein A wash at 1.0M NaCl, with 5-10mM EDTA will substantially improve overall purification performance.

Template 1, step 1&2

- **Protein A elution buffer:** Minimum conductivity
- **Cation exchange:** UNOsphere S
- **Buffers:** A 0.05M MES, pH 6.0; B = A + 1.0 M NaCl
- **Fractionation:**
- Flow rate: 300-600 cm/hr
- Equilibrate: buffer A until column effluent is pH 6.0
- Load sample: volume equivalent to 20 mg IgG per mL of gel, load by on-line dilution 1 part sample to 9 parts buffer A. (1:4 if sample conductivity is fairly low)
- Wash: 5CV buffer A
- Elute: 15 CV linear gradient to 30% buffer B
- Strip: 5CV buffer B
- **Variations:** Use Na-citrate in place of NaCl to reduce corrosivity.

Template 1, step 3

- **Anion exchange:** UNOsphere Q
- **Sample preparation**: titrate pH to 8.0, dilute until conductivity is ~5 mS.
- **Buffers**: A: 0.05M Tris, pH 8.0; B= A + 1M NaCl
- **Fractionation**:
- Flow rate: 300-600 cm/hr
- Equilibrate: buffer A until column effluent is pH 8.0
- Load sample: volume equivalent to 20 mg IgG/mL
- Wash: 5CV buffer A
- Elute: 15 CV linear gradient to 30% buffer B
- Strip: 5CV buffer B

Template 2, step 2

- **HIC,** Phenyl
- **Sample preparation:** titrate pH to 7.0. Immediately prior to sample application, dilute 1:1 with 2X concentration of buffer A
- **Buffers: A:** 0.05M Na phosphate, 1.0M ammonium sulfate, 7.0
  B: 0.05M Na phosphate, 2.0M urea, pH 7.0
- **Fractionation:**
- Flow rate: per gel manufacturer's recommendation
- Equilibrate: 5CV buffer A
- Load sample: volume equivalent to 10 mg IgG per mL of gel
- Wash: 5CV buffer A
- Elute: 15 CV linear gradient to buffer B
- Strip: 5CV buffer B
- **Comments:** urea improves recovery and reduces peak volume

Template 3, step 2

- **Protein A elution buffer:** No citrate/EDTA, no/low phosphate
- **Hydroxyapatite**: CHT™ type I 40 micron
- **Sample preparation:** titrate pH to 6.5, dilute to ~5 mS with buffer A (dilution optional, improves capacity)
- **Buffers:** A: 0.05M bis-tris propane, pH 6.5; B: 0.5 M NaPO4, pH 6.5
- **Fractionation**:
- Flow rate 300-600cm/hr
- Equilibrate: buffer A until column effluent is pH 6.5
- Load sample: volume equivalent to 20 mg IgG per mL of gel
- Wash: 5CV buffer A
- Elute: 15 CV linear gradient to 60% buffer B
- Strip: 5CV 100% buffer B

What to expect

- All of the templates will work with most antibodies but usually one will emerge as conspicuously superior for a particular antibody, and/or better suited to your manufacturing preferences.
- The “best” one will usually be adequate for producing material for investigation and toxicology studies with modest modification.

Is a 2-step platform feasible?

- **The first law of process development:** The simplicity of the final process is inversely proportional to the amount of work required to develop it.
- A 2-step process can work, but success is highly dependent on the antibody.
- Biggest challenge: virus removal
- Next biggest challenge: aggregate and leached protein A removal
- Best prospect: protein A followed by CHT

What's next

- Evaluate buffer options
- Evaluate media options
- Flow-through vs bind/elute mode
- Refine loading conditions
- Refine elution conditions
- Determine capacity

Buffer options

- An example:
- Cation exchange Chromatography: 0.05M MES vs 0.05M citrate, pH 5.5
- Citrate is USP and less expensive, but…
- MES is zwitterionic. No conductivity (conductivity from NaOH titration only).
- Low conductivity supports higher capacity.
- Higher capacity supports smaller columns.
- Smaller columns use less buffer.
- MES is more economical

Flow-through elution

- **Benefits**
- Simpler buffer requirements
- Lower net buffer volume
- Minimal sample dilution

- **Liabilities**
- No sample concentration
- One-sided fractionation
- Sacrifices protein A removal
- Likely to be sample-volume sensitive

Media: bind/elute vs flow-through

- **Bind/elute**
- Use high capacity chromatography media

- **Flow-through**
- Conventional chromatography media or filtration-based media can be used, ***but***…
- High capacity chromatography media will allow use of the smallest media volume with the result of less buffer consumption, less product dilution, and faster cycle time.

Flow-through, media selection

- **Why UNOsphere Q?**
- Poly-Q ligand -C-Q-C-Q-C-Q…
- Zipper configuration provides maximum complementarity to DNA PO4 zipper.
- Exceptional avidity and capacity for DNA.
- Capacity 3-5x conventional Q media. Supports minimal column volume, minimal buffer volume, shortest process time.
- Parallel capability with LPS, retrovirus
- Low backpressure, high flow rates.

Flow-through, conditions

- **To determine flow-through conditions**
- Run binding conditions from the template.
- Determine salt concentration at which antibody elutes. Apply sample at that concentration, or
- Determine pH modification necessary to elute antibody. Apply sample at that pH.
- Refine either as necessary.

Elution conditions

- **Linear gradients**
- Better purity
- Better process control
- Better process monitoring
- Better reproducibility
- Better conformance with new regulatory initiatives

- **Step gradients**
- Mechanically simpler
- Higher product concentration