# Application of High Throughput Resin Tips for Chromatography Development

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## Abstract

In recent years, a drive to expedite drug development timelines has led biologics process development to rely increasingly on high-throughput screening. In order to continue to improve throughput, methods have been developed using resin-filled pipet tips on a liquid handling system with a multi-channel arm. The case study that will be presented is establishment of an affinity chromatography purification method for various monoclonal antibodies, which allows for simultaneous purification of up to 96 samples in a single day. Method optimization required an understanding of how the system differed from a traditional column and included evaluations of different flow rates, contact times, and tip geometries. Comparisons with other high-throughput methods were performed to demonstrate equivalent product quality and sufficient method reliability. Additionally, the methods were compared to traditional columns to show how they differed in terms of yield and pool concentration.

## Background

### Why is high-throughput Protein A chromatography desirable?

Removal of media components and some host cell proteins is essential in order to obtain useful analytical data for monoclonal antibodies

Development for biological upstream processes is increasingly relying on high-throughput systems that generate large numbers of samples

Target outputs: ≥ 3 mg recovered ≥ 1 mg/mL concentration

A Tecan Freedom EVO 200 has a multi-channel arm (MCA) with 384 pipetting channels, adaptable to 96 for disposable tips.





*Resin-packed tips (PhyTip columns) can be used – but none were available for the MCA that contained enough resin for purification of 3 mg*

## Collaboration

PhyNexus developed a PhyTip column compatible with the MCA with a sufficient amount of resin (160 µL)

In the initial prototype, the resin was loosely retained between two screens, leaving room for the resin to expand along with the fluid during pipetting

Merck developed a method for the Tecan and tested different tip prototypes

**Thank you to PhyNexus Inc., especially Lee Hoang, for all of their hard work and support!**

## Method and Tip Development

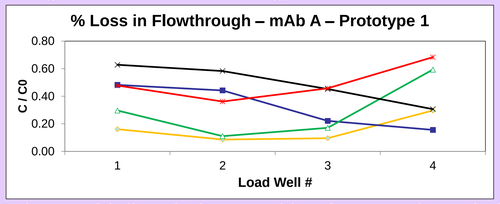
### Method developed by Merck, balancing throughput, recovery, and deck space:

Method lasts 4-8 hours depending on titer of samples

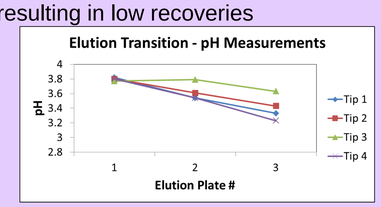
- Equilibration
- Sample Load
- Wash 1 (No Salt)
- Wash 2 (High Salt)
- Wash 3 (No Salt)
- Low pH Elution

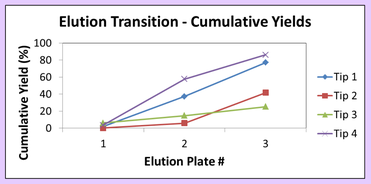
### Initial prototype produced inconsistent results:

As liquid moved through the bed, resin could clump together, or air pockets could appear, leading to inconsistent binding



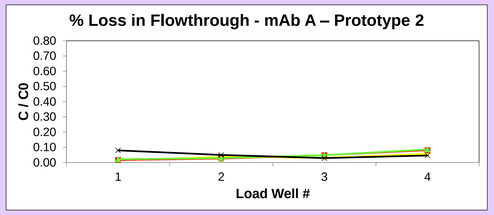

Residual liquid in tips could disturb the transition from wash to elution, resulting in low recoveries



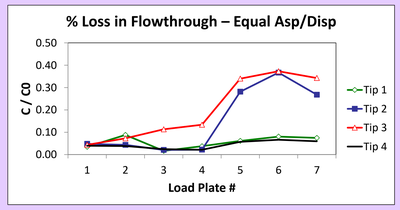



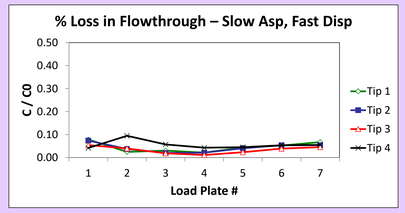

### Problems were addressed with both method optimization and tip engineering:

The second prototype tip lowered the top retaining screen, resulting in much more consistent flow behavior and better binding:




Liquid class definitions were also adjusted to increase delays and alter aspiration and dispense speeds, improving robustness:

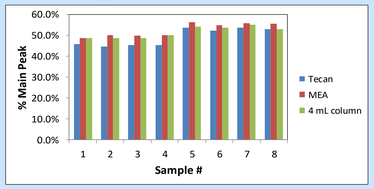


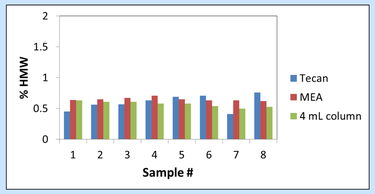

## Results

### A set of samples was purified across all available methods to ensure analytical comparability:

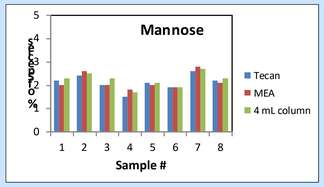
Charge Variants (HP-IEX)

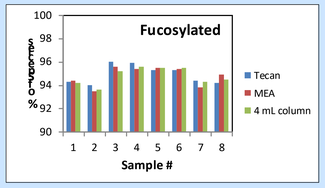



Aggregates (UP-SEC)




Glycosylation

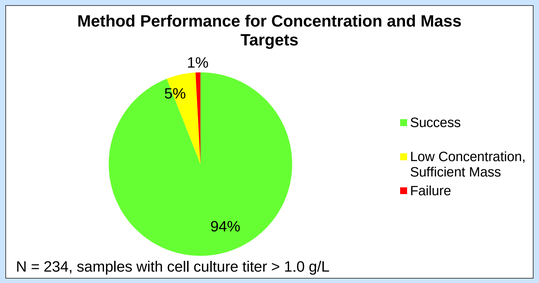



MEA = automated 12-channel pipetting system from PhyNexus

Petroff, Matthew, et al. "High-throughput purification tools for rapid upstream process development are interchangeable for biologics." *Engineering in Life Sciences.* 00 (2015): 1-10.

### The method has consistently met the target concentrations and masses since implementation:




## Conclusions

A high-throughput Protein A purification method has been developed and implemented, with 500+ samples supported to date.

Ongoing work is focusing on better integration with upstream for high-throughput harvesting. Fluidized bed may allow purification w/ some cell debris.