VACCINEINSIGHTS

ADVANCES IN VACCINE MANUFACTURING PART 2: DOWNSTREAM BOTTLENECKS & INCREASING MANUFACTURING CAPACITY

SPOTLIGHT

INNOVATOR INSIGHT

Streamlining vaccine development & production with modality-specific purification tools for mRNA & beyond

Eugene Sun

The COVID-19 pandemic has emphasized the need for readily available commercial-scale vaccine production tools to support global immunization efforts. With an increasing number of novel nucleic acid and viral vector platforms joining vaccine development pipelines, a wider range of purification methods is needed. This Innovator Insight presents case studies of implementing purification tools for rapid candidate screening and intensified downstream processing of various vaccine modalities, including viral vector, virus-like particle, subunit, and mRNA vaccines.

Vaccine Insights 2023; 2(11), 405–415

DOI: 10.18609/vac/2023.54

NEW VACCINES FOR MALARIA: AFFINITY CHROMATOGRAPHY

In 2021, there were around 247 million cases of malaria, resulting in approximately 619,000 deaths, mostly in children under 5 years [1]. However, new vaccines are bringing hope for controlling the disease. A vaccine developed at the Jenner Institute (Oxford University, UK)—R21/Matrix-M—demonstrated an efficacy of 77% in Phase IIb clinical trials [2] and has been approved for use in Ghana and Burkina Faso.

The R21/Matrix-M vaccine uses a virus-like particle (VLP), which was purified using an affinity tag during its development. Scientists at the Jenner Institute are also working on a range of malaria vaccines targeting different

life stages of the parasite. During the development of a candidate vaccine targeting the PfRh5 protein, the researchers first investigated the functionality of a polyhistidine tag (His-tag) to capture the protein. However, initial results indicated that the immobilized metal affinity chromatography (IMAC) process used to capture His-tag was not scalable due to poor yield.

The team next investigated the C-terminal tag (C-tag), which is comprised of four amino acids (glutamic acid-proline-glutamic acid-alanine, or EPEA) coupled to the C terminus of the target protein. For use in conjunction with the C-tag, Thermo Fisher Scientific has created a scalable, cGMP-compliant affinity resin (CaptureSelect™ C-tagXL), which specifically targets C-tag's four amino acids.

The CaptureSelect C-tagXL is one of many CaptureSelect affinity resins designed to capture specific targets. These affinity resins utilize the variable heavy chain (VHH) of Camelid antibodies. The small size of VHH (around 15 kDa) enables binding to difficult-to-reach epitopes and means that the domains are very robust. VHH affinity ligands are produced in an animal-origin-free production process using Saccharomyces cerevisiae (baker's yeast). CaptureSelect affinity resins allow for elution using mild pH conditions, which maintains the stability of the target molecule. Scientists working on the vaccine were able to elute the PfRh5 protein using a TRIS, tris(hydroxymethyl)aminomethane, buffer with magnesium chloride at pH 7 [3].

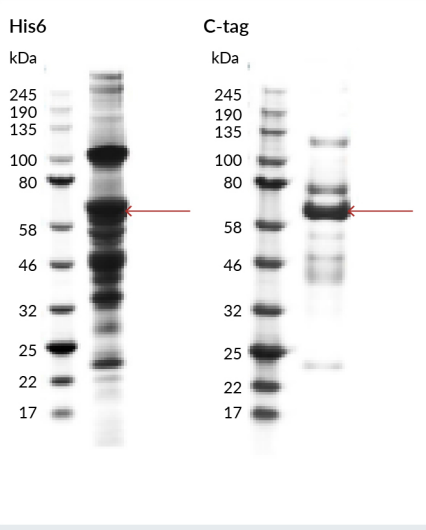
Figure 1 shows a comparison of the two purification processes for the PfRh5 protein—one using the His6-tag and IMAC, and the other using C-tag and CaptureSelect C-tagXL. Both processes involved tangential flow filtration, followed by the affinity step, followed by size exclusion chromatography (SEC). The PfRh5 purity was approximately 72% for the C-tag purification, which was much higher than the 20% purity observed using the His-tag process (Figure 1A). CaptureSelect C-tagXL also offered higher resolution (Figure 1C).

Figure 1C shows the process yield at each step. There was a 43.3% overall process yield with the C-tag, compared with a 25.5% process yield with the His-tag. In addition, the affinity step yield for the C-tag was approximately 85%, compared with the 64% step yield for the His-tag, and the SEC pool purity for the C-tag process was greater than

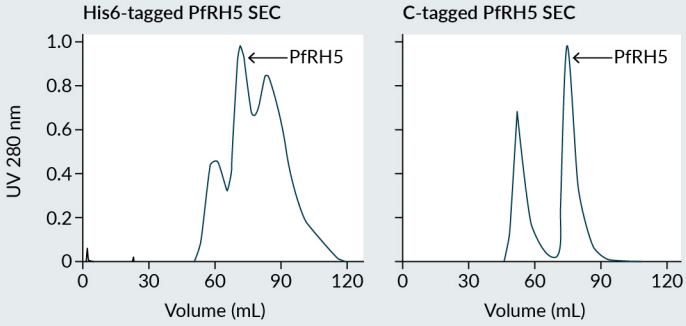
FIGURE 1

Comparison of Ctag and Histag used in a protein-based vaccine purification process.

A Purity comparison on gel



B Size exclusion comparison



C Yield comparison of both processes

Process yield (after)	His6-tagged construct (%)	C-tagged construct (%)
Culture supernatant	100	100
TFF	82.1	91.0
Affinity chromatography	52.5	77.4
SEC	25.5	43.3

TFF: Tangential flow filtration. SEC: Size exclusion chromatography

(A) Purities of the elution pools; **(B)** size exclusion chromatography (SEC) chromatograms of the eluate; **(C)** yields of both processes.

DOI: 10.18609/vac/2023.54

99%, compared with 80% for the His-tag process.

mRNA VACCINE FOR COVID19: AFFINITY CHOMATOGRAPHY

The Pfizer-BioNTech and Moderna mRNA vaccines were amongst the first to be approved for COVID19. For both of these vaccines, an affinity process was used to capture mRNA1273.

An effective affinity tool is Thermo Fisher Scientific's POROS™ Oligo (dT)25 affinity resin, which is designed to capture synthetic mRNA from an *in vitro* transcription (IVT) reaction. This process is based on the poly-deoxythymidine (poly dT) ligand being attached to a 50 µm rigid bead as shown in Figure 2.

There are 25 deoxythymidines, which can bind via hydrogen bonding to the adenosine of the polyA tail of an mRNA molecule. Since both adenosine and thymine molecules are very charged, a counter ion such as sodium chloride or potassium chloride must be used to weaken the local charge interactions. This allows for same-charge molecules such as nucleic acids to approach each other closely

FIGURE 2

Thermo Scientific™ POROS™ Oligo (dT)25 affinity resin.

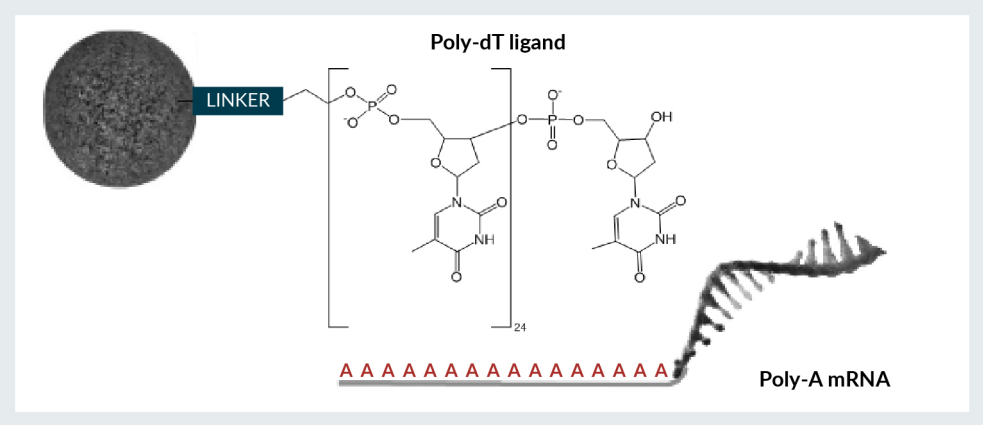
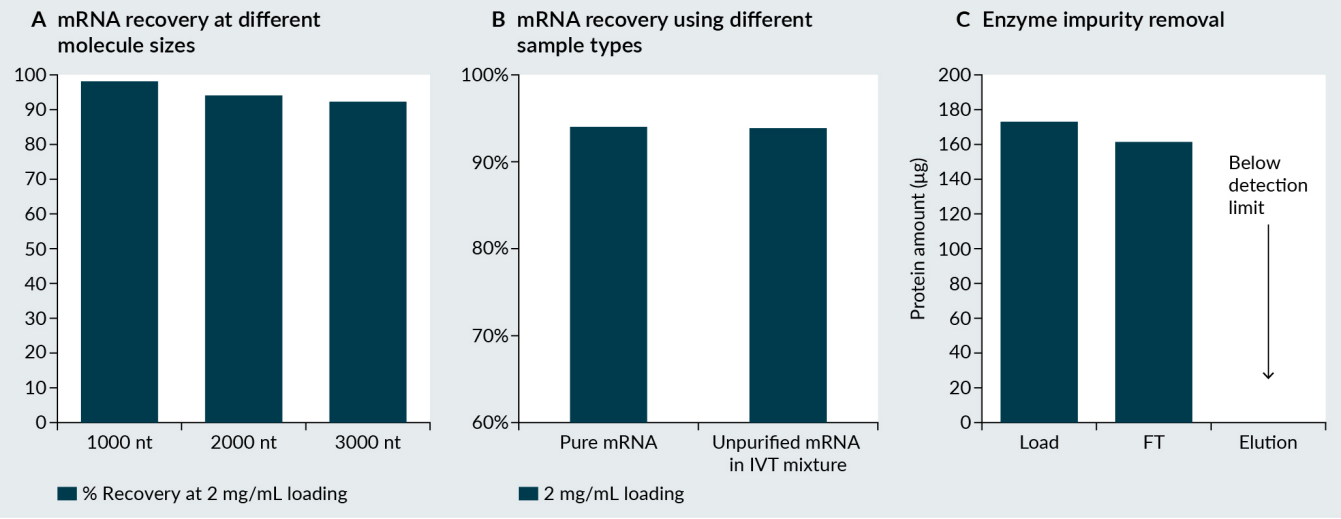


FIGURE 3

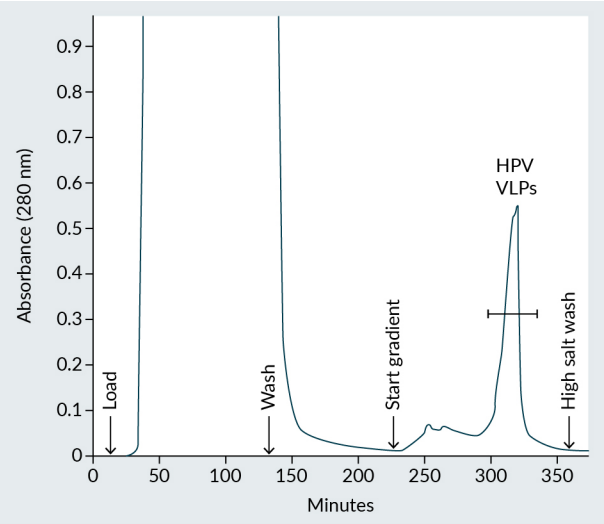
POROS™ Oligo (dT)25 application data: recovery and purity.



enough for hydrogen bonding to occur. Water or a solution with less conductivity is used to break or dissociate the hydrogen bonding between the base pairs, which enables the mRNA molecule to elute off the resin.

FIGURE 4

POROS HS50 chromatogram of the clarified lysate.



Elution with a linear NaCl gradient caused the antigen to elute between 0.9 and 1.35 M NaCl, with the peak eluting at 1.06 M NaCl.

High dynamic binding capacity for this resin has been observed, dependent on the mRNA size, concentration, and residence time during the load phase, typically resulting in a yield of greater than 90%. It also has good scalability and is non-animal derived.

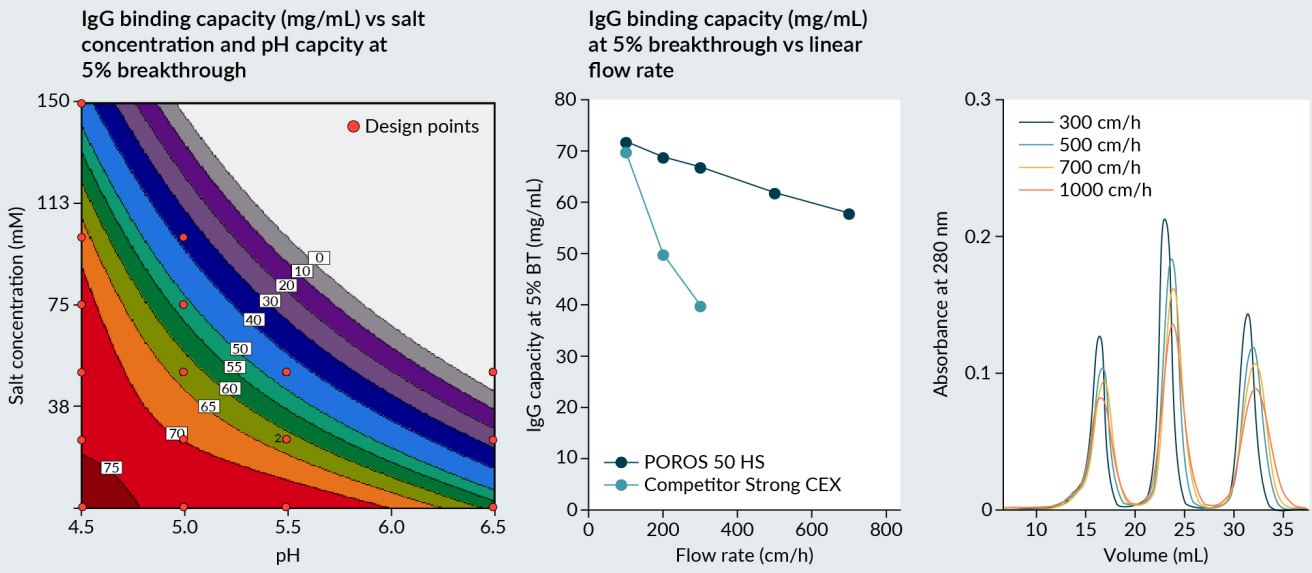
Figure 3 demonstrates application data for the Oligo (dT)25 resin. The structure of the POROS bead allows for efficient recovery across a wide range of molecule sizes (Figure 3A). The recovery for unpurified mRNA in IVT mixture versus pure mRNA is similar (Figure 3B), which indicates that Oligo (dT)25 resin can specifically bind to the target mRNA, regardless of load impurities. Regarding enzyme-impurity removal, the majority of the enzymes are in the flowthrough as expected, with none in the elution pool (Figure 3C).

VLP VACCINE FOR HUMAN PAPILLOMAVIRUS: CATION EXCHANGE CHROMATOGRAPHY

In addition to affinity-based methods, ion exchange chromatography methods can be

FIGURE 5

POROS HS50 resin delivers optimal dynamic binding capacity with high resolution.



DOI: 10.18609/vac/2023.54

an equally effective method for capturing vaccine targets, as demonstrated by VLP-based vaccines for human papillomavirus (HPV).

HPV is a common and highly transmissible sexually transmitted infection, which can cause several cancers. The first two vaccines developed for HPV were released in 2006 and 2008, and the prevalence of HPV infections has since decreased by nearly 90% in England, where vaccine coverage is high [4].

Thermo Fisher Scientific's POROS™ HS50 Strong Cation Exchange Resin was the tool used to purify the first two HPV vaccines. The recombinant HPV type 11 major capsid protein, L1, derived from baker's yeast, was able to bind the HS50 resin as shown in Figure 4. The clarified lysate was directly loaded onto the HS50 resin, resulting in 90% purity of the eluate. There were minimal measured traces of impurities such as endonuclease, RNA, and DNA, illustrating that an ion exchange step can provide specificity and impurity clearance similar to an affinity chromatography step.

Out of four resins tested, scientists selected the POROS HS50 resin due to the large (100–200 nm) throughpores of the HS50, which allowed for both high capacity and high resolution. In addition, the POROS HS50 has a small (50 µm) bead size. There is an inverse relationship between particle size and resolution, with smaller beads offering better resolution. Although this often comes at the cost of back pressure, the POROS resins can mitigate this due to the rigid polystyrene divinylbenzene polymer that is used to form the base bead. The polystyrene divinylbenzene backbone also means that the beads have very robust physical and chemical stability. The effect of these characteristics on the performance of the POROS HS50 is demonstrated in Figure 5.

ADENOVIRAL VECTOR VACCINES: ANION EXCHANGE CHROMATOGRAPHY

Anion exchange chromatography (AEX) is widely used for purifying adenovirus-based viral vector vaccines. Typically, adenoviruses are negatively charged at physiological pH and therefore have a strong affinity towards the positively charged backbone of an anion exchanger. Figure 6 provides an example of a process flow for adenovirus purification.

Many of the unit operations shown in Figure 6 are employed in purifying commercial viral vector vaccines against COVID19, including ChAdOx-1-S (Oxford/AstraZeneca) and Ad26.COV2.S (Johnson & Johnson).

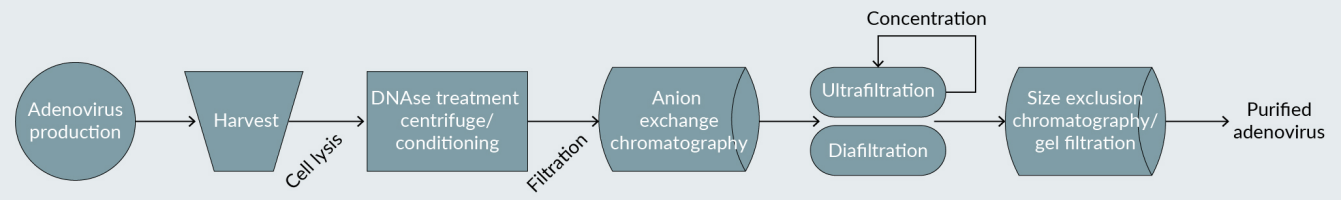
There are a number of novel viral vector vaccines currently in development, including an AAV vector vaccine for SARS-CoV-2 [5] and a lentiviral vector vaccine to be used for personalized cancer treatment [6]. Table 1 presents additional affinity solutions for viral vector vaccines.

SARS-COV-2 SPIKE ECTODOMAIN PROTEIN: WEAK ANION EXCHANGE

The POROS™ D50 Weak Anion Exchange Resin, which performs similarly to

FIGURE 6

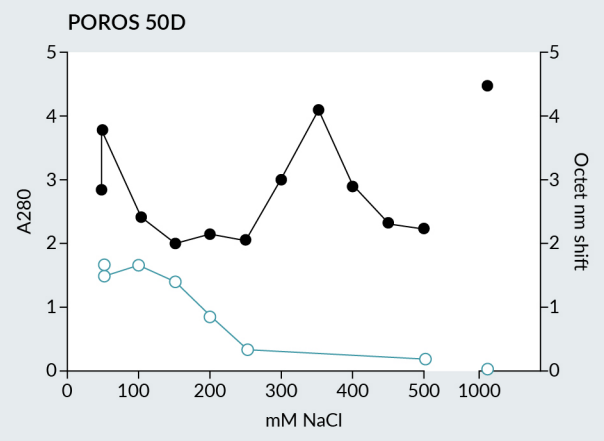
AEX is an effective capture step in the purification of adenovirus-based viral vector vaccines.



other well-known weak AEX resins such as dimethylaminoethyl, was evaluated at the NIH Vaccine Research Center for a spike glycoprotein construct of SARSCoV2 [7].

FIGURE 7

A280 UV (black) and Octet binding data (blue) for POROS D50 fractions.



In this study, six different AEX resins were evaluated, and POROS D50 was chosen based on recovery and mass balance. A 70% yield of the spike protein in the eluate was achieved, with the remaining 30% being detected in the flowthrough. Based on this observation, it was suggested that optimization of residence time and loading density could improve yield and reduce loss in the flowthrough.

Elution with NaCl caused the spike protein to elute between 300 and 400 mM NaCl (Figure 7). The run was performed using a 2 min residence time and load density of 50 g/L

While this study focused on high-throughput screening using RoboColumns, it provides an example of how an AEX resin can be used to purify spike proteins, which can then be used to develop subunit vaccines. Table 2 shows the POROS AEX resins offered by Thermo Fisher Scientific.

TABLE 1

Affinity solutions for viral vector-based vaccines.

Thermo Scientific ™ resin	Serotype affinity
POROS™ CaptureSelect™ Adv5*	Adv5
POROS™ CaptureSelect™ AAVX	AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, recombinant and chimeric vectors
POROS™ CaptureSelect™ AAV9	AAV9
POROS™ CaptureSelect™ AAV8	AAV8
POROS™ 50 HE Heparin	Some viral vectors such as lentivirus
CaptureSelect™ Lenti VSVG*	Lentivirus pseudotyped with VSVG particles

*Research use only resin. Can be developed into a bioprocess resin for commercial manufacturing.
VSVG: Vesicular stomatitis virus G glycoprotein.

TABLE 2

POROS anion exchange resins.

POROS resin	Type of AEX resin	Surface chemistry	BSA binding capacity (mg/mL)	AEX applications
D50	Weak	Dimethylaminopropyl	90	Bind/elute: Protein, virus, pDNA purification Flow through: Trace impurity removal by binding impurities (DNA, viruses, HCP, aggregates, endotoxin)
PI50	Weak	Polyethyleneimine	80	
HQ50	Strong	60% Quanternized polyethyleneimine	75	
XQ	Strong	Fully quaternized amine	>140	

AEX: Anion exchange chromatography; BSA: Bovine serum albumin; HCP: Host-cell proteins.

DOI: 10.18609/vac/2023.54

Q&A

Eugene Sun

Q With your Oligo (dT)25 resin, what kind of recovery and purity can be expected for large self-amplifying mRNA constructs?

ES: Several factors impact the binding capacity, such as low concentration, construct size, and—most importantly—residence time. At a 2 min residence time, we have seen up to 4 mg/mL binding capacity for constructs of 3000 nucleotides or smaller.

For larger constructs like self-amplifying mRNA, which can be as large as 10,000 nucleotides, the same binding capacity as smaller constructs can be achieved simply by extending residence time. In terms of yield recovery for larger constructs, if you limit the loading, you will see better yields.

Q What is the column lifetime of the Oligo (dT)25 resin?

ES: We have internal data that shows resin reuse for our Oligo (dT) resin up to 70 cycles when using 0.1 M sodium hydroxide. One could expect to see a higher number of cycles when using 0.2 or 0.5 M sodium hydroxide.

Q In addition to yield, what are the benefits of using the C-tag over the His-tag?

ES: The first major benefit is scalability. On a large scale, you do not need to use heavy metal ions to charge your IMAC resin or use imidazole to elute. Another benefit is not having to deal with clipped or truncated species of a His-tag construct. We know that His6 binds to a particular metal, but so do clipped variants, such as 5- or 4-histidine. During process development, these impurity levels would need to be controlled. This is not an issue when using the C-tag resin since all four amino acids must be present in order for the resin

to bind. So, if there happens to be any clipped variants, they should simply pass through into the non-bound pool.

Q Does the C-tag affect the confirmation or characteristics of the vaccine, and can it be placed on the N-terminus to avoid any such effects?

ES: Unfortunately, the C-tag was not designed to be added to the N-terminus. The resin only detects the full EPEA amino acid sequence at the end, and it must be free-floating.

To solve expression issues, one alternative is to use flexible linkers, added prior to the C-tag. Most end-users do not need to use linkers, but it is an option.

Q Is there a need to cleave the C-tag, and if so, can it be cleaved on the column?

ES: There is no need to cleave the C-tag. It is small, inert, and should not induce an immunogenic response.

If you do wish to cleave the C-tag, you would need to add the protease cleavage site prior to the C-tag. My recommendation would be to cleave the C-tag after purification is complete, since we do not have data concerning the on-column exposure to these different proteases.

Q What binding capacity can one expect from C-tagXL?

ES: The capacity is based on 400 nmol/mL of resin, so for a 30 kDa molecule, you will have a capacity of around 12 mg/mL of resin.

Q Does the Oligo (dT) affinity resin capture both single-stranded and double-stranded mRNA IVT products?

ES: If the resin has the poly-A tail (which it typically would since it comes from an IVT reaction), it will capture both the single-stranded and double-stranded mRNA.

Q What are the advantages of CaptureSelect Lenti VSVG over POROS Heparin?

ES: One of the major benefits of CaptureSelect Lenti VSVG is that it is animal-free. In addition, we have seen better impurity clearance.

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Q Does Thermo Fisher make custom ligands specific to a single client?

ES: Most customers ask for exclusivity to avoid issues with proprietary or sensitive information, so we often make custom ligands specific to one client.

Q Your example of POROS HS resin showed IgG capacity data. What would you expect the capacity to be for VLPs?

ES: That depends on the size of the VLP. VLPs can range in size between 100 and 200 nm, which is similar to the pore sizes for the various anion exchange resins. For example, the pore size of our POROS D and XQ resins is around 110 nm, while the POROS PI and HQ pore sizes are around 200 nm. If the VLP is smaller than the pore, it can diffuse into our resins. If that happens, the capacity is expected to be higher than that of the IgG.

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BIOGRAPHY

EUGENE SUN is the Field Application Scientist for the northeast region of North America and is responsible for providing technical support for POROS and CaptureSelect chromatography resins. Prior to joining Thermo Fisher Scientific Bioproduction Group in 2021, Eugene supported Amgen's Pivotal Drug Substance Technologies group in Cambridge, Massachusetts and was responsible for the development, characterization, and scale-up support of downstream processes to enable commercial advancement of programs from clinical trials to marketing application. Prior to that, Eugene started his career supporting MedImmune/AstraZeneca's early drug discovery/pre-clinical pipeline down in Maryland as a member of the Purification Process Sciences department. Eugene has over 10 years of extensive bench-scale experience developing both early and late-stage purification processes across all downstream unit operations for monoclonal antibodies, various formats

of bispecific antibodies such as bispecific T-Cell engagers (BiTEs), and biosimilar programs. Eugene earned his Bachelor of Arts in Biochemistry and Music from Bowdoin College, Brunswick, Maine.

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INTENDED USE STATEMENT

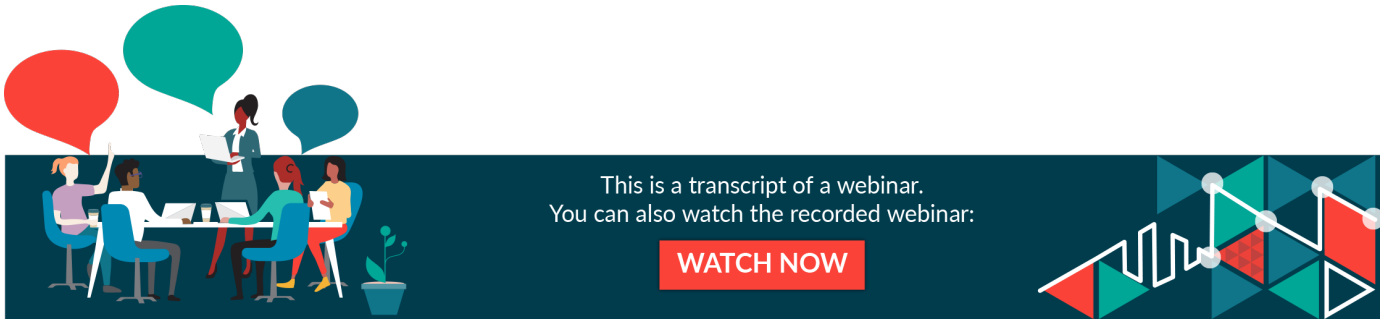
POROS resins: Pharmaceutical Grade Reagent. For Manufacturing and Laboratory Use Only.
CaptureSelect ligands and resins: For Research Use or Further Manufacturing. Not for diagnostic use or direct administration in humans or animals.

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: The named author takes responsibility for the integrity of the work as a whole, and has given his approval for this version to be published.
Acknowledgements: None.
Disclosure and potential conflicts of interest: The author has no conflicts of interest.
Funding declaration: The author received no financial support for the research, authorship and/or publication of this article.

ARTICLE & COPYRIGHT INFORMATION

Article source: This article is based on a transcript of a webinar, which can be found here.
Webinar recorded: Aug 30, 2023; **Revised manuscript received**: Oct 27, 2023; **Publication date:** Nov 22, 2023.



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