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VACCINE INSIGHTS

SPOTLIGHT ON

Advances in vaccine manufacturing part 2: downstream bottlenecks and increasing manufacturing capacity

RNA vaccines part 2: addressing ongoing challenges

INNOVATOR INSIGHT: Streamlining vaccine development and production with modality-specific purification tools for mRNA and beyond
Eugene Sun

INNOVATOR INSIGHT: Protein characterization using variable pathlength spectroscopy: high- & low concentration methods
Paul Mania and Nigel Herbert

EXPERT INSIGHT: Challenges and opportunities in sterile filtration of vaccines
Kevork Oliver Messerian, Zhuoshi Du, Neil Taylor, and Andrew L Zydney

INTERVIEW: Optimizing CMC to make vaccines for the world
Piper Trelstad

VIEWPOINT: Establishing self-sustaining vaccine manufacturing ecosystems: Africa's priority diseases
Nicaise Ndembi, Mahlet Kifle Habtemariam, Akhona Tshangela, Ngashi Ngongo, and Jean Kaseya

VIEWPOINT: What is a sustainable vaccine manufacturing footprint for Africa?
Philip Dorrell and Rishabh D Jhol

VIEWPOINT: Germany supports the African Agenda of building local vaccine manufacturing capacity
Manuel Batz

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.

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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].

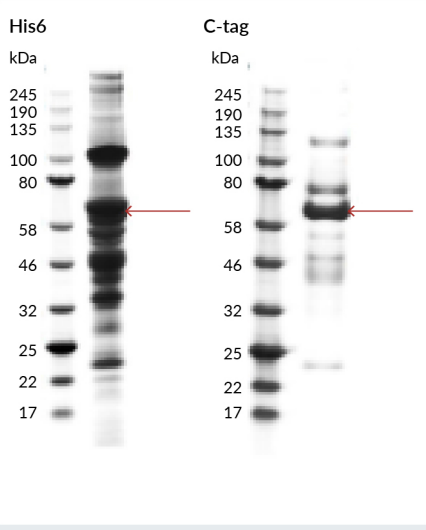
Figure 1 shows a comparison of the two purification processes for the PfRh5 protein—one using the His6-tag and IMAC, and the other using C-tag and CaptureSelect C-tagXL. Both processes involved tangential flow filtration, followed by the affinity step, followed by size exclusion chromatography (SEC). 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

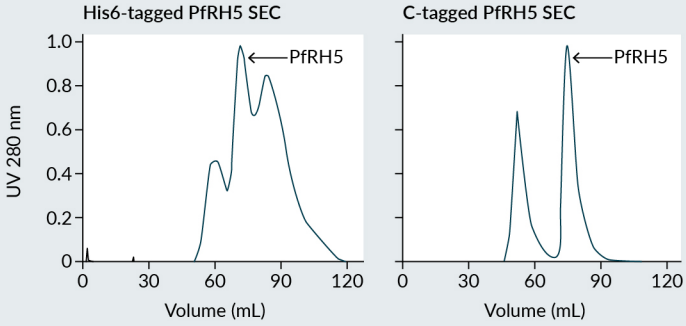
FIGURE 1

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.

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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.

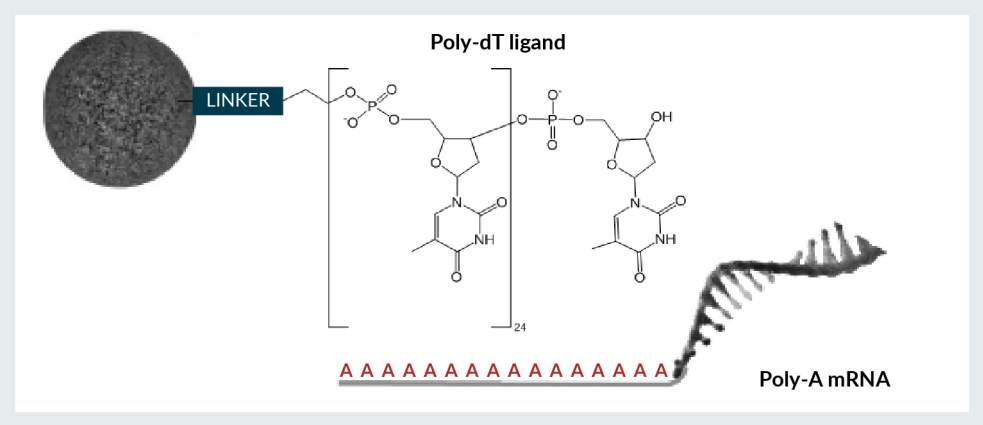
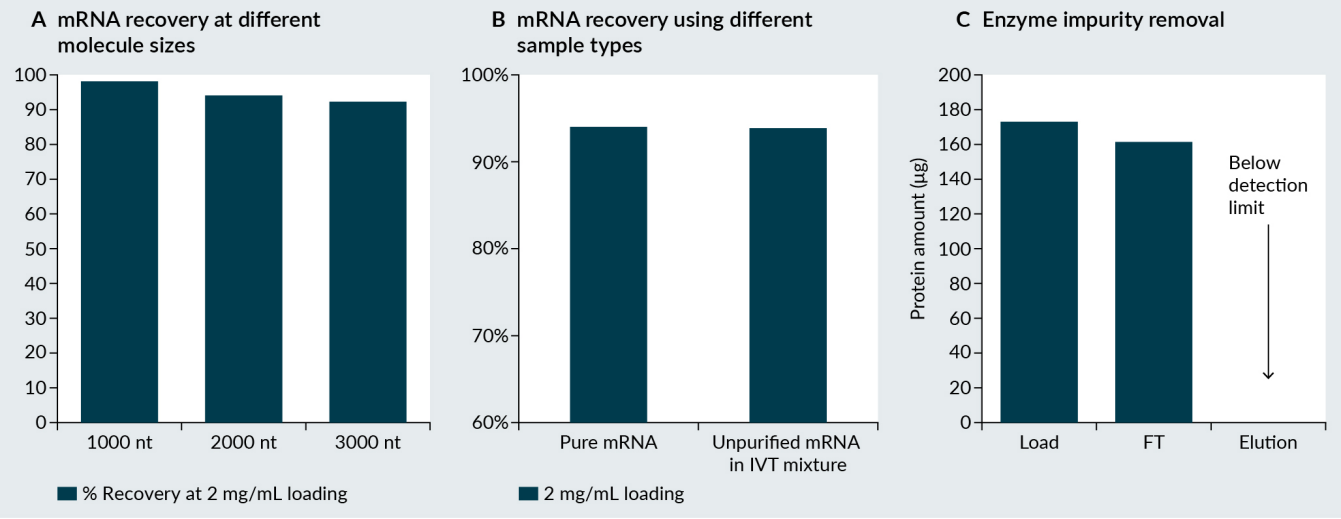


FIGURE 3

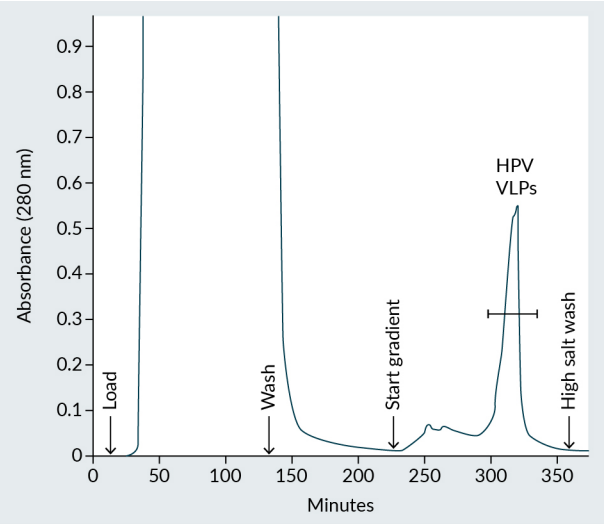
POROS™ Oligo (dT)25 application data: recovery and purity.



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.

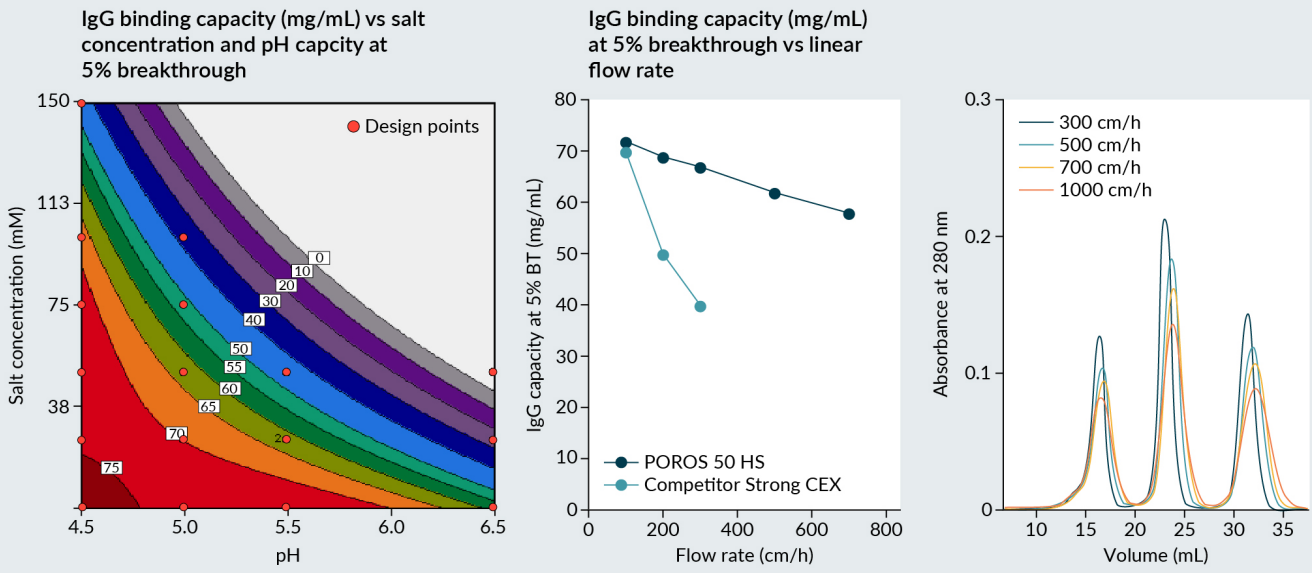
Figure 3 demonstrates application data for the Oligo (dT)25 resin. The structure of the POROS bead allows for efficient recovery across a wide range of molecule sizes (Figure 3A). The recovery for unpurified mRNA in IVT mixture versus pure mRNA is similar (Figure 3B), which indicates that Oligo (dT)25 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.



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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).

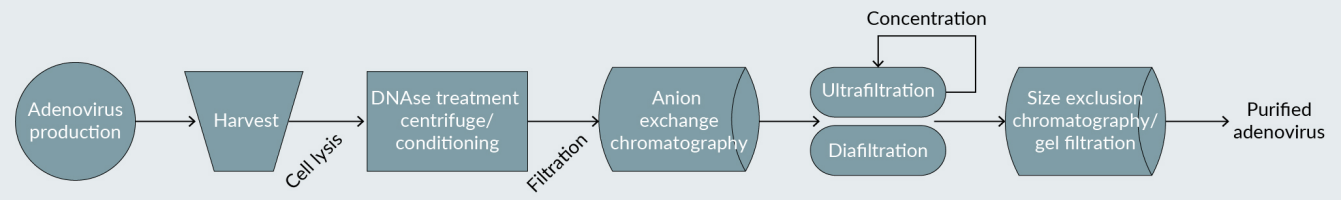
There are a number of novel viral vector vaccines currently in development, including an AAV vector vaccine for SARS-CoV-2 [5] and a lentiviral vector vaccine to be used for personalized cancer treatment [6]. Table 1 presents additional affinity solutions for viral vector vaccines.

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

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

FIGURE 6

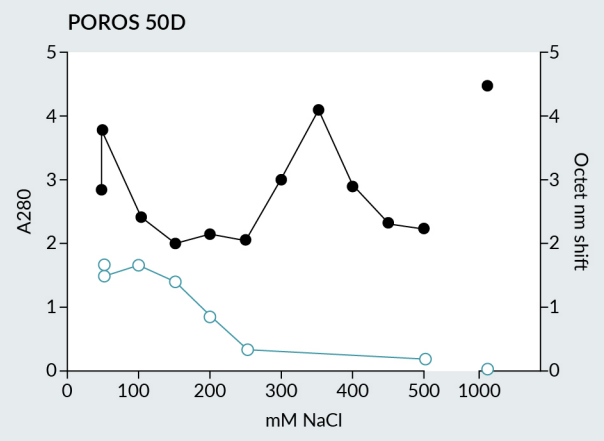
AEX is an effective capture step in the purification of adenovirus-based viral vector vaccines.



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.

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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.

AFFILIATION

Eugene Sun
Field Application Scientist,
Thermo Fisher Scientific

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

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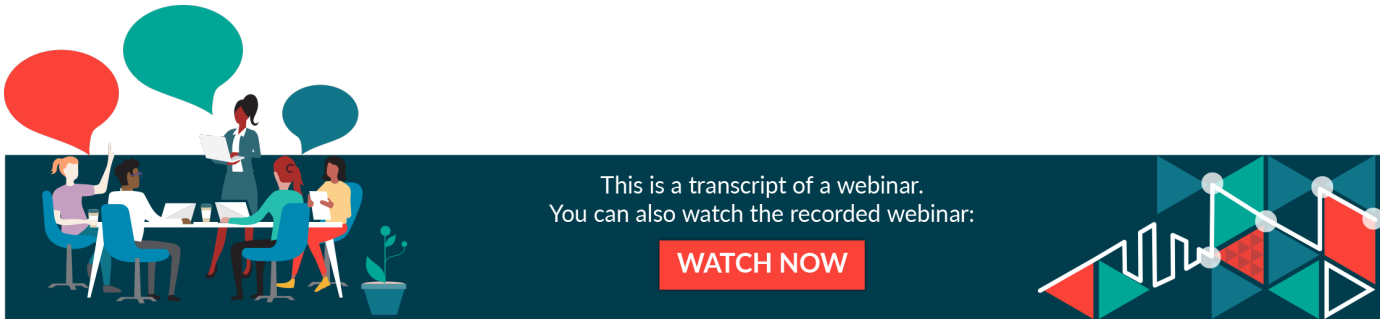

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VACCINEINSIGHTS

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

SPOTLIGHT

INNOVATOR INSIGHT

Protein characterization using variable pathlength spectroscopy: high- & low-concentration methods

Paul Mania and Nigel Herbert

The potential to tailor bioanalytical methods to specific biologic therapeutic products is critical to effectively meet regulatory CMC expectations. This article describes the use of variable pathlength technology for protein concentration measurement, with a particular focus on exploring low- and high-concentration methodologies.

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PROTEIN CONCENTRATION ANALYSIS

For all vaccine types, concentration measurement is a critical analysis at various points in the vaccine development pipeline, including quality control, process development, analytical development, and especially GMP manufacturing.

Traditionally, concentration measurements have been carried out via UV-Vis spectroscopy; however, this approach has a number of limitations. Assay time can be anywhere from 30 minutes to 3 hours, with additional time required to send samples for analysis. In addition, manual dilutions and calculations can introduce errors up to 20%. The latest ASTM International standard for UV-Vis [1]

points out the risk of carrying out dilutions to avoid saturation of the instrument, and states: "To avoid the dilution step, the instrument may contain an automatic system which will allow adjustment of the path length of the measurement cell to optimize the measured absorbance."

There are two approaches to sample preparation for traditional UV-Vis spectroscopy—volumetric or gravimetric—both of which require significant time and skill. Equipment must also be properly calibrated to avoid introducing between-user errors.

VARIABLE PATHLENGTH TECHNOLOGY

UV-based variable pathlength technology (VPT) avoids many of the pitfalls of traditional fixed-pathlength UV-Vis spectroscopy. It incorporates the Beer–Lambert law and the Slope Spectroscopy method, allowing the pathlength to be the adjusted variable instead of the concentration.

Multiple absorbance measurements are taken at different pathlengths to generate a slope, which is then used to determine sample concentration. Using this method, a wide range of concentrations can be measured in a more accurate and repeatable way than traditional UV-Vis spectroscopy.

Repligen provides two VPT spectrophotometer systems that can be used to measure concentration without sample dilution: the at-line CTech™ SoloVPE® System and the in-line CTech™ FlowVPX™ System.

Figure 1 shows the main components of the SoloVPE system.

The SoloVPE and FlowVPX can measure any substance with a chromophore in the UV-Vis range, as seen in Figure 2.

VPT has been validated for in-process and clinical manufacturing testing of monoclonal antibodies (mAb), polysorbate concentration, viral filtration, nucleic acid concentration, and plasmid purity. SoloVPE and FlowVPX Systems provide QC-grade measurements regardless of modality, which allows for alignment on sample testing from group to group, or location to location.

As a platform technology, the system reduces sample prep time, uses low sample volumes, and eliminates background correction, which results in a high-throughput assay. In a validation study performed by Regeneron [2], the use of VPT in the QC lab saved a month of labor every year, and the methods could be easily transferred to other sites with minimal training.

The default settings of the SoloVPE are suitable for many samples; however, for very high- or low-concentration samples, custom settings can be used to improve results, as illustrated by the case studies below.

CASE STUDY: HIGH-CONCENTRATION mAb

Figure 3 shows the absorbance versus pathlength for a mAb sample with a concentration of 220 mg/mL, showing the top of the slope flattening due to saturation of data at higher absorbance.

Essentially, the absorbance is too high for the detector to discern differences in absorbance, which may cause underestimation of the sample concentration. Beer–Lambert's law is typically unreliable above 2.0 AU, as illustrated in Figure 4.

To achieve linear data with an R2 value of 0.999 or higher, the number of data points can be reduced from the default ten. Figure 5 shows the slope analysis with the seven data

FIGURE 1

Variable pathlength technology principle.

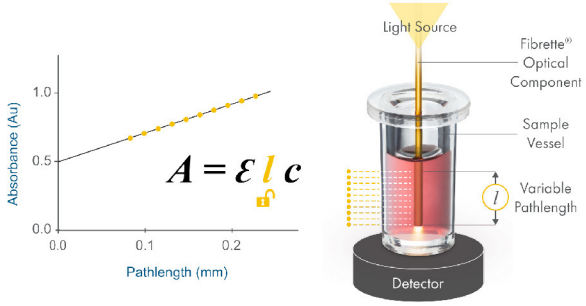

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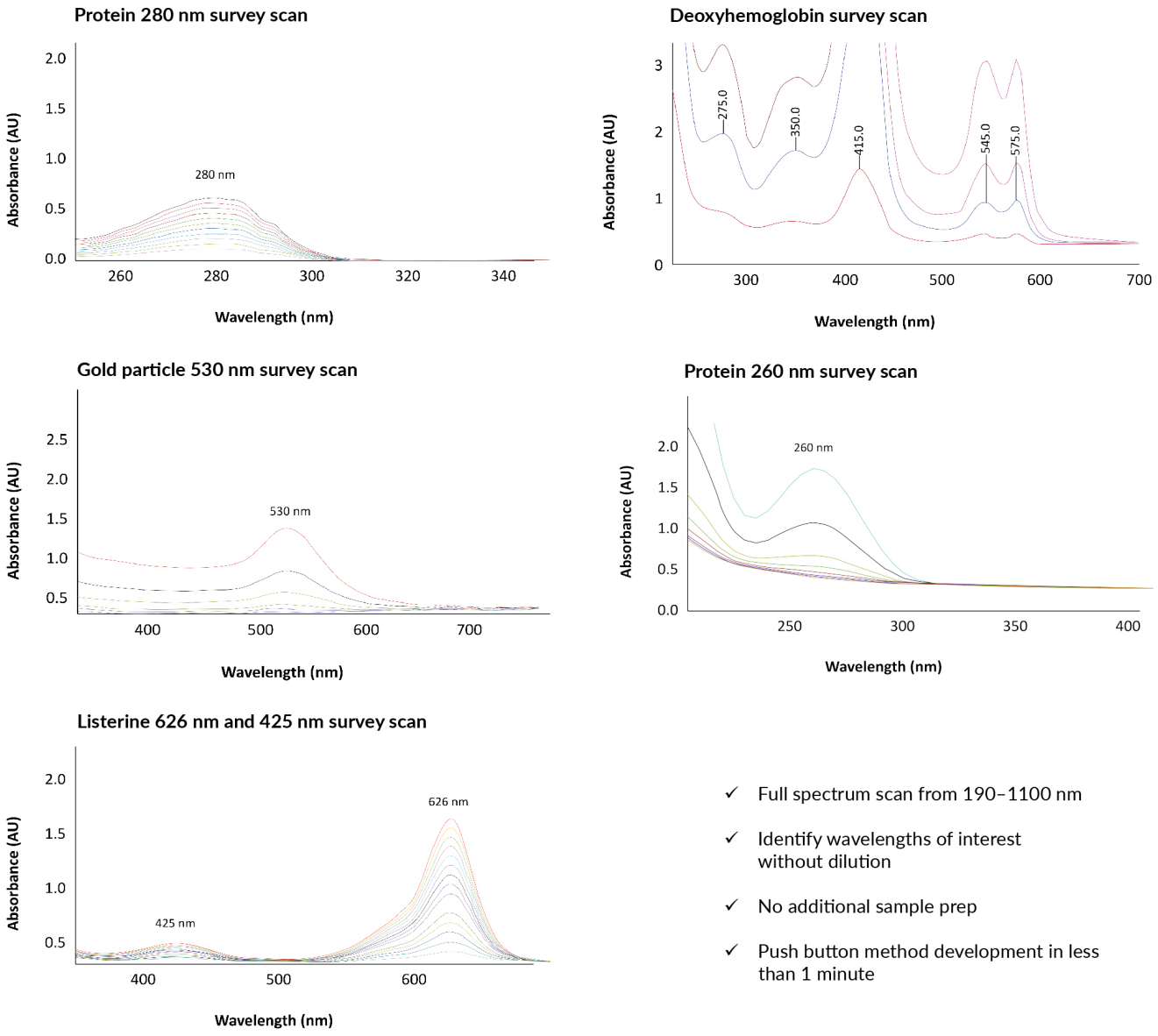
FIGURE 2

SoloVPE results for a variety of samples.



- ✓ Full spectrum scan from 190–1100 nm
- ✓ Identify wavelengths of interest without dilution
- ✓ No additional sample prep
- ✓ Push button method development in less than 1 minute

points closest to the detector, which allows the SoloVPE System to collect the most linear absorbance values and significantly increase the R2 value. The minimum number of data points is five.

Factors to ensure effective measurement of high-concentration samples include:

- Mixing: the sample must be homogenous, so proper mixing techniques are critical. It is advised to measure directly after mixing;
- Plastic vessels: cleaning is not required for plastic vessels which eliminates the risk of residual carryover. Slope measurements are independent of the vessel material;
- 6–7 data points: as demonstrated above, collecting fewer than the default ten data points helps linearize the data near extremes;
- Modified quick slope settings: concentrations over 200 mg/mL require a change

in the SoloVPE Software to allow for the best chance of acquiring linear slope data. Quick Slope methods can be saved and loaded for specific products;

- Cary averaging time of 1 second: increasing the default time the Xenon flash lamp spends on each datapoint from 0.5 to 1 second allows for better spectral resolution by eliminating outlier data.

Using the default settings for this 220 mg/mL mAb underestimates the sample concentration and reduces consistency, with a relative standard deviation of 4% (Table 1). Adopting a custom high-concentration method with fewer data points excluded the saturated values, giving an accurate concentration reading of 220, and a relative standard deviation of 0.61%. Custom settings allow the SoloVPE System to acquire the best section of linear data for regression plot.

CASE STUDY: LOW-CONCENTRATION mAb

With a low-concentration sample, absorbance values do not change greatly between readings, leading to a low slope and inconsistent results when default settings are used.

Factors to ensure effective measurement of low-concentration samples include:

- Large fused silica vessels: a 2.5 mL volume vessel gives 15 mm of pathlength range, which allows the SoloVPE System to take larger pathlength steps to ensure discernible absorbance changes that coincide with the change in pathlength;

FIGURE 3

Slope analysis for mAb sample with 10 data points.

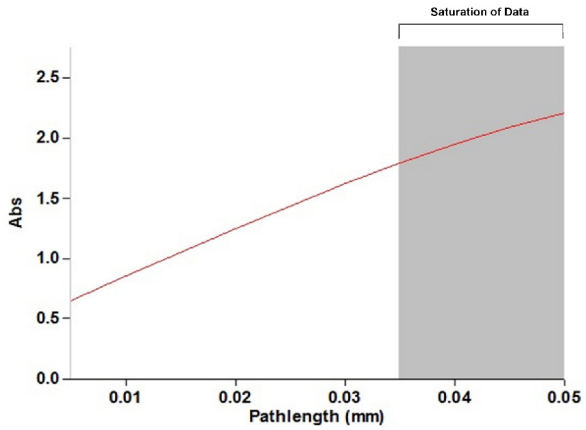


FIGURE 4

The impact of high absorbance on slope analysis.

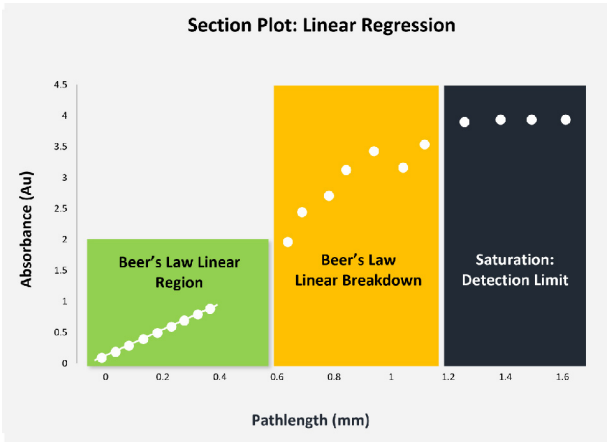
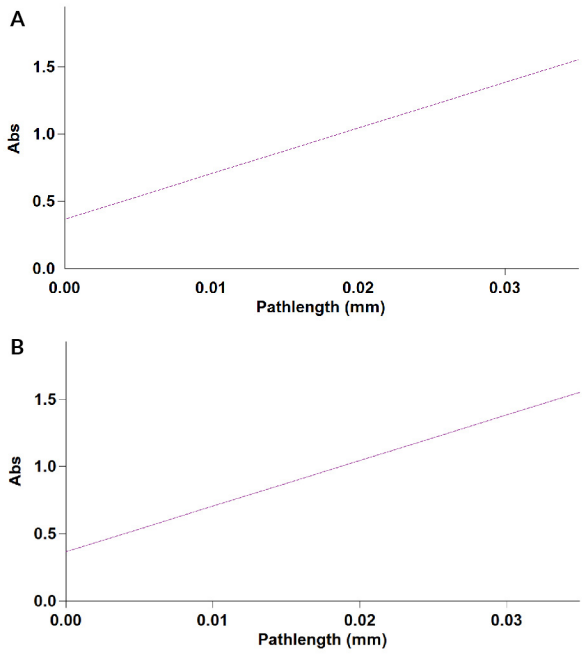


FIGURE 5

Slope analysis for mAb sample with 7 data points.



High concentration, modified settings. Antibody 220 mg/mL.

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TABLE 1

Data comparison for default and custom settings when measuring a high-concentration sample.

	Using default settings	Using custom settings
Sample 1	200.32	219.32
Sample 2	191.30	221.85
Sample 3	208.10	219.75
Mean value	199.91	220.31
Relative standard deviation %	4.21%	0.61%

TABLE 2

Data comparison for default and custom settings when measuring a low-concentration sample.

Expected concentration 0.01 mg/mL	Using default settings	Using custom settings
Sample 1	0.0205	0.0105
Sample 2	0.0132	0.0104
Sample 3	0.0185	0.0107
Mean value	0.0174	0.0105
Relative standard deviation %	21.68%	1.45%

- Baseline correction enabled: measures and subtracts the buffer from results;
- 6–7 data points: collecting fewer than the default ten data points allows larger steps, increasing linearity;
- Cary averaging time of 1 sec: as with high concentration, increasing the average time spent by the Xenon flash lamp at each data point allows for better spectral resolution, helping to eliminate outlier data.

Using default settings overestimates the concentration of a 0.01 mg/mL mAb sample and reduces consistency, with a relative standard deviation of over 21% (Table 2). Using custom settings, an accurate measurement is obtained, with a relative standard deviation of 1.45%.

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Q&A

Nigel Herbert

Q Is the SoloVPE system and software fully GMP compliant and how are the system and software qualified? How can CTech support the instrument computer and software qualification?

NH: The SoloVPE system is fully GMP compliant. We have a software package known as Secure VPT, which allows it to be 21 CFR Part 11 and Annex 11 compliant, and we offer installation qualification (IQ), operational qualification (OQ), and preventive maintenance (PM) with the system for it to be qualified.

Q Can you describe the validation process and the validation support that Repligen provides?

NH: I'm part of the applications group and my colleagues and I specialize in validation. Whatever processes you need, we can assist you. Whether it's SOPs, validation protocols, or getting the system to your location, we'll be able to provide you with assistance along the way.

Q How long does it usually take for the SoloVPE system to be installed and validated for PD work?

NH: Installation can be done in a day. Once the system is at your site, a member of our support team will come on-site to install and qualify it that day. In terms of the validation work, that varies depending on your specific needs, but we are happy to offer our assistance and any other services that are required to validate the system.

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Q Where would you typically see the SoloVPE being used?

NH: SoloVPE can be used in any functional group, and it is a GMP-grade system. We typically see it used in analytical development, quality control, and manufacturing groups to provide QC-grade concentration measurement.

Q What are the advantages of measuring drug-antibody ratio on SoloVPE compared with traditional UV?

NH: Similar to our other modalities, measuring drug-antibody ratio on SoloVPE does not require scatter or baseline correction. We can also measure high concentrations without the need for dilution, and our software allows for automatic calculation of the payload concentration, the antibody concentration, and the drug-antibody ratio.

Q Can oligonucleotides be measured using SoloVPE? What is the maximum plasmid concentration that can be measured undiluted?

NH: For oligonucleotides, we can typically measure up to around 25 mg/mL, depending on the extinction coefficient and the size of the molecule. At the low end, we have gone as low as 0.0005 mg/mL. It will take some method development to see where the absolute limits of the system are, depending on the sample.

Q Analytical ultracentrifugation (AUC) is seen as the gold standard for empty/full capsid determination for AAV products. How does the SoloVPE method compare?

NH: We do not have a direct comparison to AUC, but we do have data relating to digital droplet (dd)PCR and ELISA that show a good correlation between titers. AUC can determine partially filled capsids, whereas SoloVPE cannot. However, the advantages of SoloVPE include speed, cost, and ease of use.

BIOGRAPHIES

PAUL MANIA, CTech Bioanalytics Applications Specialist, is a seasoned professional who has been with Repligen since 2017. With a solid foundation in biological science earned from Duquesne University and substantial industry experience as a laboratory technician, Paul brings a wealth of knowledge to his role. In his capacity as a Bioanalytics Applications Specialist, Paul is dedicated to ensuring customers' success. He specializes in method validation and implementation, working tirelessly to seamlessly integrate customer solutions and expedite their functionality. His mission is to optimize his customer's applications,

harnessing the power of at-line concentration measurement and the remarkable precision of variable pathlength spectroscopy to the fullest.

NIGEL HERBERT stands as a dedicated advocate for customer success. With a BSc in Biomaterials Engineering and a Minor in Chemistry from Alfred University, Nigel brings a wealth of skills to his role as a Senior Bioanalytics Applications Specialist at Repligen. Since joining the team in 2019, Nigel has been on the forefront of advancing customers' analytical methods. He specializes in UV-vis spectroscopy, analytical processes, and method validation, offering invaluable support to ensure the highest standards of precision and reliability. One of Nigel's remarkable achievements is his collaborative spirit, resulting in numerous published application notes. Through close partnerships with customers, he has made significant strides in solving complex challenges and driving innovation in the biotechnology field.

AFFILIATIONS

Paul Mania
Bioanalytics Applications Specialist,
Repligen

Nigel Herbert
Senior Bioanalytics Applications Specialist,
Repligen

AUTHORSHIP & CONFLICT OF INTEREST

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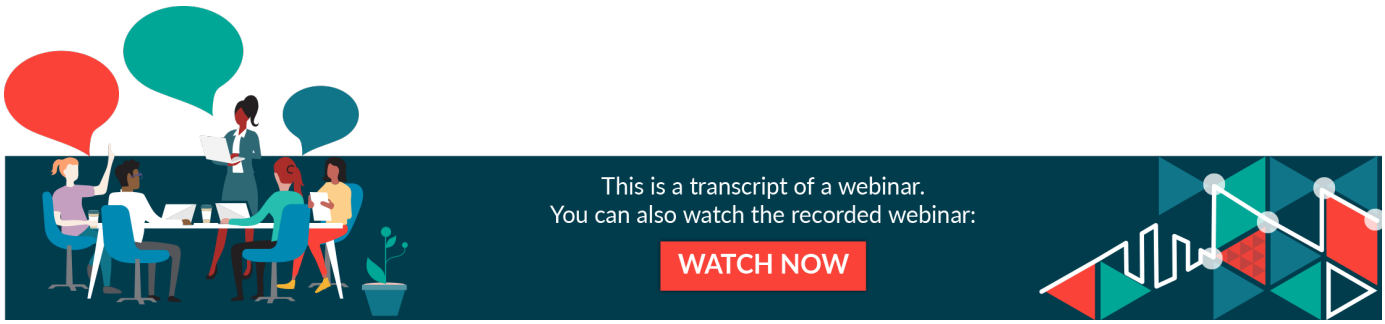

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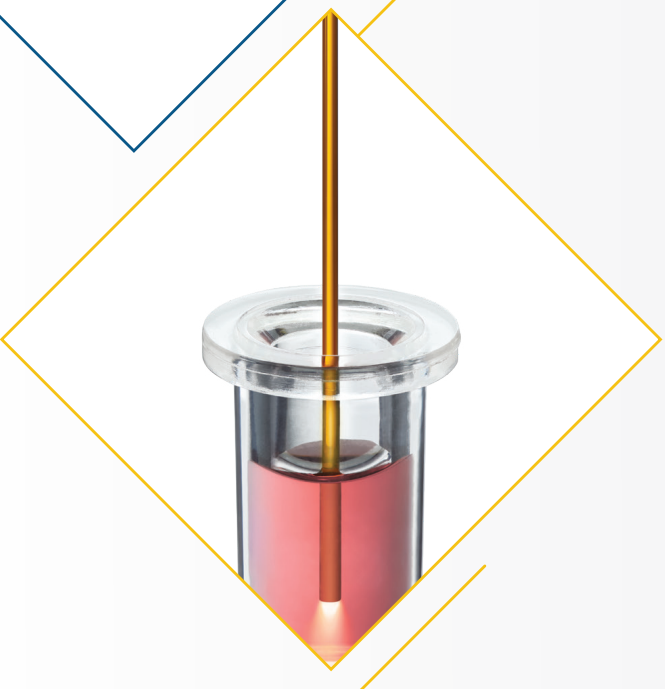
REPLIGEN



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SPOTLIGHT

EXPERT INSIGHT

Challenges & opportunities in sterile filtration of vaccines

Kevork Oliver Messerian, Zhuoshi Du, Neil Taylor, and Andrew L Zydney

Sterile filtration plays a pivotal role in ensuring the safety of nearly all injectable drug products, including vaccines. One of the greatest challenges with sterile filtering many vaccines is the overlap in the size distributions of the vaccine particles and the pores of the sterile filter. The large size of mRNA vaccines, live attenuated viral vaccines, and glycoconjugate vaccines causes membrane fouling, significantly reducing both the overall filter capacity and vaccine recovery. Sterile filters with built-in prefilters, such as dual-layer filters, can be particularly attractive for processing these vaccine products. However, it is still not possible to predict the filtration performance for any given vaccine based on the sterile filter pore structure/morphology. This review highlights recent work analyzing the unique fouling behavior observed with different vaccine products, providing new insights into the design and optimization of the sterile filtration process.

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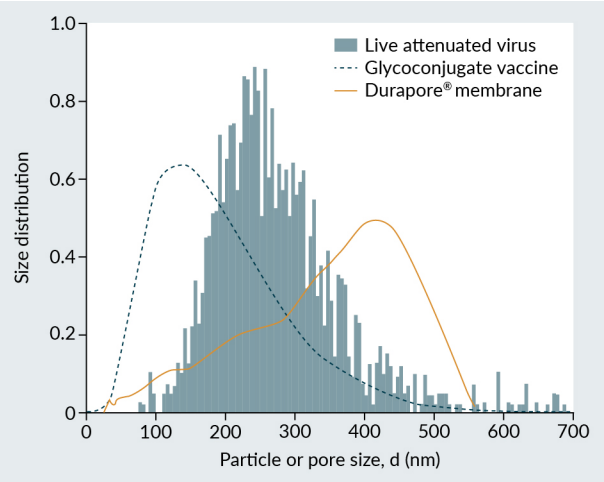
INTRODUCTION

Vaccines are an essential part of modern healthcare, significantly reducing the burden of infectious disease and providing protection against subsequent cancers [1]. The early viral vaccines were live attenuated versions of the viral pathogen [2]. The past decades have seen the development of a wide range of alternative vaccine products, including bacterial toxoids, sub-unit vaccines made from specific immunogenic proteins, glycoconjugate vaccines made from the capsular polysaccharide of pathogenic bacteria

coupled to an immunogenic carrier protein [3], and mRNA vaccines that code for an immunogenic protein and are delivered to the patient in a lipid nanoparticle (LNP) formulation that minimizes mRNA degradation and facilitates cellular uptake [4]. Bacterial toxoids and many sub-unit vaccines are quite small and can be processed using well-established technologies developed for purification of recombinant protein products. In contrast, the glycoconjugate and many live attenuated viral vaccines (LAV) are composed of relatively large sub-micron species with particle diameters as large as several hundred nanometers. This is shown in Figure 1 for both a live attenuated cytomegalovirus vaccine [5] and a single serotype of a glycoconjugate vaccine formed by conjugation to the tetanus toxoid protein [6]. This can create significant challenges for the downstream purification process, limiting the capacity and performance of many unit operations, including the use of sterilizing grade filters that are commonly employed in bioprocessing. This review highlights recent advances in understanding the behavior of these large 'particle' vaccines during the sterile filtration step used to ensure the sterility of the vaccine product [7].

FIGURE 1

Particle size distribution for a live attenuated cytomegalovirus vaccine candidate (determined by nanoparticle tracking analysis [5]), a glycoconjugate vaccine serotype (determined by dynamic light scattering [6]), and the pore size distribution for the Durapore® sterile filter (determined by mercury porosimetry [17]).



A considerable portion of the size distribution for the vaccine particles and the membrane pores overlap, creating significant challenges in effectively filtering these large particles.

STERILE FILTRATION

The most common methods of sterilization include aseptic processing, gamma irradiation, and sterile filtration [8]. Aseptic processing uses a completely closed system that requires all production steps to be housed within a clean room (a highly regulated manufacturing space in which air supply, process streams, and equipment are systematically controlled to prevent microbial contamination) [9]. Maintaining sterility throughout vaccine production tends to be expensive, creating significant challenges in the development of widely accessible vaccine products [10]. Yet, aseptic processing may be the only viable option for some very large and complex vaccine products; for example, the recombinant measles vaccine, which is close to 1000 nm in size [11]. Gamma irradiation has been successfully used to sterilize several LAV vaccines [12]; however, this has been reported to cause physical damage to some vaccines [13] and the presence of residual killed bacteria (generating endotoxin) remaining in the drug product is also known to cause side effects during administration of the vaccine [14]. Thus, the preferred method for vaccine manufacturing is sterile filtration, in which the vaccine is filtered through a membrane that has been validated for its ability to provide complete removal of all bacteria.

Membranes (or sterile filters) are classified as 'sterilizing grade' based on ASTM protocol F838-83 [15]. This requires that the filter is able to produce a completely sterile permeate (no colony-forming units [CFU]) when the filter is challenged with 10^7 CFU/cm^2 of membrane area using the bacterium *Brevundimonas diminuta*, which is approximately 0.4×1.2 µm in size [16]. These filters are usually described as having a

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nominal pore size of 0.2 or 0.22 µm, although direct measures of the pore size for different sterile filters indicate a broader pore size distribution with some pores reaching as large as 0.5 µm in diameter. The pore size distribution for the Durapore® membrane as determined by mercury intrusion porosimetry is shown in Figure 1 as an example; the pore size distributions for other sterile filters are similar [17].

The overlap in the size distribution between the vaccine and the sterilizing grade membrane creates significant challenges in the design and operation of the sterile filtration process. In particular, the retention of vaccine particles on or within the sterilizing grade filter can cause:

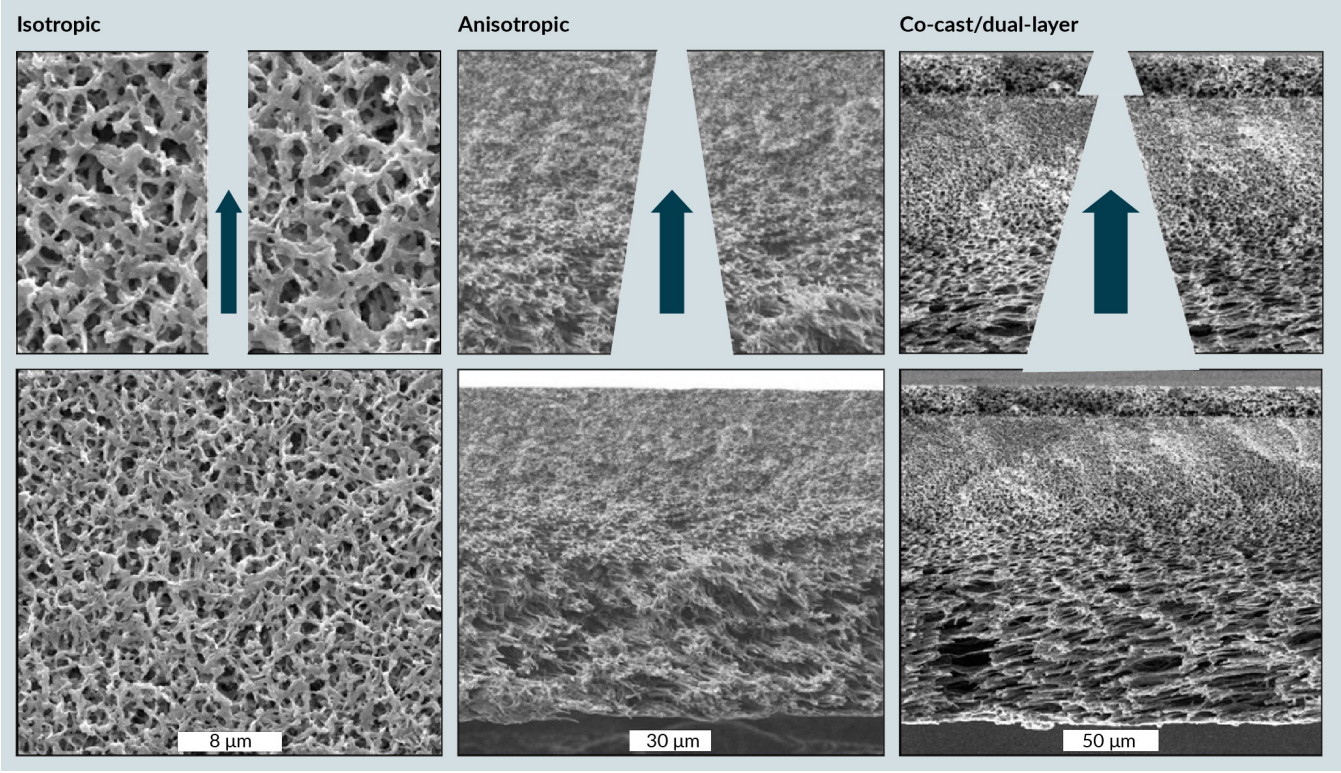
- Significant yield loss for the vaccine product
- Large increases in the resistance to filtration
- Low filter capacities (defined as the maximum volumetric throughput per unit membrane area at the point where the filter needs to be replaced, usually because the maximum pressure limit has been exceeded during filtration at a constant filtrate flow rate)

Considerable effort may be required to identify sterile filtration membranes and operating conditions that can provide the desired product yield and capacity, with different sterilizing grade filters showing dramatically different performance characteristics.

Sterilizing grade filters were originally designed as homogeneous membranes in which the effective pore size was relatively uniform throughout the depth of the filter (Figure 2, left panel). The overall resistance to flow provided by the membrane can be significantly reduced using an anisotropic (often referred to as asymmetric) structure in which

FIGURE 2

Scanning electron micrographs of cross-sections through isotropic, anisotropic, and co-cast/dual-layer sterile filters with the upper panels showing a schematic of the pore size gradient.



Images adapted from [18].

the pore size decreases as one moves through the depth of the filter (Figure 2, middle panel). The more open pores at the filter entrance can also serve as a built-in 'prefilter' that provides at least some protection against fouling in the size-selective barrier at the filter exit. Today, most sterilizing grade filters are sold as co-cast or dual-layer membranes (Figure 2, right panel) in which there are two distinct layers, each with its own pore size and gradient (homogeneous or anisotropic). The pore size of the first 'prefilter' layer (most commonly 0.45 or 0.8 μm) is chosen to maximize the overall yield and capacity, while the second layer (nominal 0.2 μm pore size) serves as the final barrier ensuring the sterility of the drug product. The membrane surface chemistry can be chosen to reduce adsorptive losses arising from both hydrophobic and electrostatic interactions between the vaccine and the membrane. More hydrophobic polymers like polyvinylidene fluoride are typically surface-modified to increase the hydrophilicity and reduce adsorptive interactions. Ultimately, the selection of sterilizing grade filter morphology and chemistry (e.g., polyethersulfone, polyvinylidene fluoride, or cellulosic) is largely empirical, requiring extensive experimental studies to identify the optimal filter and operating conditions for any given vaccine product given the very different size, deformability, and surface characteristics of the different vaccines.

Sterile filtration can be performed at either a constant filtration rate (i.e., constant flux) or at constant transmembrane pressure. In constant flux operation, the capacity is determined by the increase in transmembrane pressure during the filtration, while the capacity at constant transmembrane pressure is determined by the decline in filtrate flux. Small-scale experiments performed using circular disk filters can be used for initial assessment of the filter capacity (maximum volumetric throughput per unit membrane area). Constant flux operation is generally favored for manufacturing since it is more compatible with vial filling procedures [19,20]. These larger-scale pilot and commercial-scale filters are commonly provided as pleated membranes in a cylindrical cartridge [21]. Ideally, a linear scale-up would be expected (constant volumetric capacity per unit membrane area); however, non-uniformities in flow distribution and parasitic pressure losses in large filter cartridges can lead to scale-dependent differences in performance [22,23].

CHALLENGES IN STERILE FILTRATION OF VACCINES

Although all vaccine products tend to cause fouling during sterile filtration, recent studies have demonstrated that the underlying fouling mechanisms can be dramatically different depending on the unique properties of the vaccine. Figure 3 shows scanning electron micrograph images of the upper filtering surfaces of two sterilizing grade filters, one after filtration of a LAV candidate and one after filtration of an mRNA-containing LNP. The LAV is clearly visible as small spheres that restrict and block pores in the Sartobran® P membrane. The fairly rigid protein capsid that surrounds the LAV stabilizes the deposited virus as individual particles on the filter surface. In contrast, the LNPs form a thin amorphous layer that fully covers the filter surface, with some regions where the amorphous deposit appears to have intruded through the open pores causing a 'donut hole' to appear in the fouling layer. The drag and pressure forces during sterile filtration appear to cause the LNPs to 'fuse' into the amorphous deposit seen in the right-hand panel. This also gives rise to an unusual pressure dependence in the resistance of the fouled membrane formed during filtration of the LNPs, with a maximum in the resistance obtained at a transmembrane pressure of about 8 psi [24].

These differences in fouling mechanisms can also lead to large differences in the performance characteristics when using different sterile filters. Figure 4 shows a comparison

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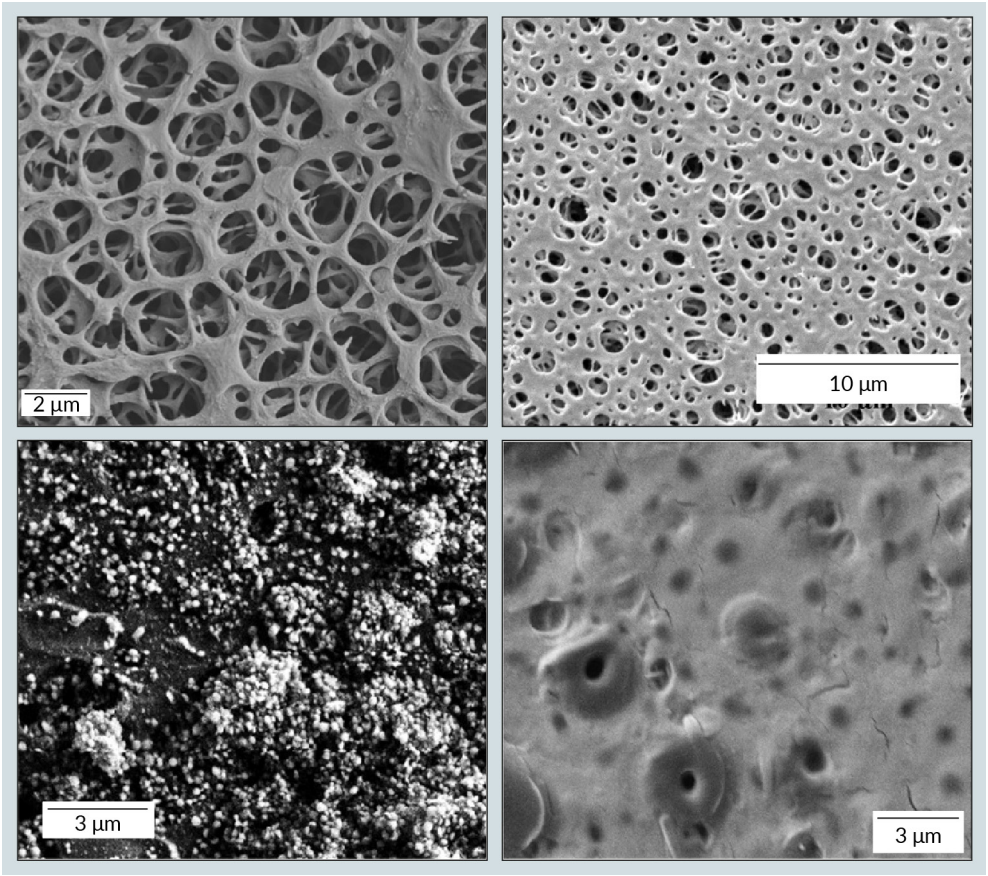
of the capacities for three different sterile filters: the Millipore Express® (asymmetric PES), Durapore (symmetric PVDF), and the Sartopore® Platinum (0.45/0.2 µm asymmetric dual-layer PES). Data were obtained using a live attenuated cytomegalovirus vaccine candidate (LAV) and a single serotype of a glycoconjugate vaccine. A more extensive analysis of the fouling behavior for filtration of the LAV through different sterilizing grade filters can be found elsewhere [26]. Capacity is defined as the volumetric throughput at a maximum transmembrane pressure of 10 psi. The LAV had the greatest capacity when using the Sartopore Platinum membrane while the lowest capacity was obtained with the highly asymmetric Millipore Express. The glycoconjugate drug substance showed the opposite behavior, with the highest capacity obtained with the Millipore Express and the lowest capacity for the Sartopore Platinum. In addition, the glycoconjugate yield was greater than 95% for all three filters, but the LAV yield was 65 and 69% for the Durapore and Sartopore Platinum, respectively, and only 1% for the Millipore Express. The very low yield provided by the Millipore Express was due to the restrictive pore structure of this membrane and the rigidity and large size of the LAV particles.

An attractive approach for increasing the capacity during sterile filtration of many vaccine products is to use an appropriate

FIGURE 3

Scanning electron micrographs of the upper surface of a 0.2 µm Sartobran® P membrane before (top) and after (bottom) filtration of a live attenuated viral vaccine candidate (left panel, from [5]) and a 0.2 µm Sartopore® XLG membrane before (top) and after (bottom) filtration of an mRNA-containing lipid nanoparticle (right panel, from [24]).



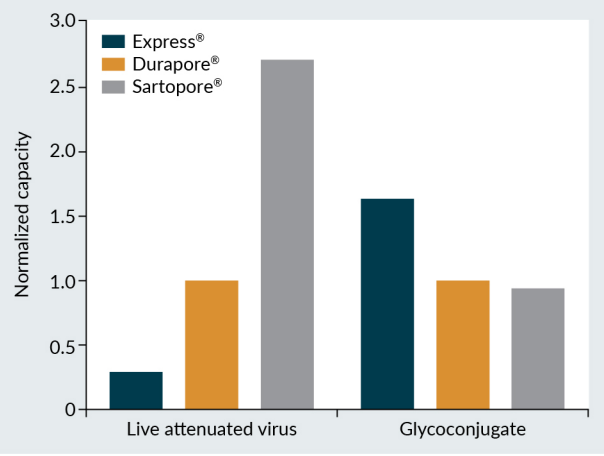
The LAV particles maintain their structural integrity (spherical shape) while the LNPs appear as an amorphous deposit likely consisting of the various lipids comprising the LNPs.

prefilter, either as part of a dual-layer membrane or using an inline two-stage filtration. The prefilter effectively removes larger foulants, including any aggregated material that may be present in the vaccine product, protecting the tighter pores in the sterilizing grade filter from fouling and thus prolonging the operational life of the filter as a whole. Messerian *et al.* [27] showed that the 0.8 µm prefilter in the dual layer Sartopore 2 XLG membrane provided a 2.5-fold increase in capacity compared to the sterilizing grade filter alone during sterile filtration of a LNP product. Du *et al.* [6] obtained more than a 100-fold increase in capacity by using a 5 µm pore size prefilter during sterile filtration of a single serotype of a glycoconjugate vaccine drug substance. In this case, the dramatic improvement in capacity was due to the removal of trace amounts of very large aggregates (>1 µm in size) as confirmed by dynamic light scattering.

The impact of a prefilter on the fouling of the dual-layer (0.45/0.2 µm) Sartobran P during sterile filtration of a live attenuated cytomegalovirus vaccine candidate can be seen in the confocal images shown in Figure 5. The captured LAV were fluorescently labeled in situ after the filtration [28]. The initial fouling (at 20 L/m^2 capacity) occurred primarily in the 0.45 µm prefilter, but as the prefilter becomes saturated a greater degree of LAV are captured throughout the depth of the 0.2 µm sterilizing grade layer. The accumulation of LAV in the 0.2 µm layer occurs much more rapidly when the filtration is performed without the 0.45 µm prefilter, significantly reducing the overall capacity of the sterile filter.

FIGURE 4

Capacity of different sterilizing grade filters (normalized by the capacity of the Durapore sterile filter) for sterile filtration of a live attenuated cytomegalovirus vaccine candidate and a single serotype of a glycoconjugate vaccine drug substance.



Capacity defined at a maximum pressure of 10 psi. The Sartopore® Platinum had the highest capacity for the LAV while the Millipore Express® had the highest capacity for the glycoconjugate. Data taken from [25,26].

TRANSLATION INSIGHT

Ongoing advances in our understanding of infectious disease and cancers has created exciting opportunities for novel vaccines. The successful commercialization of these vaccine products requires the development of cost-effective manufacturing processes, and in the case of pandemics, these processes need to be developed and transitioned from the lab to commercial-scale production on incredibly short timelines. The large size of mRNA, glycoconjugate, and LAV vaccines can create significant challenges in downstream processing, particularly for the sterile filtration step in which the vaccines need to be passed through membranes with 0.2 µm-rated pore size. Recent insights into the underlying physical phenomena governing the fouling behavior of these diverse vaccine products should enable membrane manufacturers to tailor the pore size and underlying pore morphology to develop new sterile filtration membranes that are specifically designed to meet the unique challenges in vaccine filtration. These new membranes could lead to significant improvements in vaccine yield and filter capacity, with streamlined process development timelines, enabling the industry to produce safer and lower-cost vaccines for global health.

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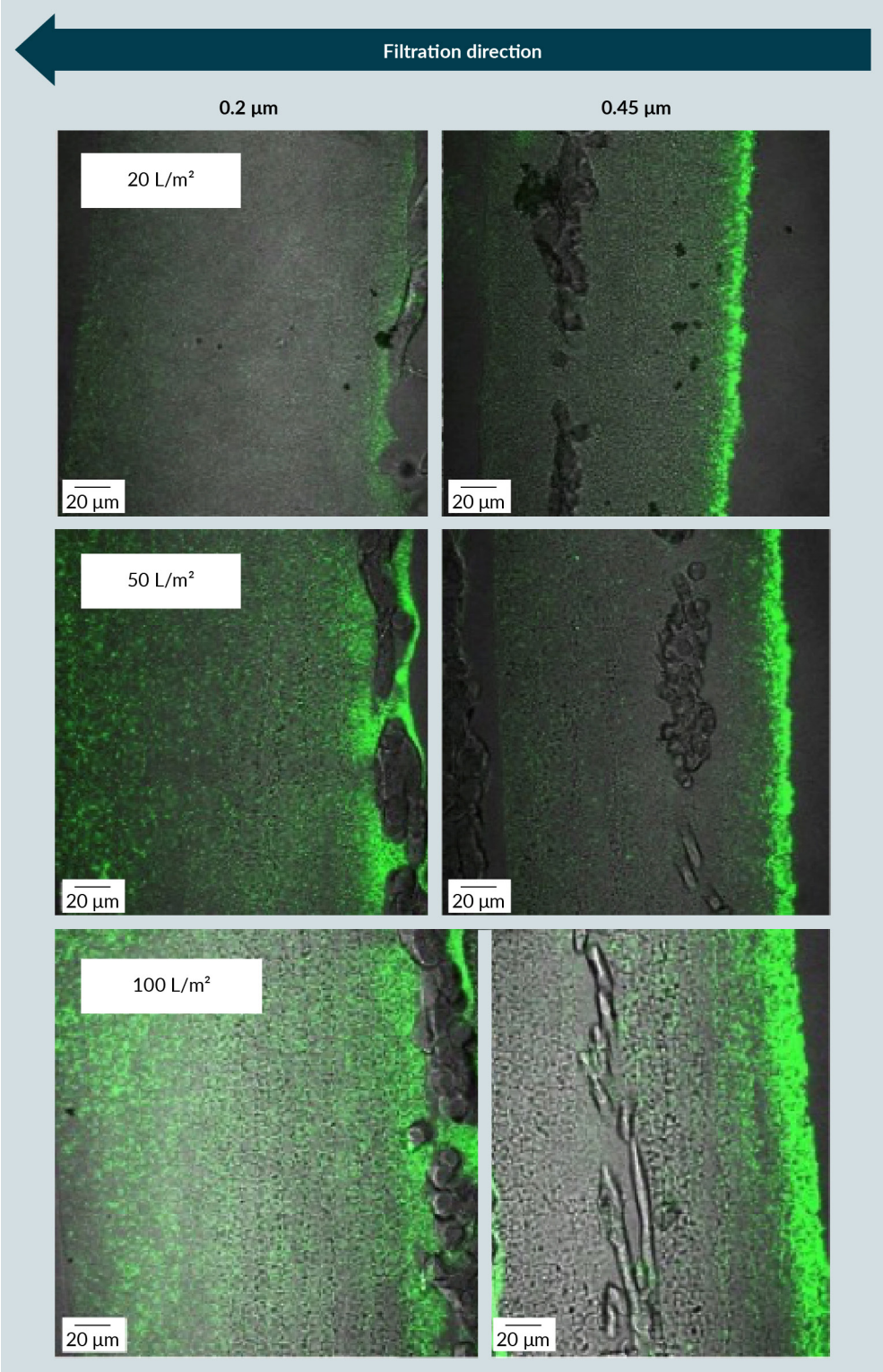
FIGURE 5

Confocal images of the 0.45 µm and 0.2 µm layers of the Sartobran P dual-layer sterile filter during filtration of a live attenuated cytomegalovirus vaccine candidate at different volumetric throughput.



The LAV capture in the prefilter protects the downstream (sterilizing grade) layer from key foulants.

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AFFILIATIONS

Andrew L Zydney
Department of Chemical Engineering, The Pennsylvania State University, University Park, USA

Kevork Oliver Messerian
Department of Chemical Engineering, The Pennsylvania State University, University Park, USA

Zhuoshi Du
Department of Chemical Engineering, The Pennsylvania State University, University Park, USA

Neil Taylor
Eli Lilly and Company, Indianapolis, USA

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Correspondence: Communication concerning this manuscript should be addressed to: Andrew Zydney, Department of Chemical Engineering, The Pennsylvania State University, University Park, PA 16802, USA. Phone: 814-863-7113; Fax: 814-865-7846; zydney@engr.psu.edu.

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INTERVIEW

Optimizing CMC to make vaccines for the world

Charlotte Barker, Editor, *Vaccine Insights*, speaks to **Piper Trelstad**, Head of CMC, Bill & Melinda Gates Medical Research Institute about her long career in vaccine manufacturing and recent move to the nonprofit sector. They discuss CMC challenges, new technologies, and the key to a successful partnership.

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Q What first drew you to the vaccine field?

PT: I went to graduate school with the intention of pursuing environmental work and found myself in the vaccines field! Merck offered me a position that met my desire for my work to have a societal impact—I took that job and never looked back.

I love the impact that these products can have. While I was working at Merck, I supported the measles, mumps, and rubella vaccine, which led to a huge drop in the incidence and mortality of these diseases. Vaccines have a huge impact across the board, and it feels amazing to contribute to providing them.

Q What led you to make the move from the pharmaceutical industry to the nonprofit sector last year, and what does your current role entail?

PT: Several of my former colleagues were working in global health nonprofits, and I knew it was something I wanted to do in the next stage of my career. When the opportunity came up at the Gates MRI, I could not turn it down. The impact and potential of the work done here is incredible.

For me, it was also a chance to expand from the vaccines I have always worked on to other modalities. Right now, we are working on monoclonal antibodies, a small molecule TB drug regimen, and even a probiotic trial, so there is a lot of diversity. From a learning perspective, this has been a nice challenge.

I lead our Chemistry, Manufacturing, and Controls (CMC) team, which holds key responsibilities at the Gates MRI. First, we provide the material that will be used in the clinic. We produce that material and—via our clinical supplies team—ensure it gets where it needs to be on time. We are also responsible for developing robust and cost-effective manufacturing and analytical processes.

The scope of the Gates MRI is product development from pre-clinical to all stages of clinical, but not commercial. We transfer our manufacturing processes to the companies responsible for commercializing them and getting them to people who need them. In CMC, we must ensure we have robust processes and cost-effective products for this transfer. Our targets are diseases that impact disproportionately low and middle-income countries (LMICs), so keeping costs down is critical.

Q What projects at the institute are you most excited about right now?

PT: I am excited about our TB vaccine, originally developed by GSK (who we continue to partner closely with). In the coming months, we will be starting a large phase 3 trial to bring this to the world as the first product meeting the WHO target product profile for a TB vaccine. This will allow us to prevent inactive latent TB—which impacts 25% of the world's population—from developing into active TB. Right now, there are 10 million cases of active TB annually, leading to over 1.5 million deaths each year. This vaccine could have a tremendous impact.

We have scaled our manufacturing processes up to commercial scale, produced the material, and are now thinking about getting this product ready to reproduce commercially. Our teams are working on understanding our design space for this product, and ensuring we have robust, well-characterized processes so that when a partner takes this on, they can have the confidence to produce the vaccine reliably at the required cost.

It is a reasonably straightforward manufacturing process. It is a recombinant protein vaccine produced in *E. coli* and a lyophilized drug product, but as with many vaccines, we do not fully know the mechanism of action. As we scale, our role is to ensure we have a high level of control as we ready ourselves for phase 3 and commercial production.

Q What lessons can you carry forward from your career in big pharma to your current role?

PT: The Gates MRI is structured like a typical biotech or pharma company—98% of our employees have a history within industry, and those learnings are applicable.

When I joined Gates MRI, we only had a small CMC team, and over the last 1.5 years, we have concentrated on growing that team to be able to support our programs with multiple different studies as we move into late-stage development.

From my experience in pharma, I knew that we had to build an analytical development group. We live and die by our assays; they can make or break a program. Having a group focused on ensuring our assays are robust, reliable, and interpretable is critical. We now have an analytical development team that spans all modalities.

I also wanted to ensure we had people getting the systems, processes, and governance structure in place to manage all this work, so we created a CMC operations team.

Q What differences have you noticed between sectors?

PT: One key difference is that we do not have any labs or manufacturing facilities ourselves so we must manage and maintain good relationships with our suppliers. One of our biggest challenges is identifying the strategic partners we can work with on multiple programs.

As our organization is small (around 160 employees plus partners and contractors) the diversity of our modalities can pose a challenge. The skill sets needed within the team vary widely between our small molecules, biologics, and vaccines.

Q What makes for a successful partnership?

PT: You start with the basics—ensuring they have the right capabilities and capacity to meet your needs. Once you check those boxes, it's building the relationship that counts. This is something that you work on and continue to drive over time. You must ensure the right flow of communication and clear governance at all levels of the team.

These relationships need to work for both sides. Typically, the companies we work with are excited about the impact of our work. They love being able to talk with their own teams about this work, but it needs to work from all other areas too. They need to be able to progress products and make profits, and we need to ensure that we can develop these products in a reasonable timeframe.

Q How has COVID-19 changed the vaccine manufacturing space?

PT: The COVID-19 vaccine situation has made it clear that the lack of vaccine manufacturing capabilities in LMICs has a material impact on the availability of vaccines for those countries. We are seeing a great push to enable and support vaccine manufacturing in these countries on the manufacturing side, the financing side, and the policy side. Many elements need to come together, but we have seen a good pivot point in how vaccines are produced around the world.

Q What are the main roadblocks in vaccine production right now? How do we overcome these?

PT: Production in LMICs continues to be a big challenge and requires a lot of focus. As we look at the back-end manufacturers for our products, we think a lot about where we will produce these products. Cost of goods is such an important element in enabling access. As we move our programs, including our TB vaccine, into late-stage development, it is paramount that we factor that in. Achieving equitable access to vaccines and where they are manufactured is a big need for the industry.

Q Have any new technologies or techniques caught your eye recently?

PT: As we think about delivering these vaccines and therapeutics, we find that we often leverage either existing or new technologies to meet the needs of these indications in LMICs.

Our shigella vaccine, which is in the preclinical stage, was developed by The Institut Pasteur and is an example of where we are trying to do things differently. Shigella vaccines have historically been tricky to develop. The age group that is most impacted by shigella and has the highest risk of disease and mortality is infants. There have been vaccines developed that work well in adults or children but struggle to show the same efficacy in infants.

Conjugate vaccines with oligosaccharides attached to a carrier protein are typically used for infants. An animal study performed by The Institut Pasteur showed that if you tightly control the oligosaccharide length and density, a significant boost in immune response is seen in animals. For this vaccine, rather than harvesting the lipopolysaccharides from organisms, we are chemically synthesizing them to have absolute control over the length and density. This could be a game-changer in terms of efficacy. It is still early days, but we are working towards getting these into toxicity studies and ultimately into the clinic. As a new variation on the classic glycoconjugate vaccine, we think it could make a difference.

We are also working on increasing levels of characterization, for instance for our TB vaccine. There is more we can do to understand our product and process, and we are looking at what tools we can apply in that space.

Q What's next—for you and your team, and the wider field?

PT: Our goal at Gates MRI is to continue to progress our programs. Our products sit within a wide range of product development stages, including our late-stage TB vaccine and our preclinical shigella vaccine. We will continue to build our capabilities as an organization as we move into late-stage, and identify the strategic partners that can enable our work. We are making a lot of progress, and it is an exciting time for us.

In the wider field, there continues to be interest, excitement, and progress with mRNA vaccines. We are even seeing work with these in the malaria and TB areas. The production of vaccines in LMICs will continue to evolve, and we will continue to engage with this. These continue to be important products for global health.

BIOGRAPHY

PIPER TRELSTAD is Head of Chemistry, Manufacturing, and Controls (CMC) at the Bill & Melinda Gates Medical Research Institute. Piper leads a team responsible for the

development of robust, innovative, and cost-effective manufacturing processes to ensure clinical supply availability for the institute's drug and vaccine candidates. Piper brings over 20 years of experience in vaccine development, manufacturing, and supply chain management, during which time she provided strategic and technical support for a variety of vaccine products at differing stages of development. Prior to joining the Institute, Piper served as Vice President of Technical Development for Takeda's Vaccine Business Unit, where she provided essential shared services and overall leadership for the cross-functional teams responsible for process, formulation, and analytical development of vaccine products.

AFFILIATION

Piper Trelstad PhD
Head of Chemistry, Manufacturing, and Controls,
Bill & Melinda Gates Medical Research Institute

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

SPOTLIGHT

Establishing self-sustaining vaccine manufacturing ecosystems: Africa's priority diseases

Nicaise Ndembi, Mahlet Kifle Habtemariam, Ngashi Ngongo, and Jean Kaseya
Africa Centres for Disease Control and Prevention




"The challenges in access to vaccines on the continent underscore the urgent need for locally produced and accessible vaccines as a public health security measure."

VIEWPOINT

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Infectious diseases threaten Africa's aspiration to achieve its 2063 developmental blueprint: 'Agenda 2063: The Africa We Want.' The rise of global protectionism and vaccine nationalism has highlighted the necessity to address African problems with African solutions. This has led to increased support from African governments, African and global public health institutions, foundations, philanthropists, and the private sector to expand manufacturing of health products in Africa while prioritizing vaccines.

Today, Africa's total public market vaccine sales are approximately US$1.3 billion, equivalent to about 4% of the approximately US$33 billion global market, while Africa's demand is about 25% of global volumes. Africa represents 17% of the world's population but has 0.1% of the world's vaccine production.

As has been witnessed with the COVID-19 pandemic, over-reliance on external sources and procurement for vaccines renders the continent vulnerable to existing and future disease outbreaks [1]. A lack of manufacturing is one of the reasons for the late vaccination of the African population during the pandemic and even now less than 40% of the African population is fully vaccinated against COVID-19. The challenges in access to vaccines on the continent underscore the urgent need for locally produced and accessible vaccines as a public health security measure. Africa's leaders are on a path to ramp up vaccine development and manufacturing capacity and strengthen the entire ecosystem to support a sustainable vaccine manufacturing sector [2].

From April 12–13 2021, African Union (AU) Heads of State and over 40,000 participants attended a virtual summit on 'Expanding Africa's Vaccine Manufacturing for Health Security', convened by the Africa Centers for Disease Control and Prevention (Africa CDC). During the summit, African leaders pledged to increase the share of vaccines locally manufactured in Africa from 1–60% by 2040. This vision will be achieved by establishing a sustainable vaccine development and manufacturing ecosystem underpinned by research and development, IP and technology transfer, innovative and sustainable financing, strategic partnerships, and commitment by African and global procurement agencies to purchase vaccines produced on the continent.

Approximately US$30 billion will be required to implement the continental Framework for Action (FFA), of which around US$5 billion is needed to fund capex and other one-off costs (primarily the set-up of the required vaccine manufacturing plants, the related cold-chain infrastructure, and the operationalization of key FFA programs). The remaining US$25 billion is needed to fund recurring costs over 20 years, including investments into R&D for priority diseases and continual process improvement for vaccine manufacturing, royalties paid out for technology transfers, and additional spending on increased vaccine procurement on the continent.

The Partnerships for African Vaccine Manufacturing has prioritized getting the support and alignment of relevant organizations, which is key to successful and sustainable vaccine manufacturing and supply to the continent in the short, medium, and long term. These entities include Gavi (through the new financial instrument, the African Vaccine Manufacturing Accelerator, which aims to provide support for sustainable procurement and long-term vaccine manufacturing on the continent), UNICEF, the Global Fund for HIV/AIDS, TB and Malaria, Bill & Melinda Gates Foundation, Master Card Foundation,

and the Coalition for Epidemic Preparedness Innovations [3]. They can all play a pivotal role in fostering alignment and support for local manufacturers to get access to technology, products, systems, and resources to enable participation in vaccine supply to the continent [4,5,6].

The Partnerships for African Vaccine Manufacturing was launched in April 2021 and the following year the continental FFA, with its eight bold programs, was endorsed by the AU policy organs in February 2022 [7]. Africa CDC defined its 22 priority diseases that will benefit from local vaccine manufacturing. Together with the Clinton Health Access Initiative and in consultation with Africa CDC, Gavi analyzed potential antigens for prioritization through the lens of market health and market-shaping needs. Preliminary results indicate that commercial sustainability and market health can be maximized by focusing on priority antigens where new manufacturers are most beneficial to markets. Dynamic market circumstances notwithstanding, priority antigens might include antigens with a need for additional suppliers or capacity, either today or in the future, as well as antigens with the greatest potential for epidemics on the continent. Examples could include oral cholera, measles-rubella, yellow fever, malaria, and Zaire ebolavirus.

Growth in the vaccine industry will also need to be accompanied by growth in supporting industries to provide raw materials and inputs, including active ingredients for the different types of vaccines, inactive ingredients (including buffer, media, and other excipients), and consumables (including vials, sterile bottles, syringes, and rubber stoppers). Africa should support end-to-end value and production chains to ensure that the required input materials are also produced locally. This will be critical to unlocking key benefits such as self-reliance and health security.

The FFA also prioritizes seven vaccine manufacturing technologies to provide sufficient flexibility to produce these vaccines. These include traditional technologies such as live attenuated virus technologies, which will be critical in manufacturing vaccines with high demand, and novel technologies such as mRNA, which are likely to grow in scale as the science and investment supporting the technologies advance.

The FFA recommends that the African vaccine manufacturing ecosystem focus on strengthening eight enablers, which will require it to roll out eight bold programs. To start with, an ambitious pooled procurement mechanism will drive sustainable and reliable volumes through economies of scale, and a deal preparation facility will mobilize the considerable investment needed. Efforts in technology transfers, regulation, R&D, and infrastructure will strengthen the enabling environment required for success. Regional Capability and Capacity Centers will be established to enhance human capital in the ecosystem, ensuring adequate skills in all key enablers. Overall, a strong focus on continent-wide strategy delivery and oversight will ensure that all programs are implemented effectively and harmoniously.

Demand certainty is a key accelerator of vaccine manufacturing in Africa and a focus area of great urgency for African vaccine manufacturers to commit to investing and ensuring sustainability. Without advanced market commitments guaranteeing the offtake of vaccines produced locally in Africa and pooled procurement mechanisms to ensure economies of scale, investments in scaling up vaccine manufacturing capacities will be at serious risk. Efforts to ensure that Africa does not remain vulnerable in accessing vaccines when they are needed most would be negated. Market guarantees from African governments, supranational procurement agencies, and international partners (Gavi, African Vaccine Market Accelerator, WHO, UNICEF, Team Europe Initiative, Master Card Foundation, Bill & Melinda Gates Foundation, World Economic Forum, World Bank, Coalition for Epidemic Preparedness Innovations and the International Vaccine Institute) are necessary to incentivize investments in and sustain

vaccine manufacturing operations at scale across the continent.

Boosting the establishment of continental regulatory systems through the African Medicines Agency (AMA) will further support a sustainable vaccine manufacturing industry in Africa. AMA aims to provide a platform for coordinating and strengthening ongoing medicines regulatory harmonization initiatives across the Continent. It plans to ensure optimal use of scarce resources by pooling expertise capacities and strengthening existing networks. A network of well-established National Regulatory Authorities (NRAs) from countries with the intent to produce vaccines and other NRAs with advanced maturity levels, using the African Medicines Regulatory Harmonisation structure with close coordination with the AU Development Agency—New Partnership for Africa's Development will promote acceptance of products approved by the NRAs as a short-term solution. In addition, as we wait for AMA to be fully operationalized, strengthening NRAs with maturity level 3 to reach maturity level 4 will facilitate regional regulation to optimize the use of limited resources [8,9,10]. This will be implemented in collaboration with the WHO.

In summary, investing in a local vaccine manufacturing industry in Africa will result in significant benefits, including increased sovereign health security, harmonization of regional regulatory and trade policy, a boost in manufacturing, technological expertise, and talent development, a strategic contribution to global vaccine supply chains during public health emergencies as well as non-emergency situations, and improved economic growth. The continent has adopted a strategy of respectful local and global partnerships, to achieve the goal of having a sovereign vaccine supply to its people.

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BIOGRAPHIES

NICAISE NDEMBI is a Senior Advisor to the Africa CDC Director General, and currently Associate Professor, Division of Epidemiology and Prevention at the Institute of Human Virology, University of Maryland School of Medicine, Baltimore, USA. Ndembi is a graduate of Kanazawa University School of Medicine in Japan, Department of Viral Infection and International Public Health, and a Research Professor within the same institution. He is a Principal Investigator on numerous grants including the US National Health Institute. He has authored/co-authored more than 200 publications in peer-reviewed journals. He is the Editor-in-Chief of the *Journal of Public Health in Africa*, *AIDS Research and Therapy*, and serves on various WHO and US NIH Advisory Groups.

MAHLET KIFLE HABTEMARIAM is Special Advisor and Deputy Chief of Staff at the Africa CDC. Her role encompasses overseeing the Executive Office's operations and spearheading key initiatives. With a background spanning over 15 years in public health, Mahlet is experienced in developing and implementing health policies, strategies, and programs at both national and international levels. In her tenure at Africa CDC, she has held key positions, initially as a Principal Program Officer and subsequently as a Senior Regional Engagement Advisor, where she led significant institutional initiatives. Before that, Mahlet held progressively responsible positions at the Ministry of Health of Ethiopia, including the Chief of Staff role in the Office of the Minister. She holds a Doctor of Medicine from Addis Ababa University and a Master of Public Health from University College Dublin. Mahlet is a Takemi Fellow of the Harvard T. H. Chan School of Public Health and an African Public Health Leaders Fellow at Chatham House.

NGASHI NGONGO (ALAIN) is a Public Health physician with 33 years of national and international experience. He is currently the Africa CDC Chief of staff and head of the Executive Office. He is the founder of the International Centre for Health Systems Strengthening, USA, and served as International Centre for Health Systems Strengthening Executive Director from 2019–2023. Before that, he served as Team Leader of the UNICEF Global Malaria Program, New York, USA from 2010–2012, Head of UNICEF Prevention of Mother-To-Child Transmission and Pediatric HIV Care Programs, New York, USA from 2003–2006; Head of the UNICEF Health System Strengthening department, New York, USA from 2013–2016; Team Leader, UNICEF Ebola Health Response, Liberia from 2014–2015, Democratic Republic of Congo in 2018; Head of UNICEF Child Survival and Development Program for West and Central Africa from 2016–2019; Regional Polio Team Lead for West and Central Africa from 2016–2019; Head of UNICEF Health and Nutrition Program in South Africa from 2006–2009; National Coordinator of the COVID-19 Vaccination in Democratic Republic of Congo in 2022; Board Member, PROSAMI, Virginia, USA from 2010 to present; Board Member of MMV, Switzerland from 2022 to present, Observer Board Member of Gavi in 2023, and Lecturer at the School of Public Health, University of Lubumbashi, Democratic Republic of Congo. Ngashi has conducted and participated in several studies and surveys.

JEAN KASEYA is the first Director General of Africa CDC, appointed by the Heads of State and Government during the 36th Ordinary Session of the Assembly of the African Union. Kaseya is a public health physician with a Master's degree in Epidemiology and Community Health and over 25 years of national and international experience. His extensive career includes leadership roles with WHO, UNICEF, Gavi the Vaccine Alliance, Clinton HIV/AIDS Initiative and others, equipping him with exemplary experience in managing public health programmes, policy development, negotiation, coordination, and grant management. Kaseya's expertise spans political, strategic, and technical realms and his association with significant organizations coupled with international roles reflects his strategic leadership and impact. Currently he is serving as a technical review panel member of Global Fund to Fight AIDS, Tuberculosis and Malaria for Malaria and Pandemic Preparedness, and Board member of Santé Rurale. At the helm of Africa CDC, Kaseya leads the delivery of core functions and objectives, shaping initiatives that enhance global health security across Africa through improved disease surveillance, health system strengthening, and crisis response.

AFFILIATIONS

Nicaise Ndembi
Africa Centres for Disease Control and Prevention,
African Union Commission,
Addis Ababa, Ethiopia

Mahlet Kifle Habtemariam
Africa Centres for Disease Control and Prevention,
African Union Commission,
Addis Ababa, Ethiopia

Ngashi Ngongo
Africa Centres for Disease Control and Prevention,
African Union Commission,
Addis Ababa, Ethiopia

Jean Kaseya
Africa Centres for Disease Control and Prevention,
African Union Commission,
Addis Ababa, Ethiopia

AUTHORSHIP & CONFLICT OF INTEREST

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VACCINEINSIGHTS

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

SPOTLIGHT

What is a sustainable vaccine manufacturing footprint for Africa?

Philip Dorrell and Rishabh D Jhol
Clinton Health Access Initiative

VIEWPOINT

"...it is critical to ensure that [vaccine manufacturing] progress is sustainable and meets the needs of Africa (and the world) in the long term."

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Given the inequities revealed through the COVID-19 pandemic, significant interest and funding have been directed at boosting vaccine manufacturing capacity in Africa. However, this growth must be commercially sustainable and avoid excessive negative externalities on the wider vaccine ecosystem. A new white paper from the Clinton Health Access Initiative proposes a sustainable, balanced goal for African vaccine manufacturing capacity and highlights the most important funding and policy interventions to achieve it.

INTRODUCTION

The COVID-19 pandemic highlighted the importance of local manufacturing to ensure vaccine supplies during times of emergency. Export restrictions, hoarding, and nationalism meant that even relatively wealthy countries struggled to procure COVID-19 vaccine doses from outside their borders.

African nations were some of the last to receive sufficient COVID-19 vaccine doses. Less than 1% of all the routine vaccine doses administered in Africa in any given year are manufactured on the continent, leaving African countries in a vulnerable position in terms of pandemic preparedness. To mitigate these risks and spur economic growth, the African Union and The Africa Centres for Disease Control and Prevention (Africa CDC), through the Partnership for African Vaccine Manufacturing initiative, have set an ambitious target for 60% of vaccine doses administered in Africa to be manufactured on the continent by 2040.

There has been significant support for boosting vaccine manufacturing capacity on the continent. Over US$4.5 billion, including some soft commitments and existing lending ceilings, has been pledged by stakeholders, while the Gavi Alliance, the region's largest vaccine financer, has updated its global healthy market criteria to put more consideration into regional supply. Around 30 new vaccine manufacturing projects have been commissioned on the continent, with a total capacity of close to 2 billion doses already installed or ordered, and a planned capacity of up to 4 billion doses.

However, while momentum for change is welcome, it is critical to ensure that progress is sustainable and meets the needs of Africa (and the world) in the long term.

With funding from the Bill & Melinda Gates Foundation, the United States Agency for International Development, and support from Africa CDC, the Clinton Health Access Initiative set out to define a sustainable, fit-for-purpose target pathway for African vaccine manufacturing and identify the most effective interventions to achieve that goal. We hope that the resulting white paper [1] will act as a roadmap for all stakeholders.

WHAT IS A SUSTAINABLE TARGET FOR AFRICAN VACCINE MANUFACTURING?

By speaking with a wide range of stakeholders, we identified three key goals for African vaccine manufacturing:

- Pandemic preparedness and response;
- Healthy global vaccine markets; for example, markets that sustainably ensure access to quality-assured affordable vaccines without supply disruptions;
- Long-term commercial viability.

There is a need for compromise between these goals; for example, if considering only pandemic preparedness, the target would be to develop end-to-end vaccine manufacturing capacity for enough doses to vaccinate all of Africa. However, this would be commercially unsustainable and not consider the market at large. From a global health perspective, the ultimate goal is to have a sustainable supply of affordable vaccines.

We found that a balanced target by 2030 would be approximately 170 million doses of end-to-end manufacturing capacity, plus approximately 460 million doses of antigen-agnostic drug product capacity,

giving a combined output of 630 million doses. This represents close to 40% of vaccine doses administered in Africa (and aligns with the Partnership for African Vaccine Manufacturing target of 60% by 2040).

The sustainable target capacity outlined is well below the 2 billion (mostly drug product) dose capacity installed or ordered, leading to overcapacity and a risk of unsustainable 'white elephant' projects. Drug substance capacity, meanwhile, remains low. In addition, more than 60% of the installed drug product capacity lacks the technology transfer partnerships essential for commercialization.

Based on this target, we estimate that the continent can support a maximum of three to five geographically dispersed manufacturers with a diversified portfolio of antigens.

These manufacturers will face a number of challenges, including structural cost disadvantages compared with manufacturers in other developing countries, particularly India. To compete, a regional market approach will be needed, with African governments committing to preferentially buy vaccines from manufacturers across the continent (not just domestically). In addition, many national regulatory bodies in the region lack the capacity to regulate vaccine production, preventing timely market access.

HOW CAN WE ACHIEVE THE TARGET?

We identified five key areas for intervention to help achieve a meaningful target of approximately 630 million doses produced in Africa by 2030. First, if stakeholders with an interest in African vaccine manufacturing can align investments and support towards a common target, the chances of achieving a sustainable African vaccine manufacturing footprint can be maximized.

Second, funding from the Gavi African Vaccine Manufacturing Accelerator and other initiatives should be used to offer financial incentives to enhance manufacturers' cost competitiveness.

Third, technology transfer partnerships between African manufacturers and originators must be prioritized since they are vital to making use of existing drug product capacity and expanding drug substance capacity longer-term.

Fourth, while challenging, it is critical to enact a collaborative procurement policy to ensure demand from African governments for African-made vaccines, which is currently being championed by Africa CDC.

Finally, an appropriate enabling environment must be created by investing in policy, education, and regulatory capacity to support vaccine manufacturing.

We believe that these interventions are best placed to balance the immediate need for rapid capacity-building in Africa with the long-term need to maintain commercial stability and deliver sustained access to affordable immunization products. It is heartening to see that many of these efforts are already underway, and the target pathway can serve as a 'north star' for stakeholders to align their existing and planned initiatives for further development of the African vaccine manufacturing ecosystem.

Given the energy, intention, and funds being directed to the space, there is potential for a thriving vaccine industry in Africa, particularly in the areas of Africa-endemic diseases and new vaccine platforms, where African manufacturers can carve out a unique niche. We hope the insights presented in our white paper will help guide stakeholders toward that bright future.

Finally, there are valuable lessons to be learned from how India and China have built their vaccine manufacturing ecosystems over the past 20 years. Our next white paper will take a deep dive into the critical factors behind the success of these LMIC manufacturing ecosystems, and how these learnings could be applied in Africa.

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BIOGRAPHIES

PHILIP DORRELL leads the work of the Clinton Health Access Initiative to support African Vaccine Manufacturing with market-shaping initiatives to create a sustainable vaccine manufacturing ecosystem. He studied at the Universities of Oxford and Cambridge, with a focus on the philosophy of medicine and African politics, and has subsequently worked on solving strategic health problems in the public and private sector in the UK, India, and South Africa.

RISHABH D JHOL is a Technical Advisor at the Clinton Health Access Initiative, where he focuses on supporting African Vaccine Manufacturing through market-shaping initiatives to establish a sustainable vaccine manufacturing ecosystem. He studied social impact strategy at the University of Pennsylvania and has a background in founding and leading non-governmental organizations in the public policy and public health space.

AFFILIATIONS

Philip Dorrell
Program Manager,
Clinton Health Access Initiative

Rishabh D Jhol
Technical Advisor,
Clinton Health Access Initiative

AUTHORSHIP & CONFLICT OF INTEREST

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VACCINEINSIGHTS

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

SPOTLIGHT

Germany supports the African Agenda of building local vaccine manufacturing capacity

Manuel Batz
Head of Component Vaccine Manufacturing TEI MAV+,
GIZ Global Programme BACKUP Health

"While the acute phase of the COVID-19 pandemic has ended, there remains a strong political commitment from the African Union, the EU, and others to support local vaccine manufacturing and ensure more equitable access."

VIEWPOINT

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Africa's vaccine manufacturing capacities are currently very limited, which makes the continent critically dependent on imports. The COVID-19 pandemic has illustrated the risk that this can entail when African countries are unable to source a sufficient number

of vaccine doses. This Viewpoint article highlights the work of the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, in particular the global program BACKUP Health, to support the improvement of framework conditions for local vaccine manufacturing in Africa at the African Union level.

In 2020, the African Union committed to address the lack of local vaccine manufacturing on a continental level through the Africa Centres for Disease Control and Prevention (Africa CDC). They established the Partnerships for African Vaccine Manufacturing (PAVM) secretariat, which coordinates all efforts to build vaccine manufacturing capacity.

The German Federal Ministry for Economic Cooperation and Development (BMZ) is investing €550 million towards this important goal. This is part of the Team Europe Initiative on Manufacturing and Access to Vaccines, Medicines, and Health Technologies with a total volume of €1.2 billion. In April 2021, BMZ commissioned the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH, a global service provider in the field of international cooperation for sustainable development, supporting the German government in achieving their international cooperation goals, to contribute to the PAVM agenda via the global program BACKUP Health. Since December 2022, BACKUP is also part of the multi-country partner agreement Team Europe Support Structure (TESS) between Germany, France, and Belgium, co-financed by the EU.

The program works with partners in Africa and Europe to foster improvements in the framework conditions for vaccine production in Africa. BACKUP does this by focusing on a number of different action areas, all fully aligned with the PAVM and its workstreams (Figure 1).

Within these areas, GIZ BACKUP Health provides direct support to the PAVM Secretariat in two main ways. Firstly, it works as part of the TESS to provide embedded experts within the PAVM secretariat and facilitate study tours for PAVM staff and others to Europe or Asia to learn more about vaccine manufacturing technologies. The embedded experts are provided by GIZ, work directly for the PAVM secretariat, and are funded by the TESS.

Secondly, BACKUP provides further dedicated support to PAVM workstreams, specifically in the areas of capacity building, market shaping, and private sector engagement. The team aims to identify 'missing links'—areas where there are gaps in knowledge that they can help to fill.

Priority areas where BACKUP is providing support include:

- Implementing a pooled procurement mechanism to strengthen demand;
- Creating stringent cross-continental regulatory standards and processes, including establishing regional centers of regulatory excellence;
- Boosting talent development with a comprehensive competency framework, training program, and R&D centers of excellence;
- Bringing together local and international companies to explore the potential for collaboration and/or tech transfer; and
- Stakeholder coordination to ensure labor is not duplicated, and resources are not wasted.

While the acute phase of the COVID-19 pandemic has ended, there remains a strong political commitment from the African Union, the EU, and others to support local vaccine manufacturing and ensure more equitable access.

Beyond vaccines, there is also strong support for within-continent manufacturing of a range of medical products and diagnostics.

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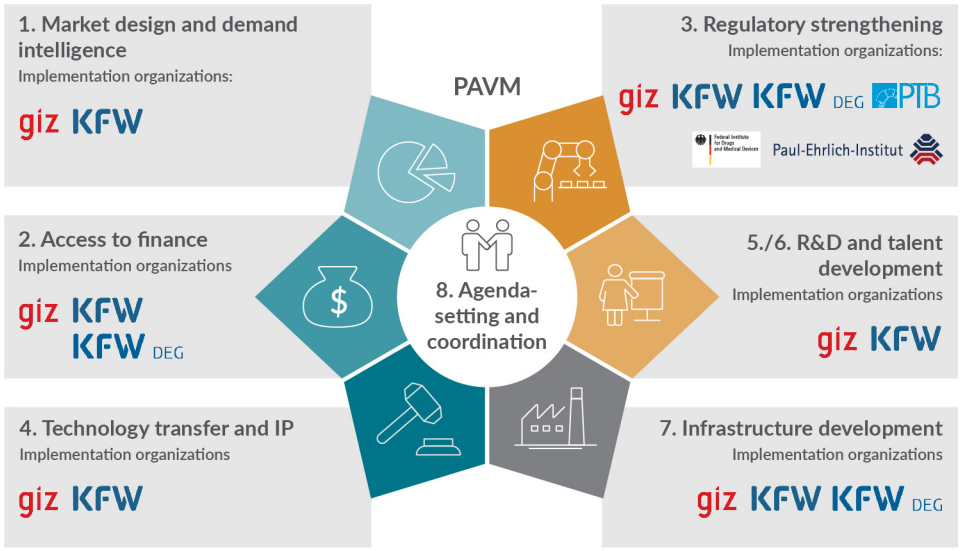
FIGURE 1

German contribution to vaccine and pharmaceutical manufacturing in Africa.



The infrastructure and talent being developed for vaccine manufacturing will be largely transferable to other products, particularly biologics.

Technology innovations will play an important role in achieving that goal, including modular and flexible facility design and intensified processing. The best opportunities for African manufacturers lie in novel vaccine modalities, such as mRNA and viral vectors, where the playing field is more level.

Since PAVM was established, strong progress has been made. More African countries have attained the minimum level of regulatory capacity required to host manufacturing, training schemes have been launched for capacity building on a continental level, and discussions are advancing to build a pooled procurement mechanism. Roadblocks remain, including a shortage of skilled staff, high manufacturing costs, and a lack of local tech transfer partners. However, with efforts underway to address these challenges, the BACKUP team believes that PAVM and its partners will prevail and have a good chance of meeting the African Union's goal of 60% of vaccines produced locally by 2040.

BIOGRAPHY

MANUEL BATZ leads a team that supports the PAVM in the implementation of the Framework for Action. Manuel Batz joined GIZ in April 2022 as Senior Advisor Vaccine Production for Africa. Previously he held various leadership roles within the life science tools industry with focus on emerging markets, such as Latin America and Middle East and Africa. In 2016, as Director of the Middle East and Africa Bioprocessing Division at Merck KGaA, he developed and launched a vaccine manufacturing initiative for Africa, bringing together local and global stakeholders from both the public and private sector, to support local efforts to enable vaccine manufacturing capacity on the continent. His current focus, closely aligned with the PAVM secretariat, is on supporting the development of viable business models to assure sustainability of local manufacturing.

AFFILIATION

Manuel Batz
Head of Component Vaccine Manufacturing TEI MAV+,
GIZ Global Programme BACKUP Health

AUTHORSHIP & CONFLICT OF INTEREST

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