Building Process Control into Chromatographic Purification of Viruses, Part 1

Qualification of Critical Manufacturing Components

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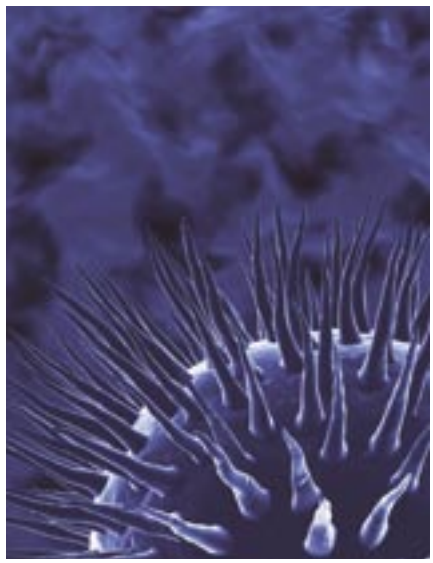
Many viral products are currently under development, including retrovirus, adenovirus, adeno-associated virus (AAV), herpes virus, pox virus (vaccinia, fowl, canary), lentivirus (HIV-based), and measles (and other paramyxovirus), as well as alpha, rhabdo, influenza, polio, and reovirus (**1–3**). Whether live viral vaccines or viral vectors for human gene therapies, viral products present a multitude of manufacturing challenges that must be overcome to deliver a product that is consistently safe and effective. Chromatography methods have a number of inherent features that make them well suited to address such challenges.

Complementarity among the many separation mechanisms supports development of multistep procedures capable of excluding contaminants to virtually any degree required to satisfy a particular application. The ability to conduct separations in closed systems that can be sanitized supports aseptic processing. Automation options make it possible to minimize human contribution to overall process variation. The materials to conduct chromatographic purification require proper qualification, and the flexibility of chromatographic methods requires that appropriate controls be implemented to ensure that a process will reproducibly yield a consistent product that is safe and effective.

Building quality into a product is fundamental to both economical manufacturing and regulatory compliance. Qualifying critical manufacturing components is an excellent place to begin that process. The methods we suggest should be considered a conceptual framework to show how material qualification issues can be addressed systematically. We base them on approaches that have been shown to have practical value in the manufacture of recombinant protein therapeutics and development of emerging viral products, but they can be adapted as necessary to meet the needs of a particular process. For regulatory guidance, please refer to pertinent documents (**4–10**).

This two-part article focuses on the ability of chromatographic methods to support manufacturing procedures that embody the high level of process control necessary to purify viruses for human applications — beginning with qualification issues.



Artist's conception of a virus
RAFAL ZDEB (WWW.ISTOCKPHOTO.COM)

PRODUCT FOCUS: ALL VIRAL PRODUCTS

PROCESS FOCUS: DOWNSTREAM PROCESSING

WHO SHOULD READ: PROCESS DEVELOPMENT AND MANUFACTURING

KEYWORDS: PROCESS OPTIMIZATION, CHROMATOGRAPHY, VALIDATION, QUALIFICATION, VACCINES, GENE THERAPIES

LEVEL: INTERMEDIATE

A Well-Controlled Process

The characteristics and abundance of contaminants relative to a viral product guide both the choice and order of methods to purify it. In addition, the sample is generally the most variable component in a purification process and, accordingly, has the greatest potential to affect reproducibility. These two

considerations together make sample qualification the foundation of a well-controlled process.

Quantitative PCR (qPCR) is frequently used to quantify a viral product, but infectivity and scanning electron microscopy (SEM) are often used as alternative or supporting methods. The concentration of helper viruses and other adventitious agents, if any, should be measured, especially if they affect product safety. Knowing the approximate helper virus concentration will also provide process developers with a preliminary idea of the overall log reduction value (LRV) that a purification process will need to achieve, as well as a baseline for calculating LRV through the various steps of the process (**7**).

Virus purification procedures commonly begin with enzymatic digestion of DNA, but many chromatographic procedures can eliminate DNA without this step. In either case, characterizing DNA levels in the supernatant or lysate provides worthwhile information. PCR and fluorescent intercalating assays such as PicoGreen (Invitrogen, www.invitrogen.com) provide adequate sensitivity at this stage. If DNAse is used in purification, protein characterization should take place after treatment so that the enzyme is accounted for as a contaminant. Gross protein content can be estimated by absorbance at UV 280 nm. Characterizing the size and charge distribution of protein contaminants by appropriate electrophoretic methods can provide valuable preliminary guidance for selection of separation methods.

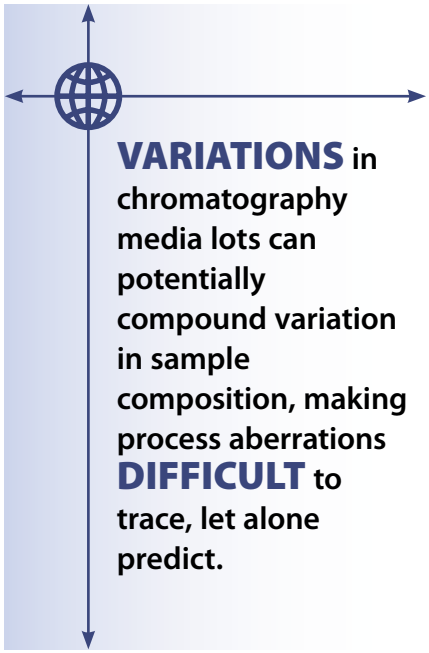
Supernatants and lysates are understood to exhibit lot-to-lot variability. It is therefore important to define acceptable ranges for a viral product and its critical contaminants to ensure robustness of an eventual purification process. Variation that can be documented not to affect the outcome of a purification process will generally be tolerable, but this highlights a challenge for developers. Cell culture and purification processes are often developed simultaneously. Thus, to the extent that a purification process is used to define an acceptable range of variation, it will not be possible to finalize requirements for sample composition until that process is established.

During evolution of an overall manufacturing process, it is advisable to retain developmental supernatant or lysate samples for future evaluation of the purification process. The range of variation is typically wider among earlier samples than it will be in the final production process. Archived samples can thereby provide a unique resource for documenting the ability of a purification process to accommodate variation outside the expected manufacturing range. However, the ability of a purification process to accommodate wide variations should not be used to compensate for lack of control during cell culture production. Consistency of sample composition is an important element of overall process control and in turn, an important factor in risk assessment (**10**).

Chromatography Media Qualification

Chromatography media are now available in an unprecedented diversity of formats, including microparticles, membranes, and monoliths; and each format offers a range of surface chemistries. Numerous formats and chemistries have been used for research purification of viruses, including affinity (**11–14**), size-exclusion (**14–18**), ion-exchange (**16–22**), hydrophobic-interaction (**17, 18**), and hydroxyapatite (**22**). Suitability of chromatography media for research does not, however, ensure suitability for manufacture of clinical-grade viral products. A practical first step in qualifying chromatography media is to use those that vendors support specifically for such applications. Such media are typically supported with either a regulatory support file (RSF) or a drug master file (DMF).

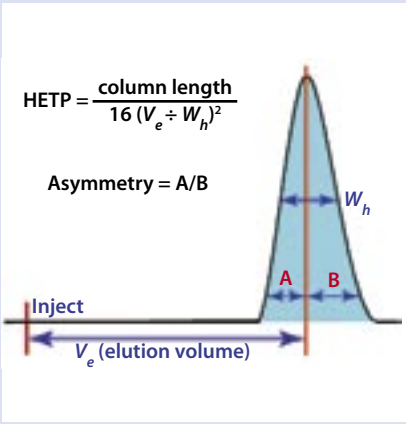
RSFs are developed by chromatography vendors for manufacturers to facilitate incorporation of media into clinical-grade manufacturing processes. Such files usually include information similar to a DMF, such as positive identification of the product, measurement of separation performance, capacity, characterization of leachables, toxicology, bioburden, lot reproducibility, and material safety data (**23, 24**). The fact that a vendor has made the substantial investment to conduct supporting studies for such documents suggests an understanding of requirements for manufacturing human therapeutics. A user should evaluate data supplied by a vendor for adequacy to support the use of the medium in a specific process. On a more practical level, the absence of such documentation places the responsibility — and expense — on users to conduct and document necessary tests.



Variations in chromatography media lots can potentially compound variation in sample composition, making process aberrations difficult to trace, let alone predict. This makes reproducibility a key factor in media qualification. Most chromatography media are supplied with a certificate of analysis (CoA) that provides data on each particular lot. This information can be useful in documenting reproducibility, and CoAs should be archived for this purpose.

Users are often advised to adopt vendors' tests for media lot

Figure 1: Evaluation of packing quality by HETP and asymmetry factor



A pulse of sample amounting to 1–2% of the column volume is injected at time zero and monitored until it completely exits the column. The sample should be a small molecule that has no interaction with the chromatography media. Acetone interacts with hydrophobic media, but not with most others. Sodium chloride (NaCl) interacts with ion exchangers but not with most others. Sorbitol interacts with boronate ligands, but not with other chemistries. Sorbitol and acetone both absorb UV at 280 nm. NaCl can be monitored by conductivity.

W_h refers to peak width at half height. A and B are measured at 10% peak height. The smaller the HETP, the higher the packing efficiency.

characterization and to periodically compare their own values with those provided by the vendors (**23**). This provides a useful check of testing consistency by both parties. However, a vendor's tests may inadequately characterize media for certain user applications. Viral particles may not behave like the test materials vendors typically use for lot characterization. Therefore, vendor tests are a good place to start, but users are responsible for ensuring that their own tests adequately characterize reproducibility for their own applications.

Bioburden and Sanitizability: Most chromatography media are delivered under biostatic conditions. Many can be sterilized, but endotoxins can persist even when source organisms have been inactivated (**25**). The most expedient qualification is to specify that chromatography media be sanitized before their first use. This is a common manufacturing practice, but it is important to know that some sanitizing reagents have contained resistant organisms that caused contaminations (**25**).

This emphasizes the point that media stability in strong sanitizing agents is necessary for media qualification, even for single-use products. Vendors usually recommend specific treatments and limits for chemical exposure. In some cases they provide supporting data documenting that the product maintains its functional characteristics over some duration or number of cycles in various sanitizing agents. Bioburden issues are also important if media are intended for reuse (addressed in Part 2).

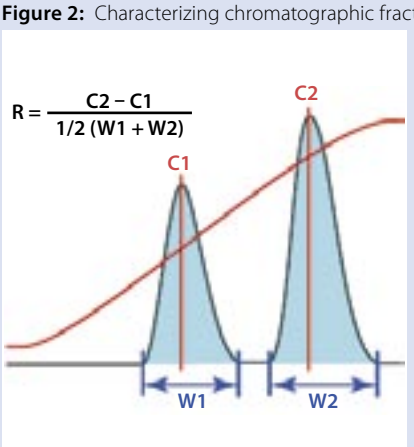
Media Integrity: Packing quality contributes directly to fractionation performance for particle-based chromatography media. A packing SOP and appropriate acceptance criteria are therefore essential for assessing performance consistency of each media lot. Most vendors provide packing procedures that are suitable for initiating assessment of reproducibility. Packing quality is commonly expressed as the height equivalent of a theoretical plate (HETP) and as peak asymmetry (A). Figure 1 provides a basic procedure and a representative result.

Membrane- and monolith-based media do not require packing, but testing is still required to document their integrity. Monoliths operate like columns, making HETP and asymmetry tests suitable for demonstrating bed integrity. Strictly speaking, such measures can be applied to membrane-based chromatography media as well, but it is more common to use a test that can detect physical flaws in a membrane or housing. Various methods have been described for measuring transmembrane pressure as air is pumped across a wetted membrane (**24, 26**). Details of these methods are highly customized to a particular membrane or housing assembly, so it is important to obtain specific instructions from its vendor.

Separation Performance: The concept of purification is based first and foremost on fractionation. Accordingly, reproducibility of separation performance is the most basic requirement for media qualification. Separation performance on most types of media can be qualified with linear gradient elution of samples containing two or more components that are well resolved from each other. This approach can be used with anion-exchange, cation-exchange, hydrophobic-interaction, hydroxyapatite, and some affinity-chromatography media. For this purpose, sample components can be a virus product and a select contaminant, or they can alternatively comprise proteins unrelated to the process. The objective is to demonstrate lot reproducibility of the media, not to model the purification process. On the other hand, the more closely the control sample simulates the actual manufacturing sample, the higher the confidence that its behavior will provide an accurate indication of how a given lot of media will behave in manufacturing.

Figure 2 provides a basic protocol for evaluating fractionation performance and illustrates a hypothetical result from an ion-exchange column. A line drawn

Figure 2: Characterizing chromatographic fractionation performance

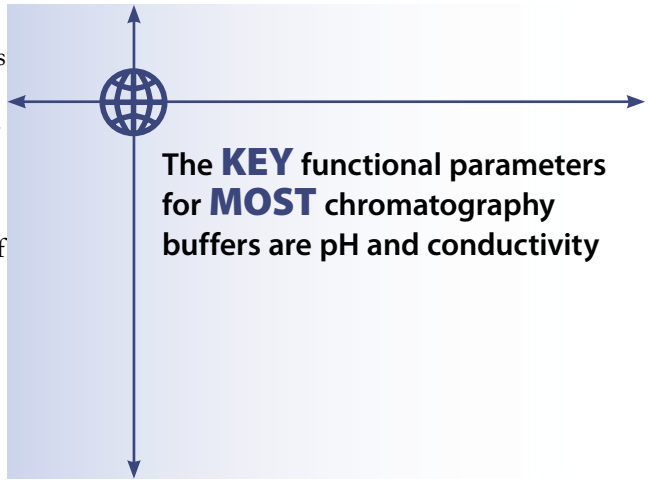


- Equilibrate the column to the selected running conditions
- Inject a sample containing the selected test components. Both components should bind to the column.
- Apply a linear gradient of sufficient amplitude to completely elute both components.
- The red trace marks the conductivity gradient. C1 and C2 mark the centers for the two peaks. The point at which each peak center crosses the conductivity curve represents its conductivity intersect. W1 and W2 mark the respective widths of the two peaks. The formula for calculating resolution is described at upper left.

vertically through each peak center and intersecting the conductivity trace provides an index for reproducibility of the surface chemistry. Variation among media lots will be evident as a lateral shift of the peaks relative to the conductivity curve. It is recommended that the peak centers not be characterized according to where they intersect the analog gradient trace (% buffer A or B). The analog trace is generated by the chromatograph independent of what it is happening in the column; a column need not even be in line. Relating peak elution to conductivity provides a direct link to the conditions inside the column. The individual peaks in a sample may respond differently to media lot variations, so it is useful to measure conductivity intersects for both. Variations among media lots can be expressed numerically by dividing the intersect conductivity for the experimental lot by the intersect for the control.

Peak separation also can provide valuable reproducibility data for chromatography media. The simplest approach is to measure the conductivity interval between peak centers. Variation between lots can be calculated by dividing the conductivity interval for the experimental lot by the conductivity interval for the control lot. This characterizes the contribution of the media surface chemistry. Calculation of resolution (R) is more informative because it integrates the contribution of media porosity. This is important because porosity and surface chemistry are usually established at different stages in the manufacture of chromatography media and can vary independently.

Lot-to-lot reproducibility of separation performance on size exclusion chromatography media can be determined by applying a three-component sample in which one of the components is so large that it will be excluded from the pores and elute in the void volume. Potential candidates include nanoparticles or extremely large molecules that absorb UV at 280 nm. This component will act as a marker for viral particles. The second component should elute within the designated fractionation range of the media. Potential candidates may include any of a large variety of commercially available proteins such as bovine serum albumin or IgG. The third component should be a very small molecule that absorbs UV, such as sorbitol, or a small molecule that can be monitored by conductivity, such as sodium chloride. The same measurements described for characterizing reproducibility of ion exchangers can be applied, except that the values will need to be expressed as mL of buffer from the point of injection.



Whatever kind of medium is being examined, it is worthwhile to eliminate as many extraneous variables as possible. More confidence can be placed in the data if testing is performed on all lots of a given medium within as brief a time interval as possible, using buffers from the same lots, sample components from the same lots, the same column configuration, the same linear flow rate, the same chromatograph, and the same operator. Documentation should show that the instrument used for testing was properly installed and qualified and that calibration was up to date for critical parameters such as flow rate, conductivity, and pH.

Binding Capacity

Binding capacity of chromatography media contributes directly to separation performance. It is also an indirect indicator of pore-size distribution and product-accessible surface area, making it especially well suited for qualifying lot reproducibility. Binding capacity is sometimes expressed as *static capacity*, sometimes as *dynamic capacity*. Static capacity values are determined by incubating a measured volume of chromatography media with excess sample, washing away the excess, and determining the amount of sample bound. Static capacity data do not accurately reflect media behavior under most conditions and provide no secondary characterization of pore-size distribution, so we discuss them no further here.

Figure 3 provides a basic method for determination of dynamic capacity and a representative profile. As with separation performance, the sample used to characterize capacity may be the virus product itself or a surrogate such as a protein. To obtain the most useful information, measure the point at which sample begins to break through at a specified level, and describe the shape of the breakthrough curve. Breakthrough provides an integrated measure of surface chemistry and porosity. Curve shape is dominantly a function of pore-size distribution. One way to characterize both is to measure the mass of sample that has been applied to the column up to the point where breakthrough sample concentration is 50% of the concentration being applied to the column. Slope can be measured at the same point.

Leachability

Chromatography media gradually break down under use, creating leachates that may include components of the support matrix,

the spacer arm, the ligand, or all three (**23**). Leaching can occur during cleaning, sanitization, or storage, in which case it potentially reduces media life. It also can occur during product elution. This is a greater concern because leached ligand in a virus product may have clinical consequences such as enhancing formation of neutralizing antibodies (**23, 27**). In either case, characterization of leachables is an essential aspect of media qualification.

Vendors typically perform basic characterization of leachables during the course of preparing RSFs or DMFs. Their data may be sufficient for qualifying size-exclusion media, ion-exchangers, hydrophobic-interaction, or hydroxyapatite media — not only because leaching typically occurs at very low levels, but also because leachate tends to be washed out of the column during the column equilibration phase before product loading (**23**). Affinity media will probably require additional qualification because their ligand leaching tends to occur during product elution, elevating the possibility of product contamination. Coelution of affinity ligands may be enhanced by specific operating conditions and sample composition (e.g., presence of proteolytic enzymes) (**23, 27**).

Buffer Qualification

Buffer composition is the means by which chromatography methods are controlled. Qualification of buffer components and the finished buffers themselves are therefore fundamental to achieving adequate control of the final process. The *US Pharmacopeia* (USP) provides tests and specifications for buffer components (e.g., water-for-injection, salts, and so on), but ensuring adequate process control requires that the finished buffers themselves be qualified (**23, 28, 29**). Documentation should be maintained to show that balances, pH and conductivity meters, and volumetric equipment are properly installed and calibrated.

The key functional parameters for most chromatography buffers are pH and conductivity. Buffer qualification requires that a range of values be determined, within which the overall purification process is shown to perform reproducibly. Because the objective is to ensure reproducibility of the process, it makes sense to use fairly stringent buffer specifications rather than exploit the tolerance of a robust purification process to support broad buffer specifications. Consequently, it is common practice in the field of commercial protein purification to set initial pH specifications at ±0.05–0.10 pH units and conductivity at ±1–2% of the target value. It is also worthwhile to keep in mind that even if a purification process will tolerate broader buffer specifications, they may amplify the range of variability in the process — which could be interpreted as a loss of process control.

Process buffers have traditionally been prepared by users, but premade buffers are now sold commercially and delivered ready-to-use in single-use plastic bags. This simplifies manufacturing operations and reduces the validation workload, but it does not relieve users of responsibility to document adequate qualification. As with chromatography media, relying on an external vendor for a critical manufacturing component increases the need to document that the vendor uses qualified components and adheres to approved SOPs in a properly maintained facility. Effective communication with the vendor is essential, and an audit may be prudent.

Vendor Audits

Vendors of chromatography media and finished buffers should have QA/QC programs in place that clearly define the protocols and SOPs for manufacture, testing and delivery, and change control. But it remains the responsibility of end users to document that those vendors have properly addressed those issues — typically by means of a vendor audit. Useful guidelines for vendor audits are published by the Parenteral Drug Association (PDA) (**30**).

The scope of an audit can include any issue that could affect media function or availability over the lifetime of a user's purification process. Focal points typically include inspection of the manufacturing facility, including a review of lot production records, assay validation records, data used to support the RSF, DMF, or MSDS, product safety stocks, and back-up manufacturing capability in the event of disaster. Discussion of change control is important for assuring users that they will be adequately notified before changes occur, giving them the opportunity to evaluate the potential effects on their purification process.

It is a good idea to discuss expectations before an audit to ensure a successful outcome for both parties. One effective preparation is for users to share lot reproducibility data with the vendor, even if their tests are different. If the data support the same estimate of reproducibility, that creates confidence for both parties that the vendor's routine practices will provide media consistently able to support the user's needs. If not, these data can provide an objective basis for discussion and understanding the cause of any discrepancies, as well as their potential significance to the purification process. Vendors are generally receptive to such input because it helps them develop more competitive products.

Another important preparation is to send the right people. The team should minimally include people who are highly experienced with purification, highly experienced with development of assays for regulated materials, and highly proficient in regulatory affairs. Under ideal circumstances, the vendor will commit parallel staff to make the audit as efficient as possible.

An Evolutionary Process

Qualification of critical manufacturing components is the foundation on which successful virus purification procedures are developed. It represents a substantial amount of work, but it is fundamental to achieving the level of process control necessary to ensure a high-quality product. Every virus purification process will pose unique challenges,

and component qualification may require some inventiveness, but this is understood to be an evolutionary process. Early and regular discussions with regulatory agencies can help ensure that necessary inventiveness conforms to current regulations, and thereby help to move the development process along as quickly as possible.

Part 2 of this article will address approaches to process development that exploit the ability of purification methods to further enhance process control.

References

1 Dudek T, Knipe DM. Replication-Defective Viruses As Vaccines and Vaccine Vectors. *Virology* 344(1) 2006: 230–239.

2 Biffi A, Naldini L. Gene Therapy of Storage Disorders By Retroviral and Lentiviral Vectors. *Hum. Gene Ther.* 16(10) 2005: 1133–1142.

3 Russell SJ. RNA Viruses As Virotherapy Agents. *Cancer Gene Ther.* 9(12) 2002: 961–966.

4 US Food and Drug Administration, Center for Biologics Evaluation and Research. *Guidance for FDA Review Staff and Sponsors: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs);* Draft Guidance, 2004: www.fda.gov/cber/gdlns/gtindcmc.htm.

5 US Food and Drug Administration, Center for Biologics Evaluation and Research. *Guidance for Industry: INDs — Approaches to Complying with CGMP During Phase 1;* Draft Guidance, 2006: www.fda.gov/cber/gdlns/indcgmp.htm.

6 International Conference on Harmonisation (ICH). Guidance Q5D: Quality of Biotechnological/Biological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products. *Fed. Regist.* 63(182) 1998: 50244–50249; www.fda.gov/cber/gdlns/qualbiot.pdf.

7 International Conference on Harmonisation (ICH) Guidance Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell lines of Human or Animal Origin. *Fed. Regist.* 63(185) 1998: 51074–51084; www.fda.gov/cber/gdlns/virsafe.pdf.

8 US Food and Drug Administration, Center for Drugs and Biologics and Center for Devices and Radiological Health. *FDA Guideline on General Principles of Process Validation,* 1987; www.fda.gov/cber/gdlns/validation0587.pdf.

9 International Conference on Harmonisation (ICH). *Guidance for Industry Q7A: Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients* (August 2001); www.fda.gov/cber/gdlns/ichactive.pdf.

10 International Conference on Harmonisation (ICH). *Guidance for Industry Q9: Quality Risk Management* (June 2006); www.fda.gov/cber/gdlns/ichq9risk.pdf.

11 Aurichio A, et al. Isolation of Highly Infectious and Pure Adeno-Associated Virus Type 2 with a Single Step Gravity Flow Column. *Hum. Gene Ther.* 12(1) 2001: 71–76.

12 Aurichio A, et al. A Single Step Affinity Column for Purification of Serotype-5 Based Adeno-Associated Virus. *Hum. Mol. Ther.* 4(4) 2001: 372–374.

13 Slepushkin V, et al. Large Scale Purification of a Lentiviral Vector By Size Exclusion Chromatography or Mustang Q Ion Exchange Capsule. *Bioprocessing J.* September–October 2003: 89–95.

14 Segura M, et al. A Novel Strategy for Retrovirus Gene Therapy Using Heparin Affinity Chromatography. *Biotechnol. Bioeng.* 90(4) 2005: 391–404.

15 Transfiguracion J, et al. Size Exclusion Chromatography of High Titer Vesicular Stomatitis Virus G Glycoprotein-Pseudotyped Retrovectors for Cell and Gene Therapy Applications. *Hum. Gene Ther.* 14(12) 2003: 1139–1153.

16 Boratynski J, et al. Preparation of Endotoxin Free Bacteriophages. *Cell. Mol. Biol. Lett.* 9(2) 2004: 253–259.

17 Vellekamp G. Empty Capsids in Column Purified Recombinant Adenovirus Preparations. *Hum. Gene Ther.* 12(15) 2001: 1923–1936.

18 Huyghe B, et al. Purification of a Type 5 Recombinant Adenovirus Encoding Human p53 By Column Chromatography. *Hum. Gene. Ther.* 6(11) 1995: 1403–1416.

19 Kaludov N, Handelman B, Chiorni J. Scalable Purification of Adeno-Associated Virus Type 2, 4, And 5 Using Ion Exchange Chromatography. *Hum. Gene. Ther.* 13(10) 2002: 1235–1243.

20 Kramberger P, et al. Concentration of Plant Viruses Using Monolithic Chromatography Supports. *J. Virol. Methods* 120(1) 2004: 51–57.

21 Spech R, et al. Densonucleosus Virus Purification By Ion Exchange Membranes. *Biotechnol. Bioeng.* 88(4) 2004: 463–73.

22 O'Riordan C, et al. Scaleable Chromatographic Purification Process for Recombinant Adeno-Associated Viruses. *J. Gene. Med.* 2(6) 2000: 444–454.

23 Sofer G, Hagel L. *Handbook of Process Chromatography: A Guide to Optimization, Scale-up and Validation.* Academic Press: New York, NY, 1997.

24 Campbell J. Validation of a Filtration Step. *Process Validation in Manufacturing of Biopharmaceuticals: Guidelines, Current Practices, and Industrial Case Studies.* Rathore A, Sofer G, Eds. Taylor and Francis: Boca Raton, 2005.

25 Nims R, et al. Adventitious Agents: Concerns and Testing for Biopharmaceuticals. *Process Validation in Manufacturing of Biopharmaceuticals: Guidelines, Current Practices, and Industrial Case Studies.* Rathore A, Sofer G, Eds. Taylor and Francis: Boca Raton, 2005

26 Rathore A, Sofer G. Life Span Studies for Chromatography and Filtration Media. *Process Validation in Manufacturing of Biopharmaceuticals: Guidelines, Current Practices, and Industrial Case Studies.* Rathore A, Sofer G, Eds. Taylor and Francis: Boca Raton, 2005.

27 Gagnon P. *Purification Tools for Monoclonal Antibodies.* Validated Biosystems: Tucson, AZ, 1996.

28 Hill D, Beatrice M. Facility Requirements for Biotech Plants. *Pharm. Eng.* 9, 1989: 35–41.

29 United States Pharmacopeia. *USP 30–NF 25* (2006); ww.usp.org/products/USPNF.

30 Vendor Certification Task Force. *Vendor Certification: A Model Program.* PDA J. 43, 1989: 151–157.

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