Considerations in

Downscaling Purification Processes for Biologics

BioReliance® by **SAFC**

by SAFC

About BioReliance

BioReliance Corporation is a leading provider of cost-effective contract services to the pharmaceutical and biopharmaceutical industries, offering more than 1,000 tests or services related to biologics safety testing, specialized toxicology and animal health diagnostics. Founded in 1947, BioReliance is headquartered in Rockville, Maryland, with laboratory operations in Rockville and Scotland and offices in Tokyo, Japan, and Mumbai, India. The Company employs more than 650 people globally. For more information, visit www.bioreliance.com.

Key Services:

- **Custom Assay Development** to fulfill your exact requirements
- **Biosafety Testing** of biologicals for viruses, bacteria, mycoplasma, fungi
- **Cell Line Characterization** including identity testing, genetic stability, EM, sequencing
- **Final Product Testing** including biopotency testing, residual DNA, host cell proteins, cross-reactivity
- **Virus/TSE Validation Studies** for all biological products
- **Contract GMP Production and Testing** of viral vectors and cell banks
- **Veterinary Vaccine Services** including characterization/identity, extraneous agent testing
- **Regulatory and Consulting Services**

All work undertaken by BioReliance is in compliance with appropriate GLP or GMP standards.

Further information is available by visiting our website at **www.bioreliance.com**

Contents

Introduction

Validation of the downstream process for its ability to remove or inactivate contaminants represents an essential stage in ensuring that the final product is free of adventitious agents or contaminants.

The basic principle of a viral clearance study is that a downscaled version of the purification process is constructed and the input material is spiked with a known amount of virus. The product fraction is then assayed for residual virus infectivity. This enables a reduction factor to be calculated.

$$\text{Viral reduction} = \text{Log}_{10} \frac{\text{Total Virus Measured in Spiked Load}}{\text{Total Virus Recovered in the Product Fraction}}$$

Generally, it is not practical to perform viral clearance studies on the full manufacturing process due to the large scale involved and the requirement to keep manufacturing equipment and areas free from virus. Therefore it is necessary to downscale the purification process in order to obtain a laboratory-scale version of the process. As a general guideline, downscaled purification processes are 1/100 to 1/500 of the full manufacturing process although this may not always be possible due to constraints on sourcing suitable equipment or the size or type of the original process. The critical aspect to consider when designing the scale down process is to demonstrate that the process used accurately mimics the full scale manufacturing process.

Design of Downscaled Purification Process

The validity of the downscale is an essential prerequisite for clearance studies for viruses, mycoplasmas and DNA. The acceptance criteria for a valid scale down of the process must be defined. These typically include that the purity and yield of the product obtained from the scale down process is equivalent to that of the full-scale process. Additional criteria may be defined based on the functionality of the specific step under investigation.

Where the step to be scaled down is relatively straightforward i.e. inactivation steps such as heat, low pH and solvent/detergent treatments, then downscaling is relatively straightforward involving a proportional reduction in the volumes for example. All other properties such as buffer composition must be maintained. Care must be taken not to oversimplify the step and all operational parameters should also be considered when scaling down the process.

For viral clearance studies the volume of material used is important and will contribute to the log reduction factor that can be achieved for a given step. In general the virus spike volume should be kept to a minimum, typically less than 5% (v/v) to ensure that the composition of the starting material is not significantly different to that processed at manufacturing scale. For large volumes of starting material it may be necessary to optimize the spike volume to minimize the volume of virus added and maximize the potential log reduction factor. Consideration of the volume of the output sample is important. Where the final sample volume is large, then the ability to detect virus at low concentrations should be considered (see Appendix).

Where the purification involves more complex manipulations, other considerations need to be taken into account.

The source of the starting material to be used for the scale down study should be identified. The material does not necessarily have to be derived from the commercial / manufacturing process but it must be representative of the material to be marketed. Typically material is taken from pilot scale processes or from the first manufacturing run of the process.

All raw materials used for the scale down process should be sourced to be of an identical specification to that used for the manufacturing process. Buffers should be prepared to the same specification as those used for the manufacturing process (e.g. chemical formulation, pH and conductivity).

Filtration, Ultrafiltration

Filtration

Downscaling of filtration is relatively straightforward. For virus reduction, filtration downscaling would take into account the size of the filter necessary to permit the required flow rate. This should be maintained upon downscaling. Consideration should also be given to operational pressures and capacity (the volume of load per unit area of the filter). In addition, appropriate controls must be included in the process to remove potential viral aggregates that may form on the addition of virus to the feed stream.

Ultrafiltration

Ultrafiltration is typically used within a process where a sample concentration and or a buffer exchange (diafiltration) is required. Generally in these circumstances viral reduction would not be tested for in this step. Where such a study is required special consideration should be given to the scale down system used. Ultrafiltration and diafiltration on a large scale usually involves the use of a cross-flow system. Scale down cross flow systems are now available but care must be taken to demonstrate the validity of the scale down model.

Chromatography

Most purification procedures involve chromatographic steps, which can be divided into three classes:

1) batch/adsorption/desorption

2) isocratic chromatography

3) gradient methods

The most commonly used methods are shown in Table 1.

There are a number of models used to scale down the chromatography columns. The simplest and most widely used is achieved by decreasing the radius of the column while maintaining the column height and linear flow rate. The effect of process parameters on resolution and throughput in chromatographic procedures is shown in Table 2.

Table 1. Chromatographic operations for large-scale purification proteins

Physico-chemical Property	Operation	Characteristics
van der Waals forces H-bonds Polarities Dipole moments	Adsorption	Good to high resolution, good capacity, good to high speed
Charge (titration curve)	Ion-exchange	High resolution, high speed, high capacity
Surface hydrophobicity	Hydrophobic interaction	Good resolution speed and capacity can be high
Biological affinity	Affinity chromatography	Excellent resolution, high speed and high capacity
Molecular size	Size exclusion chromatography	Moderate resolution, low capacity, excellent for desalting
Hydrophilic and hydrophobic interactions	Reverse-phase liquid Chromatography	Excellent resolution, Intermediate capacity

*Note: Generally size exclusion chromatography would not be included in a viral clearance study as this type of step typically does not contribute significantly to the overall viral reduction capacity for the process.

Scaling Down Chromatography Processes

When downscaling the manufacturing process there should be a general understanding of the step functionality and mode of viral removal. The operation parameters defined for the scale down model should be identical to those applied to the manufacturing scale process. Where possible 'Worse Case' parameters within the specified range, with respect to viral reduction, should be selected.

Downscaling chromatography steps is relatively straightforward. Downscaling a 20 liter column 200-fold requires 100mls of packing material. In general the height of the column is maintained while the diameter is decreased, although consideration has to be made of the availability of convenient columns. The actual scale down factor applied to the step will typically be determined from the available column size rather than a simple volumetric scale down.

Downscaling the flow rate is an important consideration since this will determine the residence time for the product on the chromatography column. The residence or contact time on the scale down column should be equivalent to the manufacturing column. This can be achieved by scaling down the chromatography column by reducing the column diameter whilst maintaining the same bed height and linear flow rate specified for the manufacturing scale process.

The volumes of each wash step should be maintained such that the same buffer volume to column volume ratio is maintained. Maintaining an identical ratio to that specified for the manufacturing process will ensure, in combination with flow rate, that the contact time is identical for both the manufacturing and scale down process.

The operational capacity / binding capacity for the step should be defined and selected to support manufacturing operations. It may be appropriate prior to the license application to complete a process limits evaluation on those parameters that could impact on the viral reduction capacity for that step.

Table 2. Effects of process parameters on resolution and throughput[1]

Parameter	Resolution varies with:	Throughput varies with:
Column length (L)[a]	L	1/L
Column radius (r)[b]	Some effect	r^2
Temperature (T)	Positive effect	T
Viscocity (n)	Negative effect	1/n
Sample volume (V)	$1/[V-V_{optimum}]$	V
Flow rate (J)	$1/[J_{optimum}]$	J

[a] For size exclusion and isocratic elutions, column length is a critical factor. For gradient elution in adsorption chromatography however, resolution is relatively independent of column length.

[b] Wall effects on resolution are pronounced in short-radius columns and decrease as column length increases.

Validation of the Scale Down Process

Prior to initiating the viral spiking study it is critical to validate the performance of the scale down process. This would normally be completed on the sponsor's site but BioReliance can offer assistance here if required. Acceptance criteria must be set to monitor the operation of the scale down process. The acceptance criteria set will be dependent on the functionality of the step and the forward processing criteria specified for the manufacturing process. Typically a comparable yield, purity and elution profile between the two scales would be used as acceptance criteria.

In addition to these acceptance criteria the possible effects that the viral spike may have on the start material and subsequent performance of the step must be considered. Mock spiking studies with an appropriate mock spike should be included, evaluating any impact on critical parameters such as pH and conductivity. An assessment on the impact these may have on the step must be made and any remedial actions documented.

Appendix

Probability of detection of virus at low concentrations

At low concentrations (e.g. in the range of 10 to 1000 infectious particles per liter) it is evident that a sample of a few milliliters may or may not contain infectious particles. The probability P that this sample does not contain infectious viruses is:

$$P = ((V-v)/V)^n$$

where V (liter) is the overall volume of the material to be tested, v (liter) the volume of the sample and n is the absolute number of infectious particles statistically distributed in V.

With V >> v this equation can be approximated by the Poisson distribution:

$$P = e^{-cv}$$

where c is the concentration of infectious particles per liter.

If a sample column of 1ml is tested, the probabilities P at virus concentrations ranging from 10 to 1000 infectious particles per liter are:

c	10	100	1000
P	0.99	0.90	0.37

This indicates that for a concentration of 1000 viruses per liter, in 37% of sampling 1ml will not contain a virus particle.

By extrapolation, for a concentration of 4000 viruses per liter in 1.83% of sampling, 1ml will not contain a virus particle. (0.0183 by the Poisson distribution).

Therefore, if we detect no virus in a sample, we apply a residual theoretical minimum detectable level of 4pfu ml^{-1}.

Reference

1. Sofer, G. and Mason, C. (1987). Biotechnology 5, 239.

BioReliance Corp.
14920 Broschart Road
Rockville, Maryland 20850
Tel: 800.553.5372
Fax: 301.610.2590
Email: info@bioreliance.com

BioReliance Ltd.
Todd Campus
West of Scotland Science Park
Glasgow, Scotland G20 0XA
Tel: 44 (0) 141 946.9999
Fax: 44 (0) 141 946.0000
Email: info@bioreliance.com

BioReliance Ltd.
Innovation Park
Hillfoots Road
Stirling, Scotland FK9 4NF
Tel: 44 (0) 141 946.9999
Fax: 44 (0) 141 946.0000
Email: info@bioreliance.com

BioReliance, K.K.
c/o Sigma-Aldrich Japan K.K.
Tennoz Central Tower 4F
2-2-24 Higashi-Shinagawa
Shinagawa-ku
Tokyo 140-0002, Japan
Tel: +81 (0)3 5796 7430
Fax: +81 (0)3 5796 7435
Email: jpinfo@bioreliance.com

BioReliance Ltd.
c/o Sigma-Aldrich Chemicals Pvt Ltd
102 Alpha Building
Hiranandani Gardens
Powai, Mumbai 400076
Tel: +91 22 40872364
Fax: +91 22 25797589
Email: indiainfo@bioreliance.com

www.bioreliance.com
North America Toll Free: 800 553 5372 Tel: 301 738 1000
Europe & International Tel: +44 (0)141 946 9999 • Japan Tel: +03 5796 7430
Email: info@bioreliance.com

BioReliance®
by **SAFC**