ICH: Q5A

Guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

Training Material
Modules 0–3

May 2025

International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

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ICH Q5A(R2) Training Modules

ICH: Q5A (R2)

Guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

Training Material

Module 0 – Introduction and Training Overview

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Purpose of Training Materials

- To clarify and illustrate key concepts specific to Viral Safety that have been updated in the revision.
- To support implementation of the guideline by use of examples (for illustrative purposes).
- To train target audiences including Industry and Regulators.

Disclaimer: The training materials are NOT intended to:

- Provide comprehensive information on all aspects of viral safety.
- Endorse the use of a specific technology.
- Introduce additional requirements beyond those already described in the revised guideline.
- Limit other approaches that can be justified from scientific and risk-based perspectives.
- Prescribe specific test methods that should be used.

Guideline Objectives

- Capture key scientific and regulatory considerations that promote harmonization with respect to evaluation of viral clearance, characterisation, and testing
- To describe the three principal, complementary approaches to control potential viral contamination
  - Selecting and testing cell lines and other raw materials, including media components for ensuring the absence of undesirable infectious viruses
  - Assessing the capacity of the production processes to clear adventitious and endogenous viruses
  - Testing the product at appropriate steps of production for the absence of contaminating infectious viruses
- Intended to be used in conjunction with existing guidelines, in particular ICH Q2, ICH Q5D, and ICH Q13

Overview of Key Updates in ICH Q5A(R2)

- Multiple Key Updates were included in the ICH Q5A(R2) revision
  - Key Update 1 – New Product Types
  - Key Update 2 – Section Locations
  - Key Update 3 – Continuous Manufacturing
  - Key Update 4 – New Test Methods
  - Key Update 5 – Resin Reuse
  - Key Update 6 – Prior Knowledge
  - Key Update 7 – Flexible Approach for Well Characterised Rodent Cell Substrates
  - Key Update 8 – Glossary Definitions
- Description of each Key update (including those not summarized in this training) are available in the Step 4 presentation for the Guideline

Structure of Training Materials

Module 0: Introduction and Training Overview (this module)

Module 1: New Product Types

Module 2: Prior Knowledge and Platform Validation

Module 3: Continuous Manufacturing

Module 1: New Product Types

- Includes two examples of "New Product Types" in scope of revision
  - Example 1: Adeno-Associated Virus (AAV) -Vector expressed by triple plasmid transfection in Human Embryonic Kidney (HEK)-293 cells
  - Example 2: Baculovirus expressed AAV-vector in SF9 insect cells
- Includes comprehensive evaluation of the product
  - Management of raw and starting materials
  - Testing Considerations
  - Model virus selection and evaluation of viral clearance

Module 2: Prior Knowledge and Platform Validation

- Includes evaluation of a therapeutic monoclonal antibody produced in Chinese Hamster Ovary (CHO) cell line
- Includes description three specific uses of prior knowledge:
  1. Master Cell Bank (MCB) Virus Testing Strategy
  2. Retrovirus Inactivation by Low pH
  3. Viral Clearance Studies with Used Chromatography Resin
- Highlights Opportunities for using new test methods

Module 3: Continuous Manufacturing

- Utilizes Annex III from ICH Q13 as proposed manufacturing process
- Reflects manufacturing hybrid manufacturing process that is partially continuous
- Focuses on aspects of viral clearance that are unique to continuous manufacturing (CM), including specific risks
- Where possible provides options for how to perform viral clearance studies and establish testing
- Highlights Opportunities for using new test methods

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Guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

Training Material

Module 1 – New Product Types (ANNEX 6: Genetically Engineered viral vectors and viral vector-derived products)

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Scope of ICH Q5A (R2)-Product Types[a]

Product Categories/Types	Examples
Included: • Products derived from in vitro cell culture using recombinant DNA technologies: • **Genetically-engineered viral vectors and viral vector derived products provided they are amenable to viral clearance** Excluded: • Inactivated vaccines • All live vaccines containing self-replicating agents • Products derived from hybridoma cells grown in vivo as ascites • **Genetically-engineered viral vectors provided they are not amenable to virus clearance** • Cell therapies	Included: • mAbs • Recombinant proteins • Recombinant subunit vaccines • Certain vaccines • Cytokines • **Helper-dependent recombinant AAV and recombinant AAV produced by transient or stable transfection** • **Baculovirus produced Virus Like Particle (VLP) vaccines and gene therapies e.g., baculovirus expressed recombinant AAV vector (genetically engineered viral vector)** • **Protein subunits expressed in baculovirus (genetically engineered viral vector derived product)** Excluded: • Inactivated viral vaccines • Live attenuated vaccines: Measles, Mumps, Rubella • Cell therapies • **Viral vectors not amenable to viral clearance such as Retroviral vectors e.g., Lentivirus**

[a] Bold text indicates new product types that are included or excluded in the Guideline

Introduction

The training materials for this module are provided in three sections of the presentation and describe virus safety considerations for two examples of new product types

1. AAV Vector Production Platforms and Virus Safety Considerations
2. Example 1: AAV vector expressed by triple plasmid transfection in HEK-293 cells
3. Example 2: Baculovirus expressed AAV vector in Sf9 insect cells

Introduction (continued)

- Using these two examples we will apply the three principles for assuring the viral safety of these new product types:
  - Selecting and testing cell lines and other raw materials
  - Assessing the capacity of the production processes to clear adventitious and endogenous viruses by model virus selection for cases A, B, C and F (Section 5 in ICHQ5AR2 guideline) and evaluation of viral clearance.
  - Testing the product at appropriate steps of production including cell banks, virus seeds , Limit of In Vitro Cell Age (LIVCA)/End Of Production Cells (EOPC) and in process testing
- Instances of where Next Generation Sequencing (NGS) can be used to replace or supplement conventional testing will be highlighted throughout both examples
- **Disclaimer: The testing in this training material is presented as examples only and other tests may be used as appropriate**

1. AAV Viral Vector Production Platforms and Considerations for Virus Safety

In this section we describe the platforms for AAV viral vector production and use two examples for detailing the recombinant AAV viral vector production and considerations for virus safety:

- Example 1: the plasmid expression system
- Example 2: the baculovirus expression system

Recombinant AAV Vector Production Platforms

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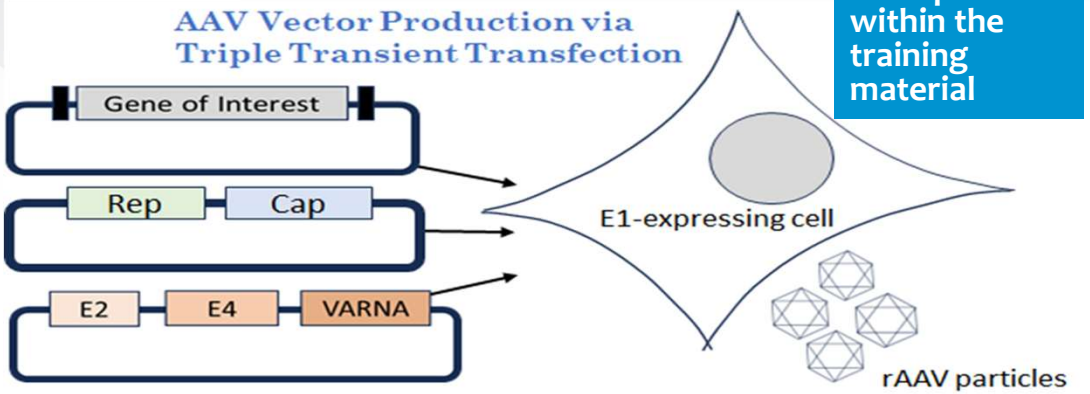
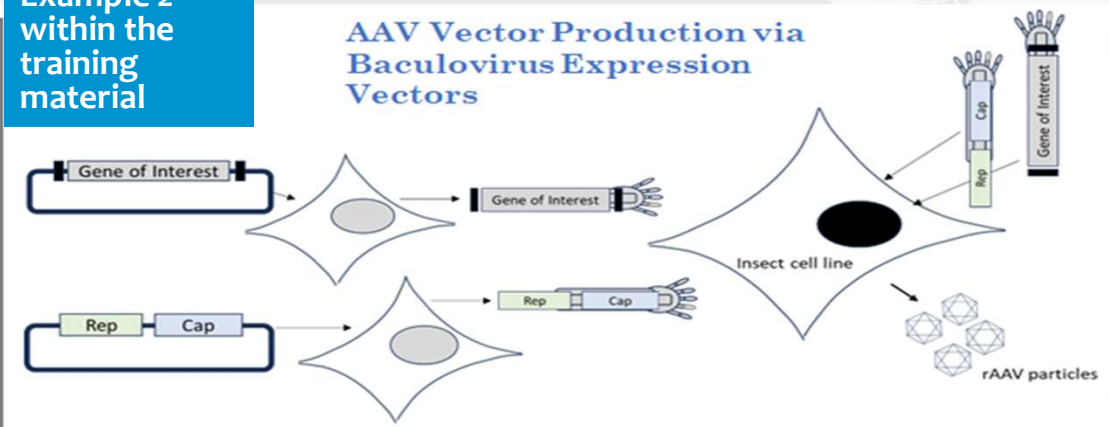
Example 1 within the training material

AAV Vector Production via Triple Transient Transfection

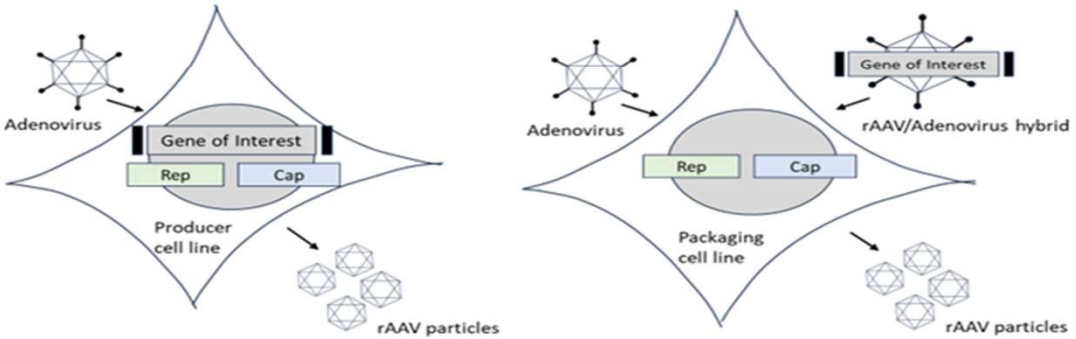


Example 2 within the training material

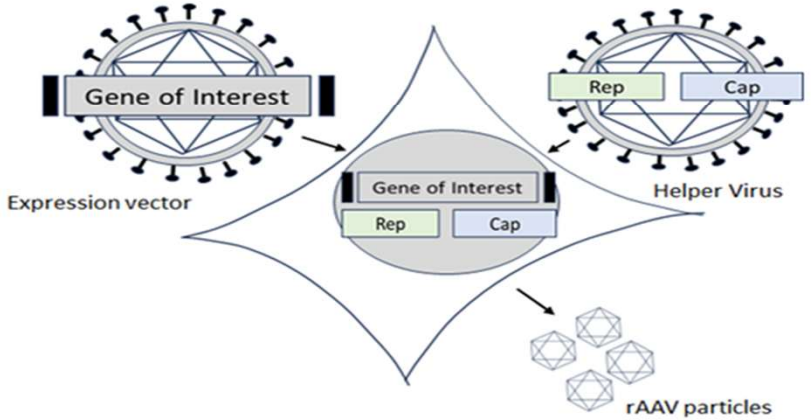
AAV Vector Production via Baculovirus Expression Vectors



AAV Vector Production via Stable Producer Cell Line or Packaging Cell Line



AAV Vector Production via Helper Virus (e.g., Herpesvirus or Adenovirus-based Vector Expression Systems



New Term: Production Virus

Production Virus: *A production virus is a process-related virus and includes a helper virus or a viral vector for protein expression*

Helper Virus: *A virus that provides helper functions allowing an otherwise replication-deficient co-infecting virus to replicate.*

Viral Vector for Protein Expression: *A recombinant virus, such as baculovirus, that can be used to express a recombinant protein or a virus like particle, or to produce a viral vector.*

AAV Vector Expression Systems and Virus Safety Approaches (Approach 1)

- Selecting and testing cell lines and other raw materials (including media components), for the absence of undesirable infectious viruses. (Manufacturers should avoid using human-and animal-derived materials in their manufacturing processes, when possible)

Example 1 (Plasmid Transfection)	Example 2 (Baculovirus Expression System)
Human Cell Line (HEK-293) • Animal-derived materials were used during the cell line history • No animal-derived materials were used during production	Insect Cell Line Sf9 and recombinant baculoviruses • Animal-derived materials were used during the cell line history • No animal-derived materials were used during production

AAV Vector Expression Systems and Virus Safety Approaches (Approach 2)

Assessing the capacity of the production processes to clear adventitious viruses, endogenous retroviruses and production virus

Example 1 (Plasmid Transfection)	Example 2 (Baculovirus Expression System)
[a]Case A using non specific model viruses but no safety factor is calculated • Retrovirus (MLV) • Bovine Viral Diarrhea Virus (BVDV) • Adenovirus Type 2 • Murine parvovirus (MMV)	[a]Case B, C and F using specific and non-specific model viruses • Retrovirus (MLV) • Production virus-high titer Baculovirus • Rhabdovirus (VSV) • Reovirus 3 • Parvovirus Provides a description of overall safety margin for production virus and specific model viruses.
Two steps evaluated and less extensive clearance regimen: • Affinity chromatography • virus filtration based on size exclusion principles for AAV vector	Four steps evaluated and extensive virus clearance regimen: • Detergent viral inactivation • Affinity chromatography • Anion exchange • Virus Filtration

[a] see slide 21; Cases B, C and F are relevant to example 2 Baculovirus Expression System because of the presence of retrovirus like particles and Sf9 rhabdovirus and production baculovirus in the insect cell line. See Section 9. Glossary for definitions of specific and non-specific model viruses.

Description of Cases A, B, C and F

- **Case A:** No virus, VLP, or retrovirus-like particles (RVLP) other than viral vector particles present, use non-specific "model" for virus clearance evaluation. If no RVLPs are detected and if the Product Enhanced Reverse Transcriptase (PERT) assay is negative, no estimation of retroviral particles per dose is required.
- **Case B**: If only rodent A-, C- and R-type RVLP present, use a specific "model" virus such as murine leukaemia virus (MLV) and a non-specific virus. Not necessary to test for the non-infectious particles in the purified bulk or drug substance in Sf9 insect cell lines that produce endogenous retroviral-like particles that have been extensively characterised.
- **Case C:** Contains a virus (other than a rodent retrovirus) for which there is no evidence of infectivity to humans (e.g., Sf9 rhabdovirus). If it is not possible to use the identified virus, "relevant" or specific "model" viruses should be used to demonstrate acceptable clearance.
  - Purified bulk tested using suitable methods with high specificity and sensitivity for detecting the virus in question. For the purpose of marketing authorisation, typically data from 3 batches or more of purified bulk manufactured at pilot plant scale or commercial scale should be provided.
- **Case F:** Clearance of production virus (helper virus or viral vector) or a specific "model" virus (e.g., baculovirus, adenovirus, herpesvirus). Testing for the absence of the production virus should be performed for each purified bulk, unless justified by robust excess clearance (Section 6.3).

AAV Vector Expression Systems and Virus Safety Approaches (3)

Testing the product at appropriate steps of production for demonstrating the absence of contaminating infectious viruses. NGS can be used to supplement or replace conventional assays.

Test Materials	Example 1 (Plasmid Transfection)	Example 2 (Baculovirus Expression System)
MCB and Working Cell Bank (WCB) and LIVCA/EOPC	• Addresses human cell line testing for general and specific viruses and characterization studies	• [a]Addresses insect cell line testing for general and specific viruses and characterization studies
Master Virus Seed (MVS) and Working Virus Seed (WVS)	• Not Applicable	• Addresses baculovirus seed testing and characterization studies
Vector Harvest	• Viral adventitious agent testing	• Viral adventitious agent and production virus testing • Quantification of the Sf9-rhabdovirus and baculovirus
Purified Bulk	• [b]Test for replication competent AAV	• [b]Test for replication competent AAV • [c]Test for baculovirus • [c]Test for rhabdovirus

[a] The sf9 cell line contains retrovirus-like particles and sf-rhabovirus [b] See Table A-5 footnote f. [c]See Table 4 Footnote h and i in guideline

Definitions of LIVCA cells/EOPC (according to Q5A R2)

Limit of In Vitro Cell Age (LIVCA) Cells: LIVCA cells are derived from production cells at or beyond in vitro cell age by expansion of the MCB or WCB. LIVCA cells may be also referred to as EOPC or Extended Cell Bank (ECB) and these terms can be used interchangeably.

The established LIVCA for production should be based on data derived from production cells expanded at small scale using a representative scale-down model and/or under pilot plant scale or commercial scale conditions to the proposed in vitro cell age or beyond. LIVCA cells are obtained by expansion of the WCB or the MCB

End of Production Cells (EOPC): Cells harvested (under conditions comparable to those used in production) from the MCB or WCB cultured to a passage level or population doubling level comparable to or beyond the highest level reached in production. In certain situations, the chronological time in culture may be measured. The EOPC are also referred to as Extended Cell Banks (ECB) and these terms can be used interchangeably with LIVCA cells.

Extended cell bank (ECB): *Cells cultured from the MCB or WCB and propagated to the proposed in vitro cell age used for production or beyond. May also referred to as EOPC.*

Application of Next Generation Sequencing in these Examples

NGS using non-targeted analysis was used to Replace the In Vivo Assays

The approach used in this example was based on the recommendations of the ICHQ5A R2 guideline: 'Non-targeted NGS can be used to replace the *in vivo* assays and supplement or replace the *in vitro* cell culture assays, without a head-to-head comparison as long as the method is demonstrated to be suitable for its intended purpose. A head-to-head comparison is not recommended due to the different end points of the assay systems and limitations of the breadth of virus detection by the *in vitro* and *in vivo* methods compared to the enhanced capability of NGS for broad virus detection of known and unknown viruses. The results of the *in vitro* and *in vivo* assays rely on virus replication and biological properties for detection in the specific target system which limits the breadth of detection. Replacement of *in vivo* assays by NGS also meets the intent of the global objective to replace, reduce, and refine the use of animals for testing'. Due to the application as a Replacement Assay, the validation package for the NGS assay is provided to demonstrate the assay suitability

- The NGS assay is demonstrated as a scientifically sound method and validated as a limit test, in accordance with, ICHQ2 and as described in the Ph.Eur. 2.6.41 draft general chapter.
- Matrix specific verification was performed to confirm the absence of interference. See Ph.Eur. 2.6.41 draft general chapter (1).
- References used to support not performing the head to head comparison include Charlebois 2020 (2), Gombold (3) and Pei-Ju Chin (2024) in progress (13)

NGS using targeted analysis was used to Replace the Species-Specific Virus Assays

The approach used in this example was based on the recommendations of the ICHQ5A R2 guideline for targeted NGS to replace the cell-based assays and antibody production assays, Mouse Antibody Production (MAP) test, Rat Antibody Production (RAP) test, and Hamster Antibody Production (HAP) for specific virus testing and Nucleic Acid Amplification Technique (NAT) virus testing, without a head to head comparison as long as the method is demonstrated to be suitable for its intended purpose. In this example NGS also complements the *in vitro* cell culture infectivity assays for general adventitious virus detection to enhance the breath of detection and potential assay limitations.

- To demonstrate the suitability of NGS to replace the species-specific virus assay, the example uses the same dataset already established for the non-targeted assay used for the replacement of the in vivo assay by applying bioinformatic analysis to the original non-targeted dataset, by interrogating the dataset for the specific viruses to be detected.
- The use of targeted NGS can help to overcome the limitation of NAT for the detection of virus variants.
- Due to the application as a Replacement Assay for specific virus testing, the validation package for the NGS assay is provided to demonstrate the assay suitability.
- Matrix specific verification was performed to confirm the absence of interference. See Ph.Eur. 2.6.41 draft general chapter (1)

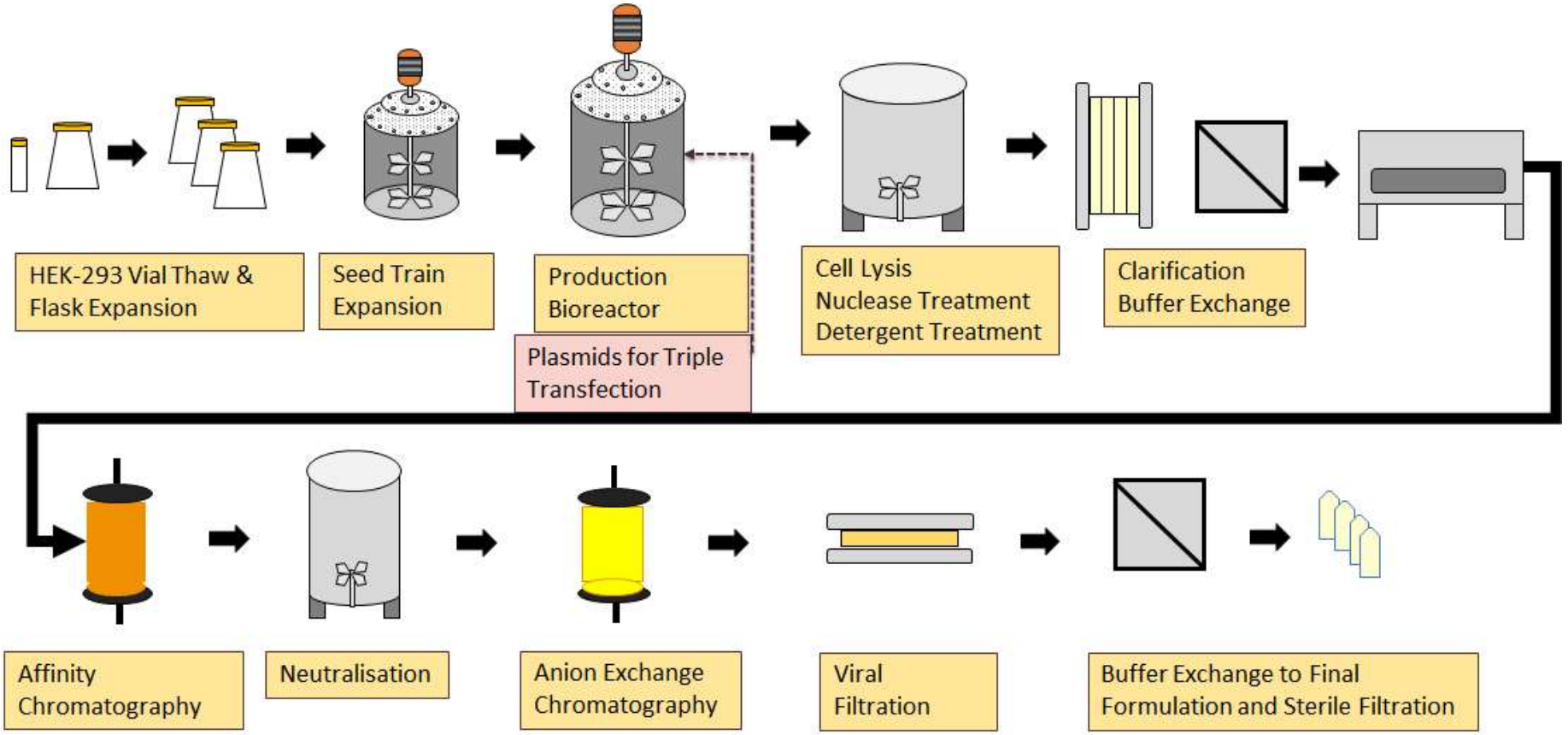
2. AAV vector expressed by triple plasmid transfection in HEK-293 cells

In this section we provide considerations for virus safety for Example 1: Plasmid Transfection Platform

- Raw materials risk assessment
- Cell Substrate testing for HEK-293 MCB, WCB and LIVCA/EOPC
  - Opportunity to replace conventional assays with molecular methods
- Virus Testing on the HEK-293 Unprocessed Bulk (Harvest) and Drug Substance
- Plan for Virus Clearance Evaluation using Non-Specific Model Viruses

AAV vector Platform: Example 1-Plasmid Transfection

- The cell substrate is of human origin (HEK-293).
- An example of the manufacturing process is shown below and includes upstream, harvest/clarification, purification and final formulation process steps



Risk Assessment for Raw Materials for Example 1: AAV Vector Plasmid Transfection Platform

Generation of HEK-293 Master Cell Bank (MCB)

- The MCB (suspension culture) has been established in chemically-defined culture medium containing no human or animal derived components
- Cholesterol was used in the cell line history. Manufacture of cholesterol involved harsh manufacturing process that is known to inactivate viruses
- Bovine serum and porcine trypsin were used in the cell line history.
- There is no available documentation for the raw materials that have been used in the history of the cell line.
- There is full documentation for the raw materials used for the establishment of the MCB.

Raw Materials used for Routine Manufacture

- No animal or human derived raw materials are used in the MCB or manufacturing process.

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1. HEK-293 Master Cell Bank & LIVCA/EOPC Testing Strategy

Assay to be Performed		Assay Information	ICHQ5A
Tests for Retroviruses and other Endogenous viruses	Reverse transcriptase assay (e.g., PERT)	• Assayed cell-free culture supernatant	Section 3.2.1
	Transmission Electron Microscopy (TEM)	• TEM to check absence of viral particles in cells	
	Retrovirus Infectivity assay	• Not required as PERT and TEM assays are negative in this example	
	Chemical Induction Study	• Not generally needed for human cell lines based on scientific knowledge & experience • A one-time characterisation study for novel/unknown viruses but not a test performed on routine basis.	
Tests for Adventitious Viruses	*In Vitro* Cell Culture infectivity assay	Consistent with existing regional regulations and guidance: • 28 day testing using 3 indicator cell lines (MRC-5, Vero and HEK-293). Indicator cell cultures were monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses	Section 3.2.2
	In vivo assay or NGS	The non-targeted NGS assay was used as a replacement assay for the *in vivo* assays in this example *Use of validated NGS method allowed for replacement of in vivo assay.*	Section 3.2.3 & 3.2.5

1. HEK-293 Master Cell Bank & LIVCA/EOPC Testing Strategy (cont.)

Assay to be Performed		Option 1 Conventional Assays were used	Option 2 Molecular Methods Used to Replace a number of Conventional Assays	See ICHQ5A
		Assay Information		
Tests for Specific Viruses[a]	Bovine virus test	• [b]Cell culture-based infectivity assays or NAT	• Targeted or non-targeted NGS was used to replace these assays. *Use of validated NGS method allowed for omission of additional virus tests relative to Option 1.*	Section 3.2.4 & 3.2.5
	Bovine polyomavirus assay	• PCR-based assay		
	Porcine virus test	• [b]Cell culture-based Infectivity assays or NAT		
	Porcine circovirus 1 and 2	• PCR-based assay		
	Human viruses	• [c]PCR-based assays		

[a] Not performed for LIVCA/EOPC based on risk assessment including testing of the MCB
[b] In this example the cell culture based test was performed according to 9cfr 113.47 and 113.53
[c] In this example the PCR based assays were performed according to (Reference14)

Cell Substrate Testing for HEK-293 WCB

Assay to be Performed	Assay Information
Tests for Adventitious Viruses	
[a]28 Day in vitro assay	• Use 3 indicator cell lines consistent with existing Regional Regulations (MRC-5, Vero and HEK-293) • The indicator cell cultures are monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance

[a]The *in vitro* virus assay is performed directly on the WCB or on LIVCA/EOPC cells directly derived from this WCB consistent with existing Regional Regulations.Table 1. Virus Tests Recommended for Characterisation of Cell Substrates (Footnote c.)

Virus Testing on the HEK-293 Unprocessed Bulk (Harvest) and Drug Substance

Assay to be Performed	Unprocessed Bulk (Harvest)	Drug Substance (purified bulk)
Tests for Adventitious Viruses		
In vitro assay	• [a]3 indicator cell lines (MRC-5, Vero and HEK-293) were used • The indicator cell cultures were monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance	Not Applicable[e]
Tests for specific viruses[b]	Not Done	Not Applicable
Tests for Retroviruses and Replication Competent Virus		
Retrovirus and Endogenous Virus[c]	Not Done	Not Applicable
Replication Competent AAV in HEK293 cells[d]	Not Done	Report test result based on detection limit

[a]Interference is not expected. Control cells cultured in parallel may be tested consistent with Regional regulations and guidance
[b]No specific virus testing is required because no additional risk factors have been introduced
[c]MCB is negative and therefore no requirement for retrovirus and endogenous virus testing
[d]Replication competent AAV testing is performed on each drug substance for maximum detection as AAV vector titers are expected to be higher at this stage than at the unprocessed bulk (harvest) stage. Cell based assay is used to include passage of the material to increase sensitivity for virus detection and test must be negative based on detection limit. See Table A-5 footnote f.
[e]Not applicable as virus testing is performed on the unprocessed bulk (harvest)

Plan for Virus Clearance Evaluation for Example 1: AAV Vector Plasmid Transfection Production System

Example of a Viral Clearance Strategy - HEK-293 Triple Transfection for rAAV viral vector production

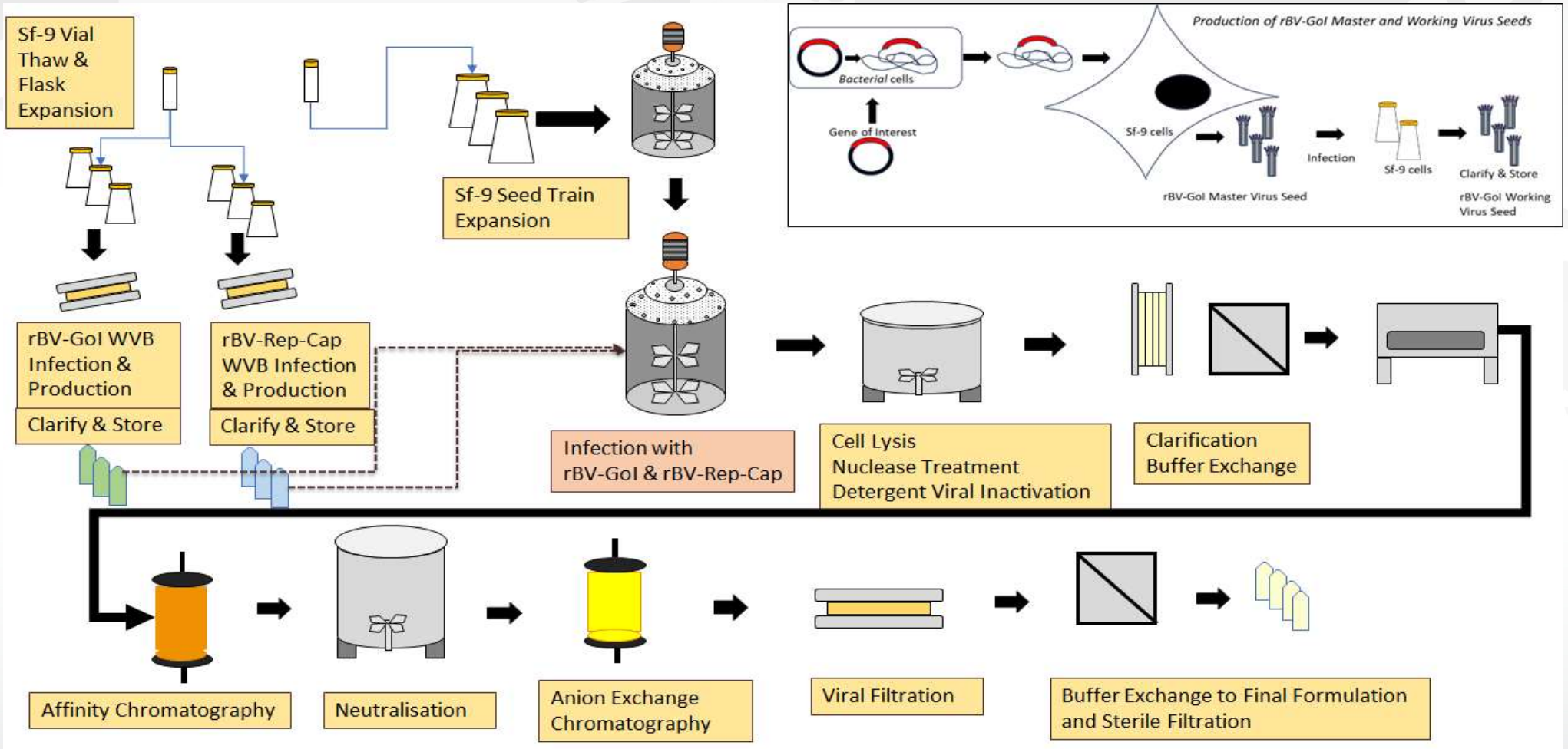
Parameters	All are Non-Specific Model Viruses due to Application of Case A			
	Enveloped Viruses		Non-Enveloped Viruses	
Model Virus	Murine Leukaemia Virus (MLV)	Bovine Viral Diarrhea Virus (BVDV)	Adenovirus Type 2 (Ad2)	Murine Parvovirus (MMV)
Physicochemical Properties	• Medium/Large size enveloped virus • 80-110nm • RNA	• Medium-size, Enveloped virus • 50-70nm • RNA	• Medium size, Non Enveloped • 70-90nm • DNA	• Small size, Non Enveloped • 18-24nm • DNA
Other Rationale for Selection	Commonly used non-specific model virus for Case A	Due to bovine origin materials used. More challenging virus for size-based exclusion	Human derived virus. HEK-293 known to be susceptible	Commonly used non-specific model virus for Case A. Unsuitable for the virus filtration.
Purification Step evaluated in this specific example				
Affinity Capture Chromatography	Evaluated	Evaluated	Evaluated	Evaluated[b]
Medium virus filtration (size-based exclusion of viruses larger than rAAV vector particles)	Not studied[a]	Evaluated	Not Studied[a]	Not studied (No clearance expected)
Total Log Reduction Value	No target for overall virus reduction has been defined in ICHQ5A for Case A			

[a]Based on Annex 5 and the application of Prior Knowledge, data from the smaller BVDV model virus was used to evaluate for retrovirus and Ad2 reduction in this example [b]Evaluation of this step with parvovirus is optional (see script)

3. Baculovirus expressed AAV vector in Sf9 insect cells

- In this section we provide a description of Example 2: AAV vector Platform: Baculovirus expression
- Raw materials risk assessment
- Cell Substrate testing for Sf9 insect cells MCB, WCB and LIVCA
- Virus Testing on the Sf9 insect cells Unprocessed Bulk (Harvest) and Drug Substance
- Virus Clearance Data for AAV vector recombinant Baculovirus Expression System using Specific and Non-Specific Model Viruses
- Calculation of Estimated Virus Particles (rhabdovirus and baculovirus) Per Dose

AAV Vector Platform: Baculovirus Expression System - Example 2



Risk Assessment for Raw Materials for Example 2: Baculovirus Expression System

Generation of Sf9 Master Cell Bank (MCB)and Working Cell Bank (WCB)

- The MCB (suspension culture) has been established in chemically-defined culture medium containing no human or animal derived components
- Non-inactivated bovine serum and porcine trypsin were used in the cell line history.
- There is no available documentation for the raw materials that have been used in the history of the cell line.
- There is full documentation for the raw materials used for the establishment of the MCB.

Raw Materials used for Routine Manufacture

- No animal or human derived raw materials are used in the manufacturing process

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1. Sf9 Master Cell Bank & Limit of *In Vitro* Cell Age/EOPC Testing Strategy

Assay to be Performed		Assay Information	ICHQ5A
Tests for Retroviruses and other Endogenous viruses	Reverse transcriptase assay (e.g., PERT)	• PERT Assay Positive	Section 3.2.1
	Transmission Electron Microscopy	• TEM was positive for the presence of retroviral particles in cells[a]	
	Infectious Retrovirus assay	• Not Done[b]	
	Chemical Induction Study	• Not done[b]	
Tests for Adventitious Viruses	*In Vitro* Cell Culture infectivity assay (28 day assay)	• Lysate of cells and their culture medium inoculated into MRC-5, Vero, Sf9 and Baby Hamster Kidney (BHK)-21 cells (for arbovirus detection) were used. • The indicator cell cultures were monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance	Section 3.2.2
	In vivo assays or NGS	A validated non-targeted NGS assay was used as a replacement assay	Section 3.2.3 & 3.2.5

[a] Positive results have been reported for Sf9 cells
[b]The infectious retrovirus assay was not conducted for this example even though the TEM and PERT assay results were positive based on prior knowledge showing that Sf9 retrovirus-like particles are non-infectious for human cell lines (Ref. 16)

1. Sf9 Master Cell Bank & Limit of *In Vitro* Cell Age/EOPC Testing Strategy (cont.)

Assay to be Performed		Option 1 Conventional Assays were used	Option 2 Molecular Methods Used to Replace a number of the Conventional Assays	ICHQ5A
		Assay Information		
Tests for Specific Viruses[a]	Bovine virus test	• [b]Cell culture-based infectivity assays or NAT	• Targeted NGS was used to replace these assays *Use of validated NGS method allowed for omission of additional virus tests relative to Option 1.*	Section 3.2.4 & 3.2.5
	Insect cell line virus tests • Nodavirus • Sf-Rhabdovirus	• Specific viruses not detected by other methods • NAT testing used for detection		
	Porcine virus test	• [b]Cell culture-based Infectivity assays or NAT		
	Porcine circovirus 1 and 2	• PCR-based assay		

[a]Not performed for LIVCA/EOPC based on risk assessment including testing of the MCB
[b]In this example the cell culture-based test was performed according to 9cfr 113.47 and 113.53 (15)

Cell Substrate Testing for Sf9 Insect cell WCB

Assay to be Performed	Assay Information
Tests for Adventitious Viruses	
[a]28 Day in vitro assay	• Lysate of cells and their culture medium inoculated into Sf9, MRC-5, Vero and BHK-21 cells (for arbovirus detection) • The indicator cell cultures are monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance

[a]The *in vitro* virus assay is performed directly on the WCB or on LIVCA/EOPC cells directly derived from this WCB consistent with existing Regional Regulations. Table 1. Virus Tests Recommended for Characterisation of Cell Substrates (Footnote c.)

Virus Testing for Baculovirus MVS

Assay to be Performed	Assay Information
Tests for Adventitious Viruses	
28 Day in vitro assay	• Lysate of cells and their culture medium inoculated into [a]Sf9, MRC-5, Vero and BHK-21 cells (for arbovirus detection) • The indicator cell cultures are monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance
Non-Targeted NGS	• Used as a replacement for the in vivo assay
Test for Specific Virus	• Rhabdovirus was detected by non-targeted NGS
Test for Retrovirus and Production Virus	
Retrovirus	• Positive PERT assay

[a]Assay interference is expected in Sf9 cells with non-neutralized baculovirus. Baculovirus is neutralized with antiserum to the baculovirus before assaying on Sf9 cells. Control cells cultured in parallel are tested consistent with Regional regulations and guidance

Virus Testing for Baculovirus WVS

Assay to be Performed	Assay Information
Tests for Adventitious Viruses	
28 Day in vitro assay	• [a]Lysate of cells and their culture medium inoculated into Sf9, MRC-5, Vero and BHK-21 cells (for arbovirus detection) • The indicator cell cultures are monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance

[a]Interference is expected in Sf9 cells. Baculovirus is neutralized with antiserum to the baculovirus before assaying on Sf9 cells. Control cells cultured in parallel are tested consistent with Regional regulations and guidance

Virus Testing on the Baculovirus expressed AAV Vector Unprocessed Bulk (Harvest) and Drug Substance

Test	Unprocessed Bulk (Harvest)	Drug Substance (purified bulk)
Tests for Adventitious Viruses		
In vitro assay[a]	• Lysate of cells and their culture medium inoculated into Sf9, MRC-5, Vero and BHK-21 cells (for arbovirus detection) • The indicator cell cultures are monitored for cytopathic viruses, hemadsorbing and hemagglutinating viruses, consistent with existing regional regulations and guidance	• Not Applicable
Tests for specific viruses	• [b]Rhabdovirus Quantification by NAT ((Quantitative Polymerase Chain Reaction (QPCR)) on 3 lots of material	• Not Applicable
Tests for Retroviruses and Replication Competent Virus		
Retrovirus and Endogenous Virus	• Not quantifiable by TEM	• Not Applicable[c]
Titration of production virus in Sf9 cells	1 x 10^9 pfu per ml	3 lots tested see footnote e (Table A-5). Significant excess virus clearance has been demonstrated in this example.
Replication Competent AAV in HEK293 cells	Not Done	Not detected

[a]Interference is expected in Sf9 cells. Baculovirus is neutralized with antiserum to the baculovirus before assaying on Sf9 cells. Control cells cultured in parallel are tested consistent with Regional regulations and guidance
[b]For virus clearance calculation
[c] The manufacturing process efficiently removes retrovirus

Virus Clearance Data for Example 2 Baculovirus-expressed AAV vector Production System using Specific & Non-Specific Model

	Specific Model Virus Clearance for Case B, C and Case F				
	Specific Model Viruses			Non Specific Model Viruses	
Model Virus	Autographa californica multiple nucleopolyhedrovirus (Baculo)	Vesicular Stomatitis Virus (VSV)	Murine Leukaemia Virus (MLV)	Reovirus 3	Parvovirus
Physicochemical Properties	Large size enveloped virus 250-300nm DNA	Medium size enveloped virus 70 x 150nm RNA	Medium/ Large size enveloped virus , 80-110nm RNA	Medium size Non-Enveloped 60-80nm RNA	Small size Non-Enveloped 18-24nm DNA
Other Rationale for Selection	Due to the use of Production Virus in the Manufacturing Process High virus titers were used in spiking studies in this example	Due to the endogenous Rhabdovirus present in the host cell line	Due to the endogenous retrovirus-like particles present in the host cell line	Commonly used non-specific model virus	Commonly used non-specific model virus
Purification Step evaluated in this specific example					
Detergent Viral Inactivation	Evaluated , 5.00 LRF	Evaluated , 5.00 LRF	Evaluated , 5.00 LRF	Not studied (No clearance expected)	Not studied (No clearance expected)
Affinity Capture Chromatography	Evaluated , 2.00 LRF	Evaluated , 2.00 LRF	Evaluated , 2.00 LRF	Not Studied in this example	Evaluated[b]
Ion Exchange Chromatography	Evaluated , 4.00 LRF	Evaluated , 4.00 LRF	Evaluated , 5.00 LRF	Evaluated	Evaluated[b]
Medium virus filtration	Evaluated , 6.00 LRF	Not Studied , 5.00 LRF[a]	Not Studied , 5.00 LRF[a]	Evaluated	Not studied (No clearance expected)
Total Log Reduction Factors (LRF)	17 LRF	16 LRF	17 LRF	Not Applicable	

[a]Based on Annex 5 and the application of Prior Knowledge, data from the smaller Reovirus-3 model virus was used to provide reduction claims for both VSV and MuLV. This approach was not applied for Baculovirus reduction in this example, as the higher Baculovirus spike titer provided even higher virus reduction for Baculovirus. [b]Evaluation of these steps with parvovirus is optional (see script)

Calculation of Estimated Virus Particles Per Dose for Baculovirus expressed AAV vector Example

Criteria	Baculovirus	Rhabdovirus
1. Assumptions Measured		
Concentration of virus entering purification process	10^9 pfu/mL	10^9 genome copies/mL
Calculated viral clearance factor	$>10^{17}$	$>10^{16}$
Volume of Culture Harvest used for one Dose	200mL (=2E+2mL)	200mL (=2E+2mL)
2. Calculation of Estimated Particles per Dose		
Starting calculation	(1E+9 virus units/mL) x (2E+2 mL/dose) =2E+11 virus units/dose	(1E+9 virus units/mL) x (2E+2 mL/dose) =2E+11 virus units/dose
Clearance Factor	>1E+17	>1E+16
Particles per Dose after Purification	<(2E+11)/(1E+17)=2E-6=2/1,000,000	<(2E+11)/(1E+16)=2E-5=2/100,000
Safety Margin[a]	<1 particle /500,000 doses	<1 particle /50,000 doses

[a]The reduction factors represented here are typical but do not represent expected targets.

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ICH: Q5A (R2)

Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

Training Material

Module 2 – Prior Knowledge and Platform Validation

International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

Introduction

Here we provide examples of the application of prior knowledge from the CHO cell line history and platform technology applied during development of a therapeutic monoclonal antibody (mAb X). Three sections will be covered as follows:

1. Master Cell Bank (MCB) Virus Testing Strategy
2. Virus Inactivation by Low pH
3. Viral Clearance Studies with Used Chromatography Resin

Note, the examples provided here are not exhaustive, flexibilities may apply to other situations.

1. Master Cell Bank Virus Testing Strategy

In this section we describe the MCB virus testing strategy for a hypothetical product, mAb X.

The Parental Cell Bank

- The CHO Parental Cell Bank (suspension cells), used to make the MCB for mAb X, was used by the same company several times to create MCBs for different products
- The CHO Parental Cell Bank has been tested by: 28 days *in vitro* virus detection assay (MRC-5, Vero, CHO-K1), *in vivo* assay, TEM, bovine viruses *in vitro**, porcine viruses *in vitro**, PCRs for Porcine Circovirus-1 (PCV-1)*, PCV-2*, BPyV* , and Minute Virus of Mice (MVM)

*Testing on bovine and porcine viruses was performed because bovine serum and porcine trypsin were used in the history of the cell line.

1. Master Cell Bank Virus Testing Strategy

Cell Line Development and Generation of MCB for mAb X

- Transfection and cell cloning were performed without animal/human derived reagents (e.g., serum).
- Generation of the MCB was performed with culture medium containing no animal/human derived components, no trypsin was used.
- There is in house documentation for all raw materials that have been used.

1. Master Cell Bank Virus Testing Strategy

Option 1: mAb X Testing

Test	Comment
In vitro adventitious virus detection	28 days e.g., MRC-5, Vero, CHO
PCR or NGS (or MAP)	See ICHQ5A (R2) Section 3.2.4
PCR or NGS (or HAP)	See ICHQ5A (R2) Section 3.2.4
MVM	PCR or 324K cells
TEM	Retrovirus-like particles expected
Retrovirus infectivity	See ICHQ5A (R2) Section 3.2.1

***In vivo* assays waived (ICHQ5A (R2) Section 3.2.3). Testing for bovine and porcine viruses not needed at the MCB level as the Parental Cell Bank was tested and no bovine and porcine raw materials (or materials with risk of bovine/porcine virus contamination) were used after this stage. PERT assay not performed as it is expected to be positive (ICHQ5A (R2) Section 3.2.1).**

1. Master Cell Bank Virus Testing Strategy

Option 2: mAb X Testing

Test	Comment
In vitro adventitious virus detection**	28 days e.g., MRC-5, Vero, CHO
NGS*	See ICHQ5A (R2) Section 3.2.5.2
TEM	Retrovirus-like particles expected
Retrovirus infectivity	See ICHQ5A (R2) Section 3.2.1

* Non targeted NGS is used to replace the in vivo test and supplement in vitro testing . The use of a validated NGS method allows for omission of additional virus specific (i.e MAP, HAP, and MVM) tests relative to Option 1.

** In addition to Options 1 and 2, a validated non-targeted NGS can also be used as replacement for in vitro testing at the MCB stage, where appropriately justified.

1. Master Cell Bank Virus Testing Strategy

Conclusion

Based on a risk assessment considering the knowledge on the CHO cell line, prior testing of the Parental Cell Bank and how the MCB was generated, both options for MCB testing presented would be adequate.

2. Virus Inactivation by Low pH

In the following example, we refer to Section 6.6 of ICHQ5A (R2) and the definitions for platform manufacturing, internal and external prior knowledge.

2. Virus Inactivation by Low pH

Platform Approach – Background Information

In this section we provide an example of external and internal (in-house) prior knowledge used to support not performing product specific virus inactivation studies with mAb X for low pH inactivation.

- Three other mAbs (A, B and C) have been previously manufactured by the same company using a similar platform process.
- The new mAb X is being manufactured using a platform process similar to that of mAbs A, B and C.
- Low pH virus inactivation data are available from mAbs A, B and C.

2. Virus Inactivation by Low pH

External Prior Knowledge

- Cell culture process parameters are not known to impact virus inactivation at the low pH conditions outlined in this example *(Biophorum publication Mattila et al. 2016)*.
- The exact composition of the process intermediate is not critical for virus inactivation at pH ≤3.6 if the mAb has undergone similar partial purification by a Protein A capture step *(Biophorum publication Mattila et al. 2016, Cai et al., Biologicals 85 (2024) 101751, Biotechnol. Prog., 2016, Vol.32, No.1, Chinniah, PDA J Pharm Sci and Tech 2016, 70 293-299, Mattila, ASTM E2888-12)*.
- ICHQ5A (R2) Annex 5 Section 5.3 states that treatment at pH ≤ 3.6, ≥ 15°C for ≥ 30 minutes at ≤ 500 mmol/L sodium chloride concentration is effectively inactivating Xenotropic Murine Leukemia Virus (XMLV). Acetate and citrate buffer are most commonly used and allow for robust XMLV inactivation.

2. Virus Inactivation by Low pH

Conditions used in Low pH Inactivation

This table shows the conditions used in viral clearance studies for mAbs A, B and C compared to the manufacturing conditions for mAb X. Viral clearance studies reflect the worst-case manufacturing conditions for mAb X.

Parameter	mAb X	mAb A	mAb B	mAb C
	Manufacturing conditions	Conditions used in viral clearance studies		
pH	3.5 ± 0.1	3.6 ± 0.05	3.70 ± 0.05	3.55 - 3.64
Temperature,˚C	15-25	14.5 ± 0.5	14-15	15
Time, min	30-60	0-60	0-60	0-120
Buffer system	Acetate	Acetate	Acetate	Acetate
Antibody concentration, g/L	22	6	10	15
Sodium Chloride, mM	0	25	0	0

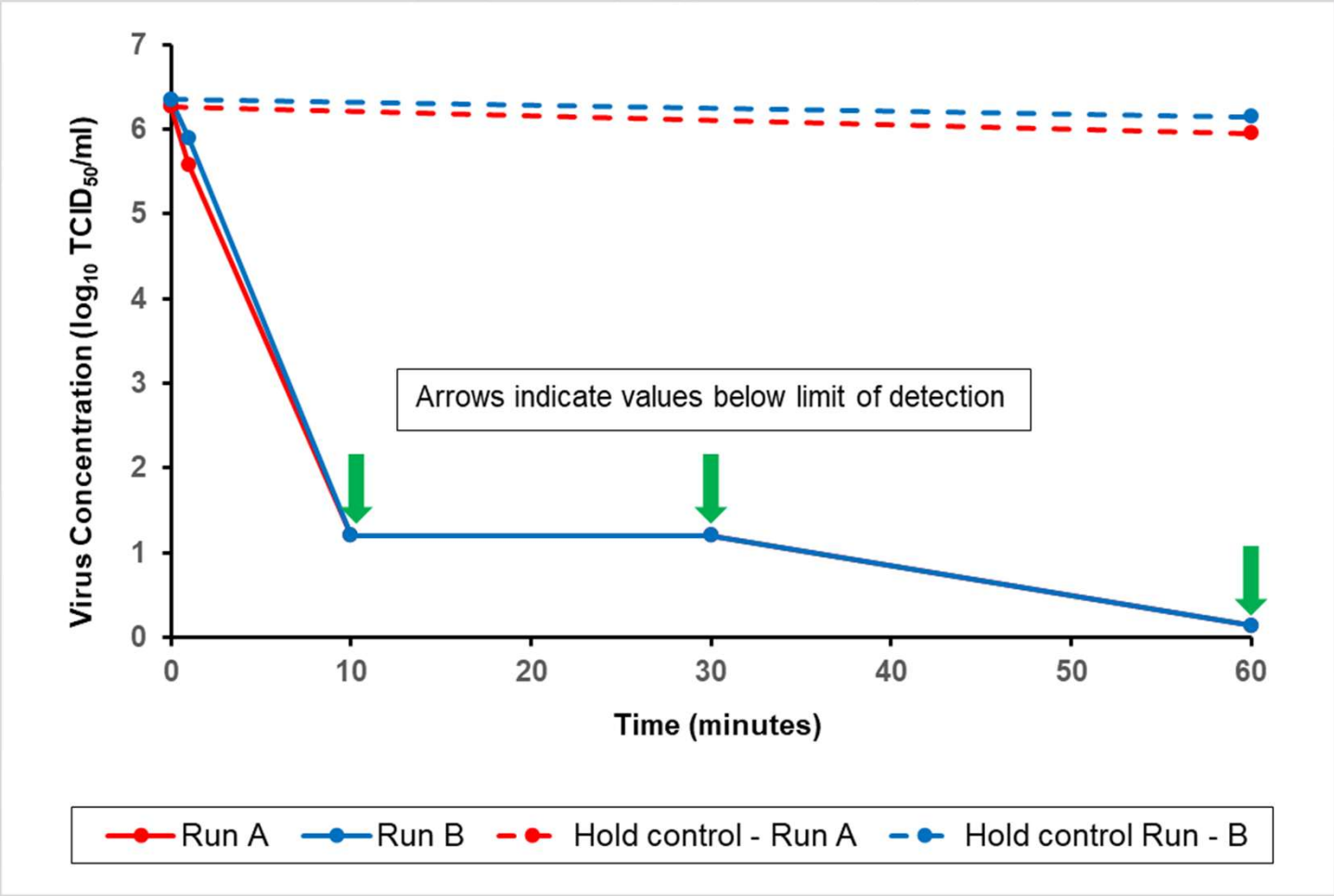
2. Virus Inactivation by Low pH

Internal Prior Knowledge on XMLV inactivation – Product mAb A

Time (min)	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%
Run	A		B	
0[a]	6.27	± 0.30	6.35	± 0.35
1	5.58	± 0.27	5.90	± 0.27
10	≤ 1.20[b]	--	≤ 1.20[b]	--
30	≤ 1.20[b]	--	≤ 1.20[b]	--
60	≤ 0.14[b]	--	≤ 0.14[b]	--
60[a] (hold control)	5.95	± 0.29	6.15	± 0.30
LRF (30 min)	> 5.07	± 0.30	> 5.15	± 0.35

[a] Virus spiked into neutralized material.
[b] The Poisson distribution was used to calculate the titre (95% minimum detection limits) where no infectious virus was detected in the sample. Large volume plating was used for 60min sample.

2. Virus Inactivation by Low pH - mAb A



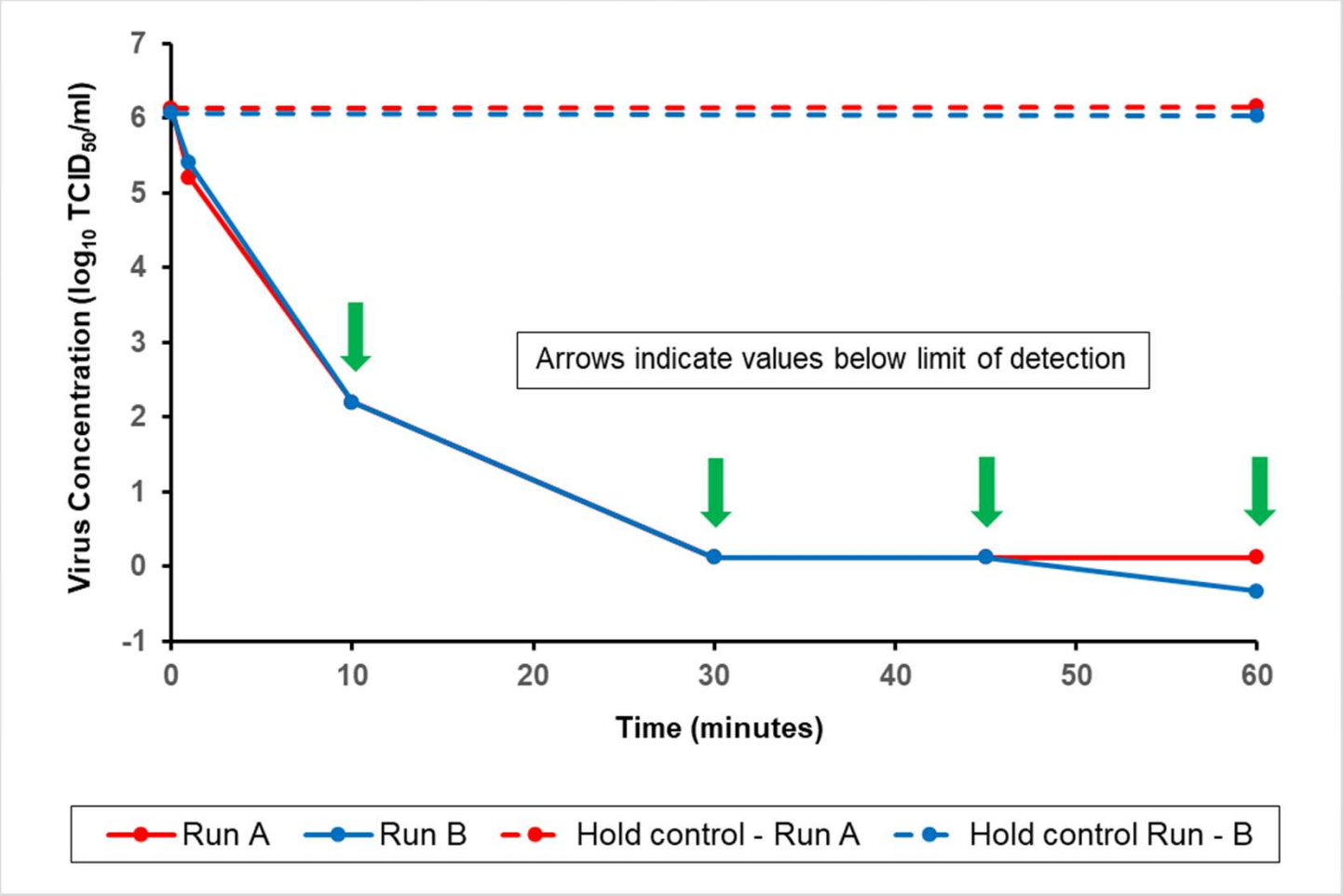
2. Virus Inactivation by Low pH

Internal Prior Knowledge on XMLV inactivation – Product mAb B

Time (min)	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%
Run	A		B	
0[a]	6.13	± 0.25	6.07	± 0.21
1	5.20	± 0.24	5.40	± 0.29
10	≤ 2.20[b]	--	≤ 2.20[b]	--
30	≤ 0.12[b]	--	≤ 0.12[b]	--
45	≤ 0.12[b]	--	≤ 0.12[b]	--
60	≤ 0.12[b]	--	-0.33[c]	0.00[c]
60[a] (hold control)	6.15	± 0.31	6.03	± 0.23
LRF (30 min)	> 6.01	± 0.25	5.95	± 0.21

[a] Virus spiked into neutralized material.
[b] The Poisson distribution was used to calculate the titre (95% minimum detection limits) where no infectious virus was detected in the sample. Large volume plating was used.
[c] 1 of 384 cultures positive using extended large volume plating

2. Virus Inactivation by Low pH - mAb B



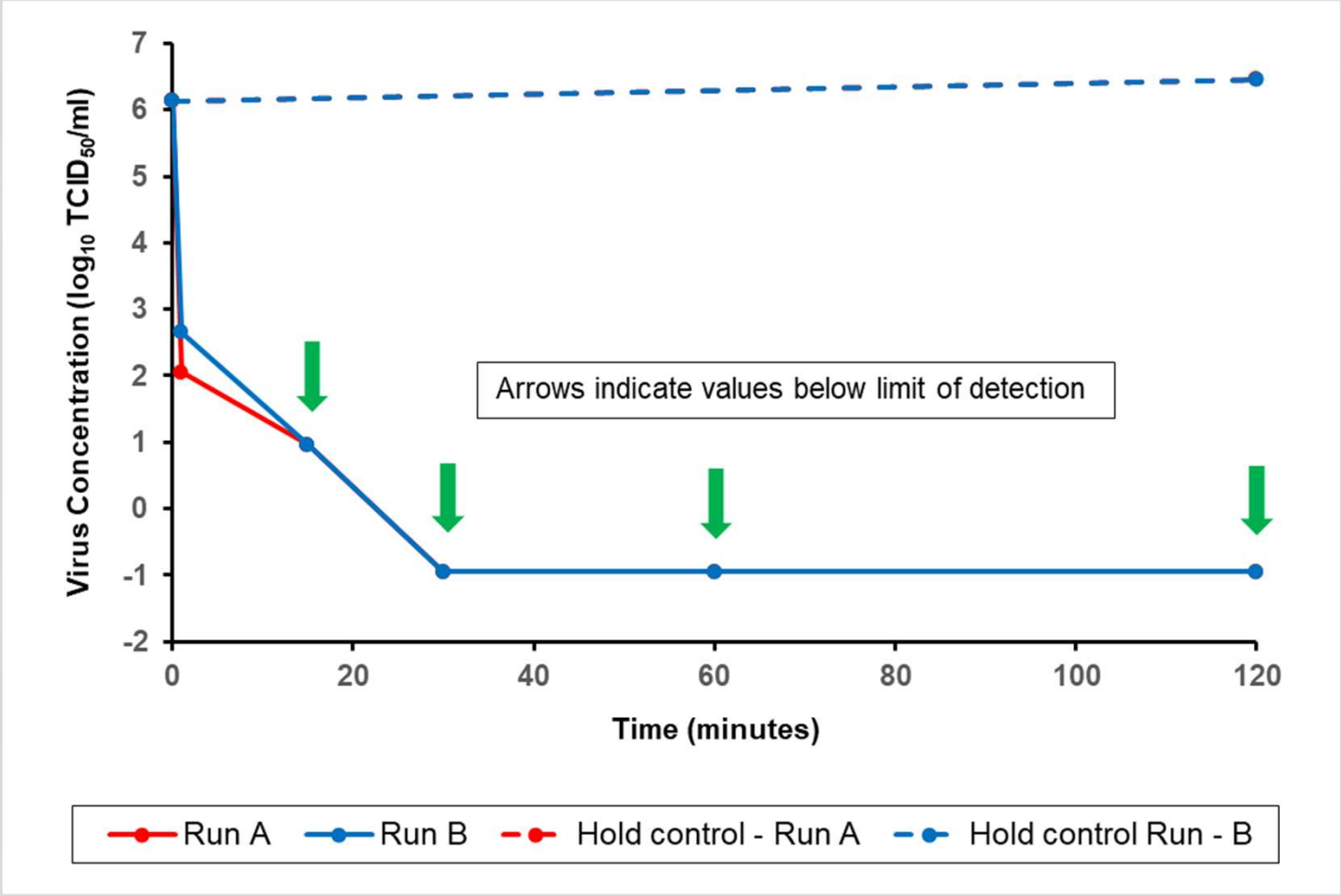
2. Virus Inactivation by Low pH

Internal Prior Knowledge on XMLV inactivation – Product mAb C

Time (min)	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%	Virus Concentration (log_{10} $TCID_{50}$/mL)	Confidence Interval 95%
Run	A		B	
0[a]	6.13	± 0.39	6.13	± 0.39
1	2.05	± 0.25	2.65	± 0.44
15	≤ 0.96	--	≤ 0.96	--
30	≤ -0.95[b]	--	≤ -0.95[b]	--
60	≤ -0.95[b]	--	≤ -0.95[b]	--
120	≤ -0.95[b]	--	≤ -0.95[b]	--
120[a] (hold control)	6.46	± 0.38	6.46	± 0.38
LRF (30 min)[c]	≥ 7.08	± 0.39	≥ 7.08	± 0.39

[a] Virus spiked into neutralized material.
[b] The Poisson distribution was used to calculate the titre (95% minimum detection limits) where no infectious virus was detected in the sample. Large volume plating was used.
[c] LRV (30min) was calculated from the values at Time 0 min and Time 30 min.

2. Virus Inactivation by Low pH - mAb C



2. Virus Inactivation by Low pH

Summary of Log Reduction Factor (LRF)

mAb A	mAb A	mAb B	mAb B	mAb C	mAb C	LRF Claim for mAb X
Run A	Run B	Run A	Run B	Run A	Run B	
> 5.07 ± 0.30	> 5.15 ± 0.35	> 6.01 ± 0.25	5.95 ± 0.21	> 7.08 ± 0.39	> 7.08 ± 0.39	**> 5.07***

*It is also acceptable to claim the average LRF from mAb A Run A and Run B (>5.11) if the difference between both values does not exceed the expectation from assay variability.

Per ICH Q5A (R2), Section 6.6, *when deriving a reduction factor claim using prior knowledge, the claim should be justified considering all reduction factors from the relevant platform data. A conservative reduction factor claim is advised to avoid the risk of overestimating the reduction capacity of the process step.* Therefore, in this example, the values from mAb A were considered as a conservative estimate of the LRF claim for mAb X.

2. Virus Inactivation by Low pH

Conclusion

Considering the information provided in the previous slides, it is acceptable not to perform product specific viral clearance studies for XMLV with mAb X for low pH inactivation.

3. Viral Clearance Studies with Used Chromatography Resin

In this section we describe the external and internal prior knowledge used to support omission of viral clearance studies on used anion exchange (AEX) resin for mAb X.

Note that viral clearance studies with used Protein A resin are not required per ICHQ5A (R2) Section 6.2.6.

3. Viral Clearance Studies with Used Chromatography Resin

Case Study - Background Information for mAb X

- For mAb X, viral clearance was evaluated for the anion exchange (AEX) chromatography step (Q Sepharose, non-binding mode) by performing product-specific viral clearance studies with naïve resin.
- In this case study, the AEX column is sanitised using 0.5M NaOH for 30 minutes.
- Viral clearance data are available from used AEX resin studies for other antibody products. These data can be provided to justify not performing viral clearance studies with used AEX resin for mAb X.
- At manufacture of mAb X, the AEX column is intended to be used for up to 50 cycles.

3. Viral Clearance Studies with Used Chromatography Resin

External Prior Knowledge

- External prior knowledge demonstrates effective virus inactivation in 0.5M NaOH that is used for resin sanitisation *(20986_10061.pdf (cytivalifesciences.com))*. Therefore, it is not necessary to repeat these studies.
- Several publications indicate that reuse of AEX resin does not affect viral clearance (PDA J. Pharm Sci and Tech. 2024, 78, 166-168, *Proceedings of the 2023 Viral Clearance Symposium, Session 3: 2023 VCS New Modalities in Chromatography and Adsorptive Filters - Removal of Carryover Studies Support by Amgen Prior Knowledge Assessment (XiaoXiang Zhu);* PDA J. Pharm Sci and Tech. 2022, 76, 324-327, *Proceedings of the 2019 Viral Clearance Symposium, Session 4: Viral Clearance Strategy and Process Understanding - Virus Reduction by Aged Chromatography Resins (A Schwantes); PDA J. Pharm Sci and Tech 2019, 73, 470-486, Retrospective Evaluation of Cycles resin in Viral Clearance Studies – A Multiple Company Collaboration, John Mattila et. al.*)

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3. Viral Clearance Studies with Used Chromatography Resin

Considerations for Internal Prior Knowledge

- Viral clearance data with used resin are provided from three other mAb products using the same parental cell substrate and same cleaning conditions.
- The AEX column resin is the same brand for mAb X and similar operating parameters are used as for the products from the in-house viral clearance studies.
- The equilibration buffer is similar regarding the chemical composition (buffer matrix, range of pH and conductivity) for mAb X and for the products from the in-house viral clearance studies.
- Similarity of process intermediates that are loaded onto the column is demonstrated. Levels of host cell protein (HCP) and DNA from the mAb X intermediate are the same or lower and the antibody concentration is comparable to the products from the in-house viral clearance studies.

3. Viral Clearance Studies with Used Chromatography Resin

Considerations for Internal Prior Knowledge

- Product specific data are provided showing that the purification (HCP and DNA reduction) works for the mAb X (after 50 cycles) as well as for the products from the in-house viral clearance studies.
- Relevant operating parameters (e.g., bed height, linear flow rate, sanitization time) for the production of mAb X are reflected by the in-house viral clearance studies demonstrating viral clearance with used resin from other products .
- Maximum number of cycles at production of mAb X is clearly defined (e.g. 50 cycles) and is equal to or lower than the number of cycles investigated at in-house studies demonstrating viral clearance with used resin from other products.

3. Viral Clearance Studies with Used Chromatography Resin

Conclusion

Considering the information provided in the previous slides, it is acceptable not to perform viral clearance studies with used resin from the AEX column for mAb X.

Reminder

The training materials presented are only examples. For individual applications, robust justifications and data packages must be presented to support the leveraging of prior knowledge to gain regulatory flexibilities with respect to virus clearance evaluation and testing.

References - Module 2 – Prior Knowledge and Platform Validation

- *(Biophorum publication Mattila et al. 2016, Cai et al., Biologicals 85 (2024) 101751, Biotechnol. Prog., 2016, Vol.32, No.1, Chinniah, PDA J Pharm Sci and Tech 2016, 70 293-299, Mattila, ASTM E2888-12).*
- *(20986_10061.pdf (cytivalifesciences.com))* - Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems
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- *PDA J. Pharm Sci and Tech 2019, 73, 470-486, Retrospective Evaluation of Cycles resin in Viral Clearance Studies – A Multiple Company Collaboration, John Mattila et. al.)*

ICH: Q5A

Guideline on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

Training Material

Module 3 – Continuous Manufacturing

International Council for Harmonisation of Technical Requirements
for Pharmaceuticals for Human Use

Introduction to Case Study 3

Here we provide an example of a virus safety strategy applied to an example of a Continuous Manufacturing process taking into account the unique aspects of this process when compared to conventional manufacturing to include testing, impact on virus inactivation steps and diversion strategies:

- Utilizes Annex III from ICH Q13 as proposed manufacturing process
- Focuses on aspects of virus safety that are unique to CM
- Where possible provides options for how to perform viral clearance studies
- Does not endorse the use of a specific technology.
- Does not limit other approaches that can be justified from scientific and risk-based perspectives.

Scope of Case Studies

- **Modality**
  - monoclonal antibody (Mab)
- **Process/Unit Operations**

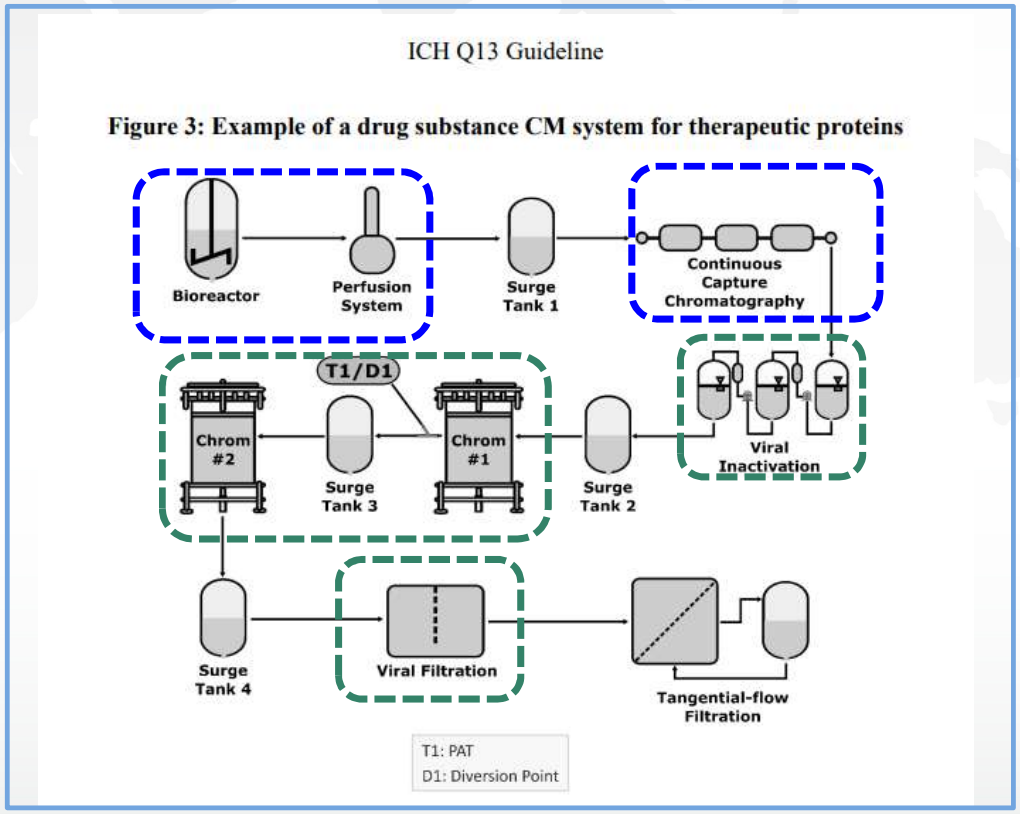
Process described in ICH Q13 Annex III is composed of

integrated

CM
- bioreactor compatible with a perfusion culture system
- continuous capture chromatography

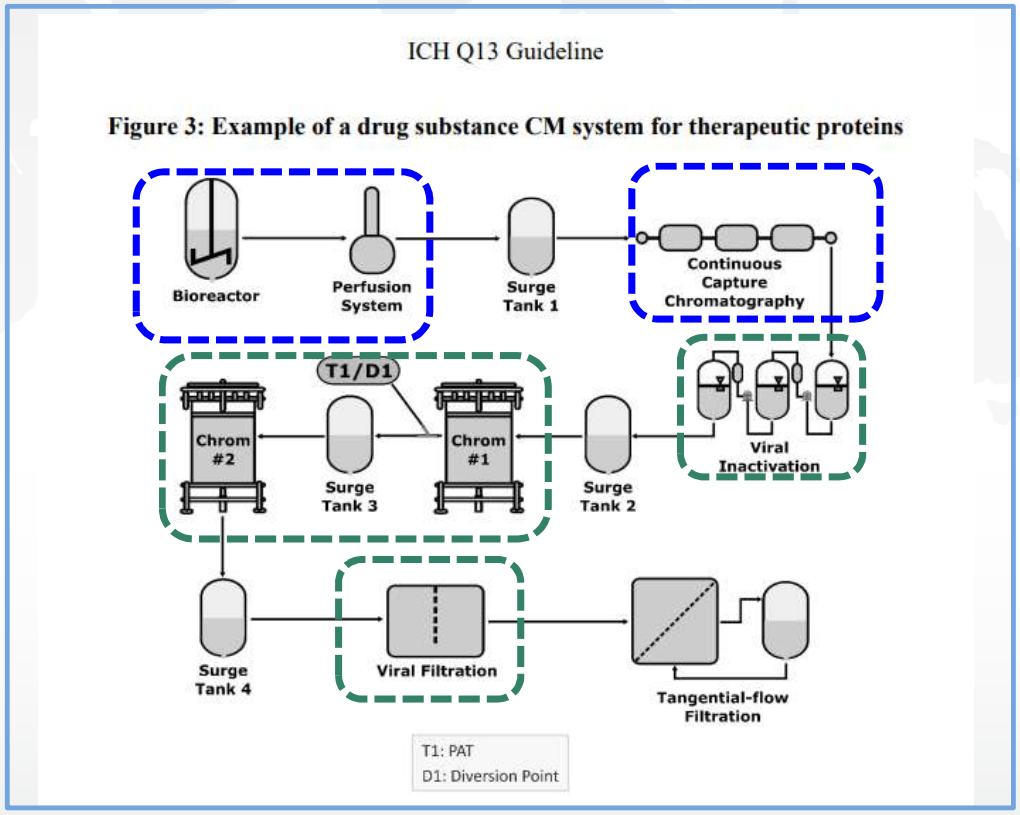
batch[1]
- virus inactivation
- polishing purification chromatography columns
- virus filtration
- buffer exchange and concentration through tangential flow filtration

[1] "Batch" reflects integrated process for which batch principles apply



Scope of Case Studies

- In addition to CM process in Q13 Annex III, following unit operations are also described.
  - Continuous virus inactivation using residence time distribution model
  - Polishing purification chromatography (no surge line or tank)
  - Extended virus filtration
- Unit operations which have little contribution to viral clearance, such as drug substance (DS) fill and Tangential Flow Filtration (TFF) are not described.
- Diversion points are not necessarily limited to that of Q13 Annex III.
- Designs of aseptic facility or closed system equipment are not described.



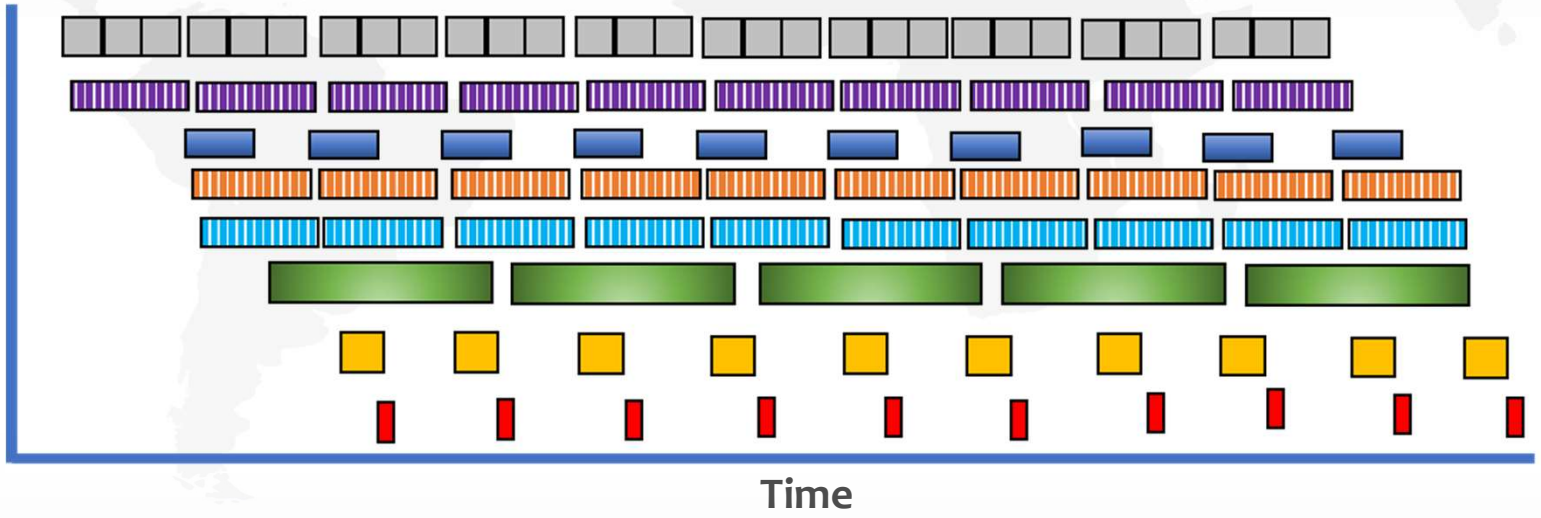
General Principles

- Most important to identify unique risk factors for process
- Scientific Risk Factors and Considerations may include:
  - Extended culture durations
  - Establishing periodicity of sampling
  - Potential for connected unit operations to introduce disturbances to subsequent steps
  - Developing appropriate diversion strategies
- Scale-down models in “batch mode” can be used where justified

Description of Process - Upstream

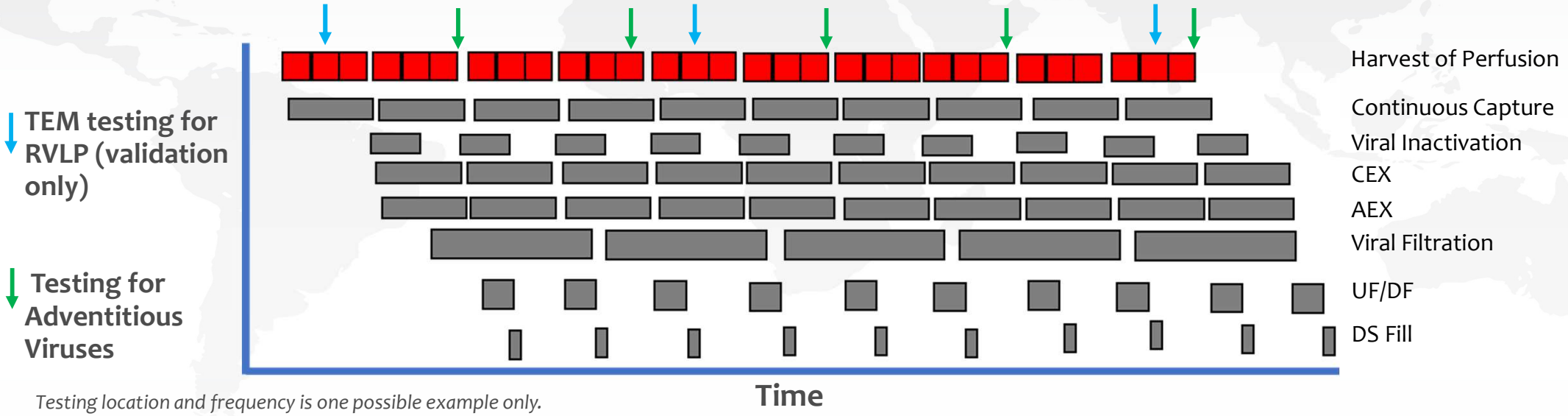
- One or more perfusion cell culture bioreactors are integrated with continuous downstream chromatography
- Each bioreactor will result in multiple harvests
- Process reflects that a run may contain multiple harvests sequentially
- Batch is defined as a quantity of protein in a cell culture harvest reflecting range of number of harvests into single DS fill
- The approach of cell line qualification including LIVCA is same as that for batch manufacturing, even when the cell culture process for CM may operate for significantly longer periods of time than that for batch manufacturing.
- LIVCA should be demonstrated for a new CM process as it may differ from existing batch process

General Description of Manufacturing Cadence



- This figure depicts the cadence of the individual unit operations and the relationships of individual cycles and batches within the process and a production run
- The x-axis depicts the time domain of a production run
- Batch reflects a quantity of protein processed from multiple harvest and filled into a single DS fill

General Considerations for Unprocessed Bulk Testing

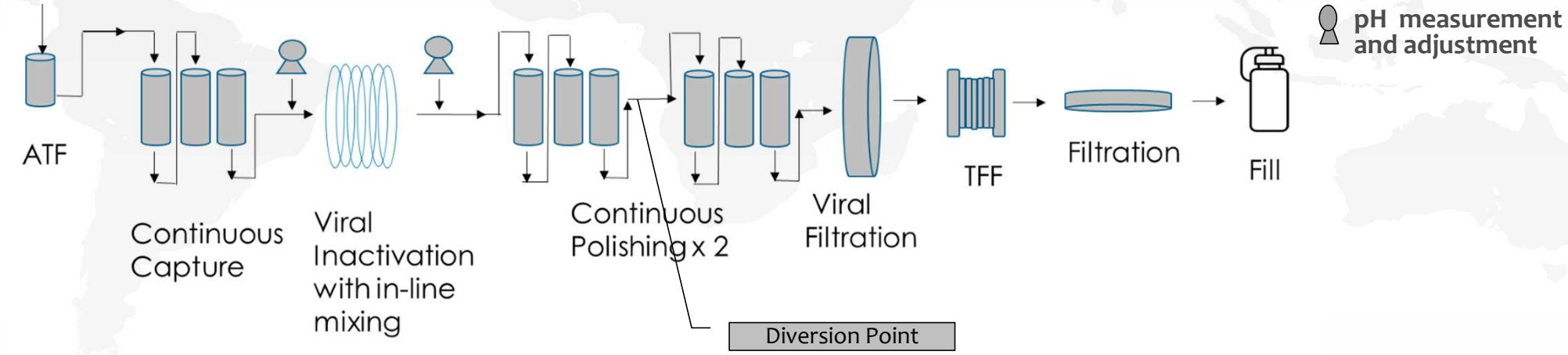


Testing location and frequency is one possible example only.

- Testing does not need to link individually to a specific harvest
- Rapid testing can support quick decision-making regarding material diversion (e.g., NGS for replacement of the in vitro assay)

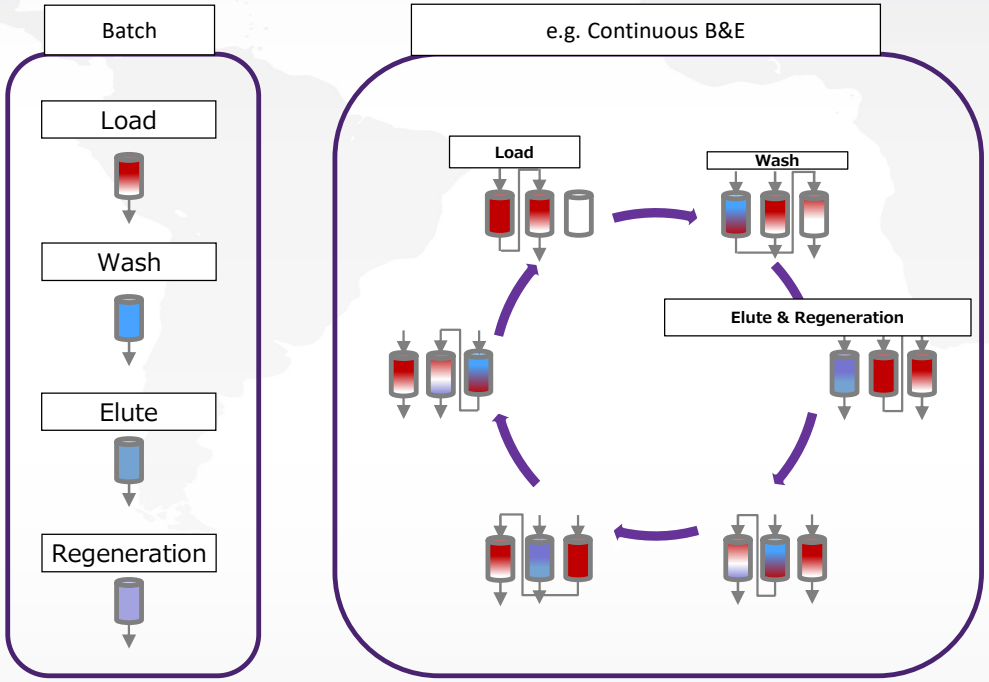
- Testing frequency balances potential loss of material due to contamination and effort (business risk)
- Should be based on process understanding for "worst case" Retrovirus Like Particles (RLVP) load
- For each batch/DS fill at least one adventitious virus test is required

Description of Process - Downstream



- Downstream purification includes multi-column capture chromatography
- Continuous virus inactivation using residence time distribution model
- Continuous polishing
- Extended virus filtration across multiple sub-batches with multiple filter changes

Multiple column-capturing loading description



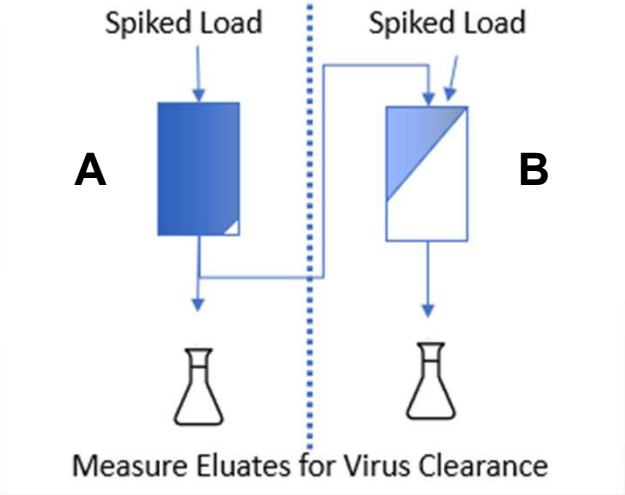
- Simultaneous to loading one or more columns, one column is by-passed and washed, eluted or regenerated.
- All columns are run using the same the same operating conditions
- Process intermediate is loaded on column in a staggered sequence
- This example focuses on the following options for routine loading:
  - Overloading (breakthrough) & switching
  - Parallel loading of columns (no breakthrough from one column rather fixed conditions for all columns)
- Duration of regeneration & sanitization phases needs to ensure efficient inactivation of residual virus

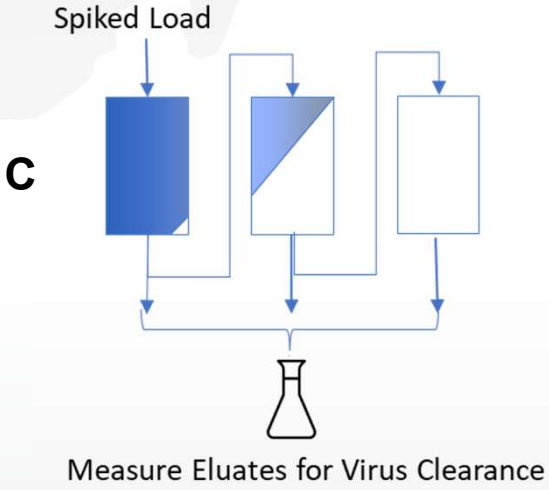
Module 3 – Continuous Manufacturing

Continuous Capture – Small Scale models for viral clearance validation

Breakthrough Mode – can be evaluated in multiple ways:

A. Overload individual column by exceeding its load density: in validation experiment maximum load density needs to be applied to reflect worst case loading (with breakthrough)
B. Retain and load product-containing effluent from first column on second column with steady state load conditions
C. Pooling: combine all elution fractions from multi-column setup



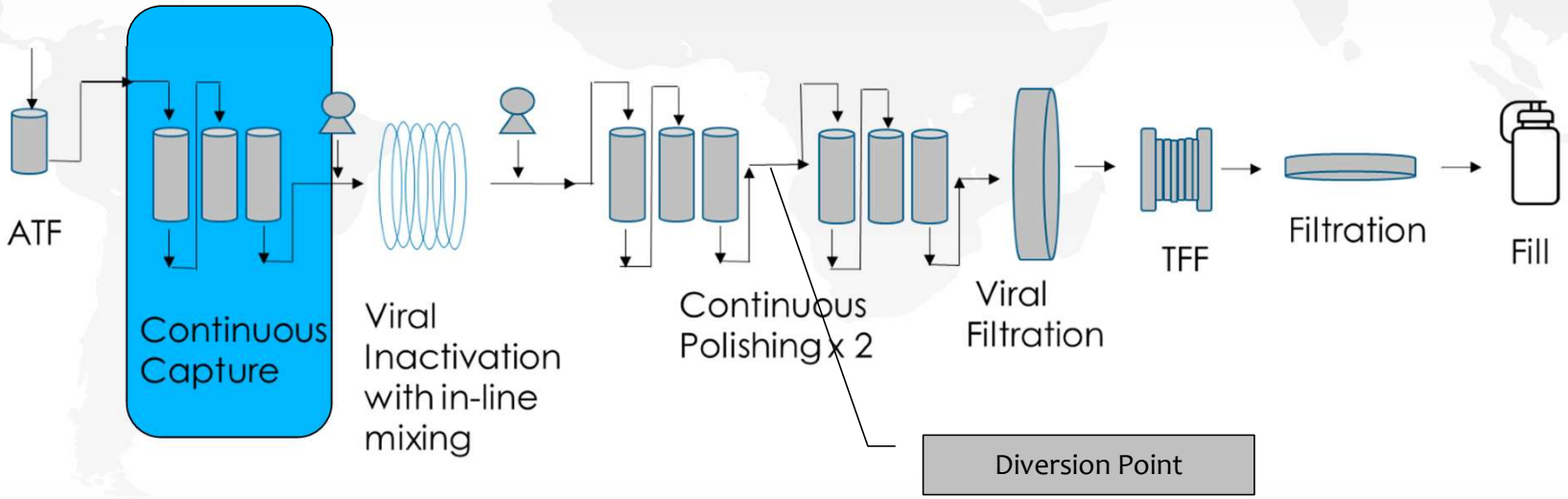


doi: 10.1002/biot.202100433* doi: 10.1002/bit.27674*

doi: 10.1002/bit.27023*

***Publications depict evaluation of bind and elute Protein A column**

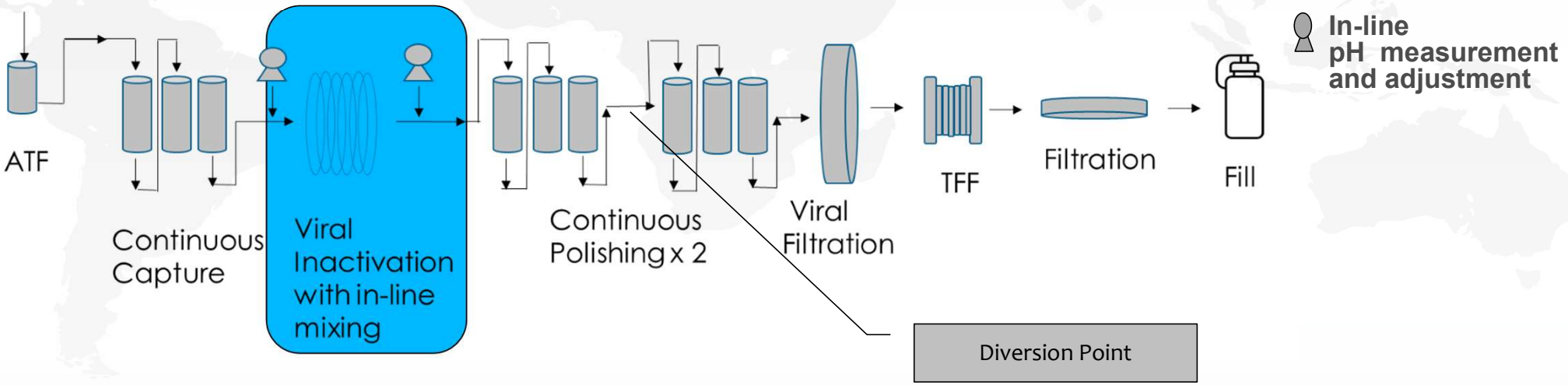
Multiple column-capturing additional considerations



Specific Virus safety considerations unique to this operation mode:

- Diversion strategy (if allowed for in the process)
- Virus carry-over: cleaning strategy (timing of loading makes sychronization more critical)
- Virus validation approach:
  - Process & feedstock variability maybe captured by testing over extended run
  - LRV may correlate with load density, thus understimation is possible, (https://doi.org/10.1002/bit.27012
  - DOI: 10.1002/bit.27674)

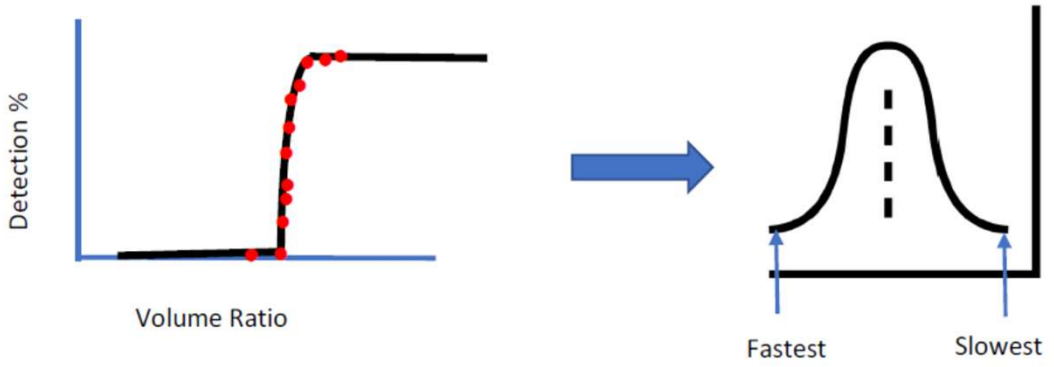
Continuous virus inactivation – introduction



Specific Virus safety considerations unique to this operation mode

- Unit operation uses a specific flow path to create inactivation duration
- Critical parameters are the same as for batch inactivation but must translate flow into time
- Must consider the establishing narrow residence time distribution, platform is commercially available
- Must consider and demonstrate distribution including worst case for system capability and inactivation

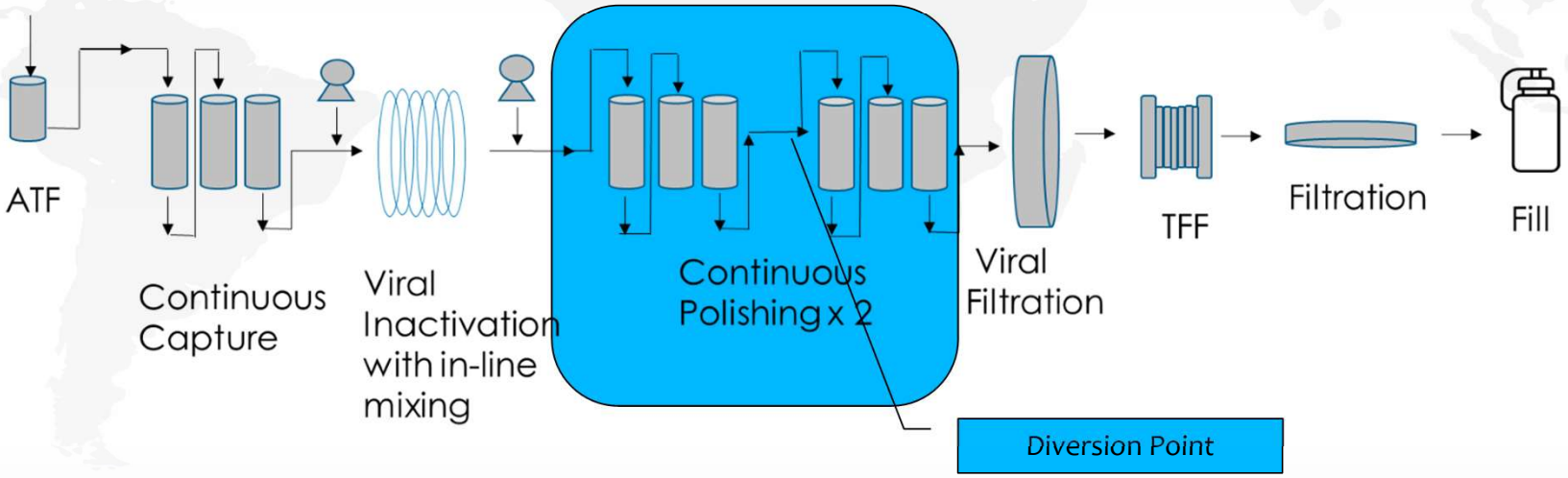
Continuous virus inactivation – Modeling Residence Time



- Tracer experiments allow characterization using a detectable molecule
- Based on the data, residence time distribution can be established and targeted
- The acceptable distribution should be justified by the submitter.
- The minimum residence time is the more critical consideration.

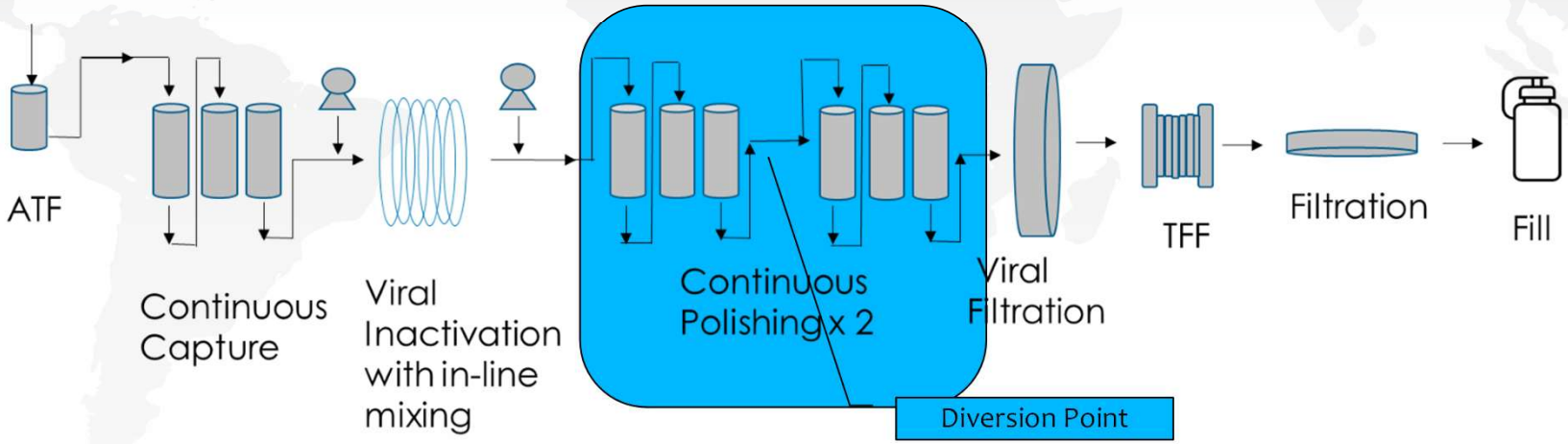
- Additionally, models could allow for real time calculation and display of the residence time distribution during routine operation. This can be potentially used and shown on inspection (e.g., Klutz et al. 2016, Orozco et al. 2017, Gillespie et al. 2019, Kateja et al. 2021).
- Monitoring of pH should be performed consistently throughout the run and material diverted in instances of excursion.
- Prior knowledge for kinetics of inactivation and use of scale down models may be leveraged to justify critical process parameters.

Connected polishing chromatography - Introduction



- Combining bind-elute chromatography and flow-through chromatography with an inline adjustment in between the steps
- Diversion point is located after chromatography (Chrom) #1 and elution from Chrom #1 other than target fraction can be diverted at Diversion Point
- Principles of virus removal remain the same in continuous/connected operation
  - Attributes of feed streams affect performance of virus clearance in the chromatography step
- Virus clearance capacity of the connected steps can be evaluated separately
- Need to consider possibilities of disturbance (e.g. Critical process parameter excursions) on Chrom#1 affect Chrom#2

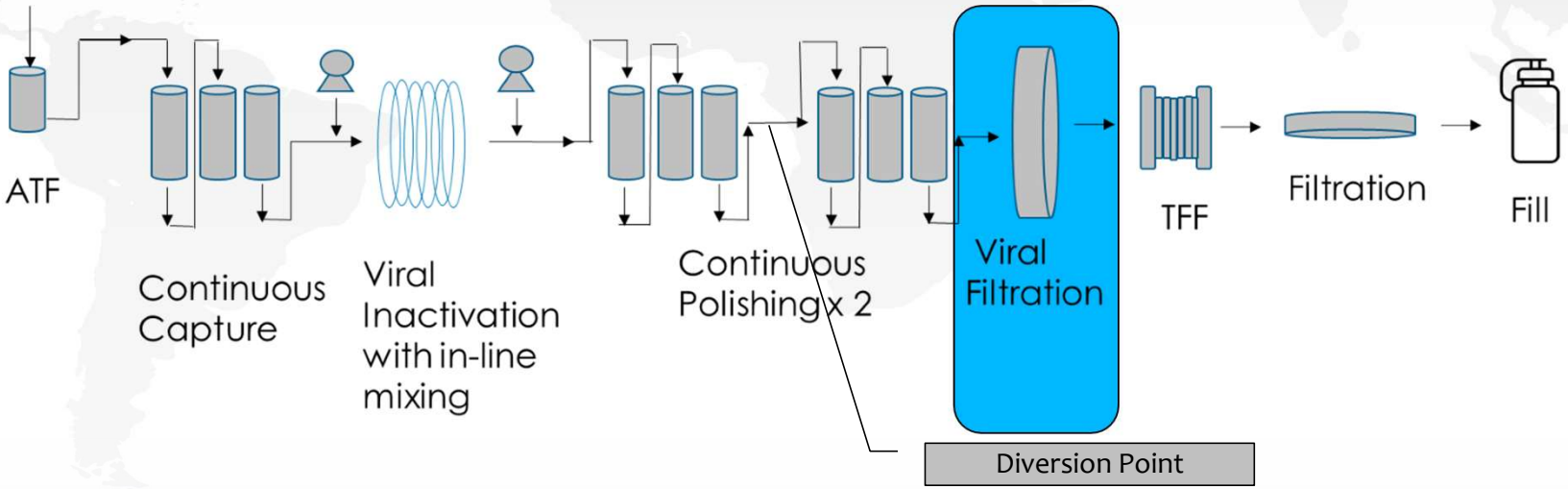
Connected polishing chromatography - additional considerations



Specific Virus safety considerations unique to this operation mode:

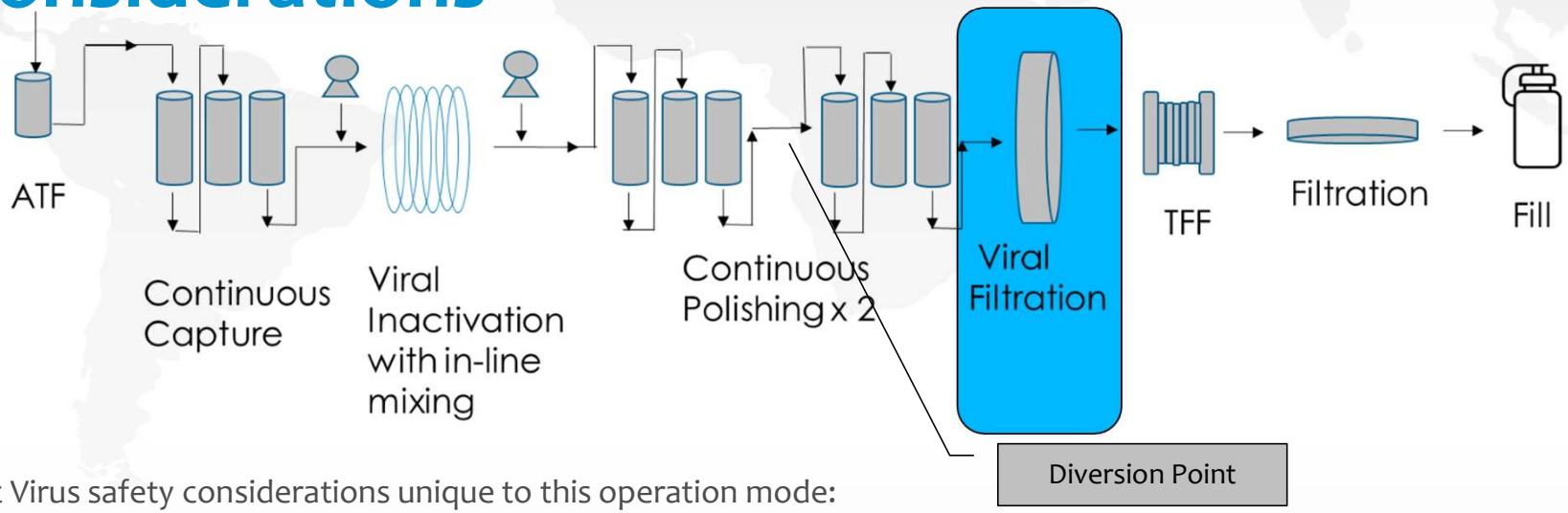
- Understanding the additional critical process parameters and impact of perturbations
- Ensure reliable control of load conditions for both chromatography steps in manufacturing
  - Fluctuation of pH, conductivity, or impurities level depend on Chrom#1 fraction => Capacity of impurity/virus clearance of Chrom#2
- There are many ways to establish inline adjustment. The method of choice depends on the equipment and automation platform used in manufacturing.
- Following the principles of batch processing, in order to design effective regeneration of the column, the risk of virus carryover from cycle to cycle should be assessed.

Continuous viral filtration - introduction



- A single virus filter directly connected to flow through chromatography used for multiple days
- Principles for viral filtration including integrity testing and virus clearance study are same as that of batch mode
- For a continuous operation during longer period campaign, the process design should enable filter change
    - Process controls should be defined to allow for filter changes and post-use integrity testing

Continuous viral filtration – additional considerations



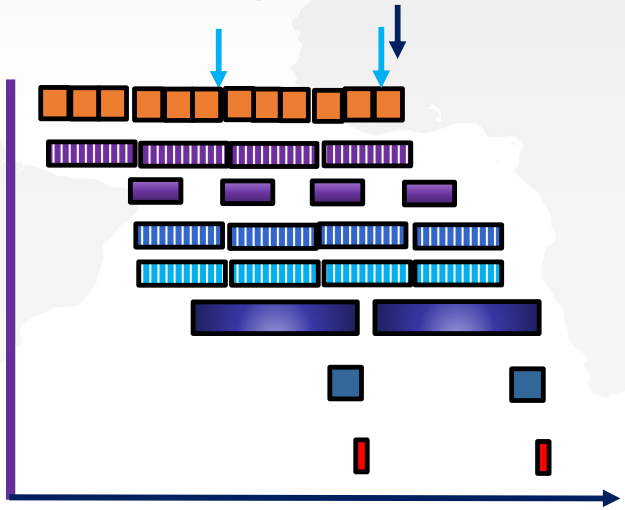
Specific Virus safety considerations unique to this operation mode:
Risk assessment: need to understand

- Impact of direct connection to chromatography step: this may lead to stronger perturbations in filter loading.
- Risk related to extended duration of process step (e.g., pressure build up associate with filter fouling, low flux, low operating pressures, pressure interruption, or depressurization)
- Validation as a batch process could be appropriate if settings of parameters which affect viral clearance do not vary beyond ranges tested in the viral clearance study (e.g., Worst case setpoint).

Continuous Viral Filtration-Spiking Strategies

- **Alternative virus spiking methods**
  - Extended time/volume may be addressed for example by bracketed spiking
  - Filter performance in terms of virus removal capacity should be established over the course of the filter life
  - This should include testing at the beginning and end.
  - Spiking approaches suggested in the literature to, for example, reduce the amount of virus spike, Johnson & Roush, 2018; Lute et al. 2020; Malakian et al. 2022; Kozaili et al. 2024,
  - Spiking strategy needs to be justified considering expected level of contamination and point of time when contamination occurs during cell culture process.
  - Need to balance level of clearance with robustness of the validation approach and effort.

Diversion strategy: Process design & Testing strategy

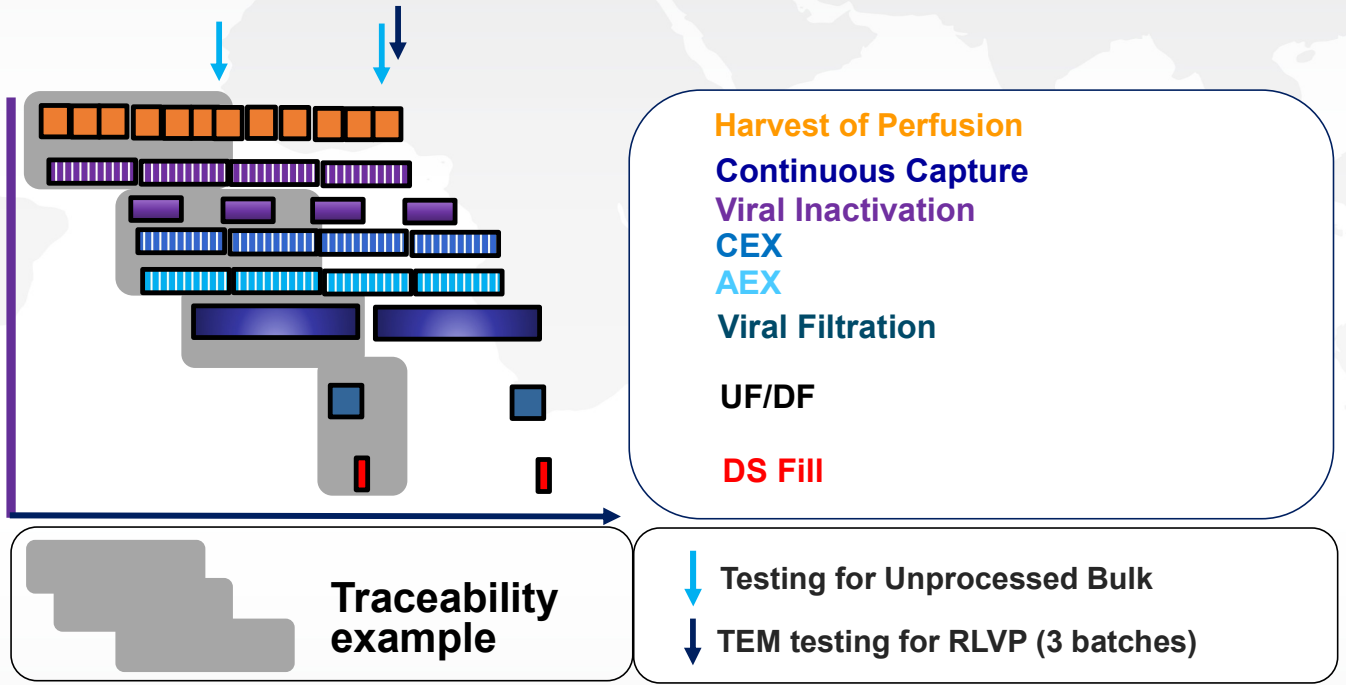


Harvest of Perfusion
Continuous Capture
Viral Inactivation
CEX
AEX
Viral Filtration
UF/DF
DS Fill

Testing for Unprocessed Bulk
TEM testing for RLVP (3 batches)

- Testing does not need to link individually to a specific batch
- Strategy of RVLP quantification should be based on process understanding for "worst case" RVLP load
- Every batch should be bracketed by negative results of adventitious virus testing
- Rapid detection methods such as NGS can be useful in decision making for adventitious agent control.

General Considerations for Diversion

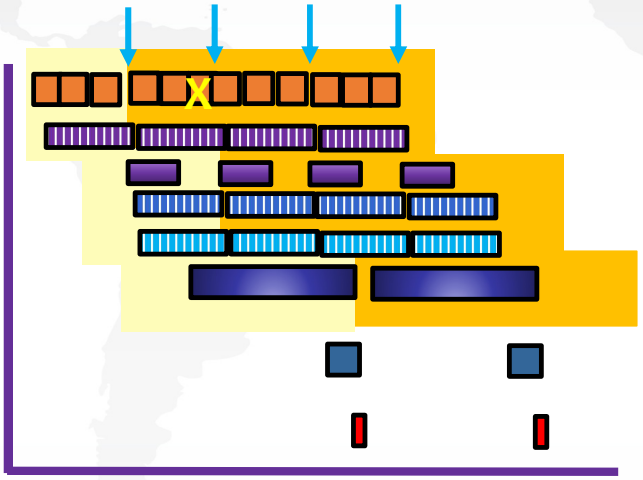


- Traceability must be maintained to consider diversion
- Requires maintaining appropriate diversion points
- No material should be released until conclusion of investigation
- Material upstream of the detected event may be considered for release if a negative test occurred subsequent to the material, depend on traceability and strategies of sampling and diversion

Diversion strategy: Testing frequency

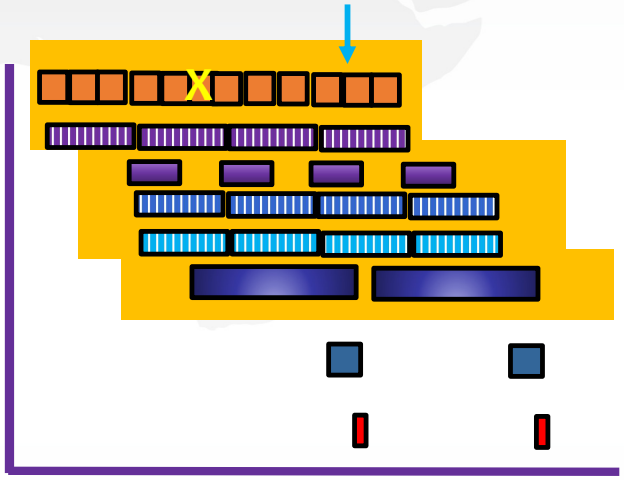
Assuming a contamination in unprocessed bulk #6
Virus filter integrity testing is available immediately after executing the step

High testing frequency



Testing frequency based on cell culture

Low testing frequency



Testing frequency based on timing of virus filter integrity testing

Harvest of Perfusion
Continuous Capture
Viral Inactivation
CEX
AEX
Viral Filtration
UF/DF
DS Fill

Testing for Unprocessed Bulk
X Virus contamination
Material quarantined
Material to be discarded

- All positive material, including subsequent harvests should be diverted and discarded.
- The impact of virus contamination can be further reduced for example by introducing one or more surge vessels.
- Materal quarantined pending investigation outcome

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