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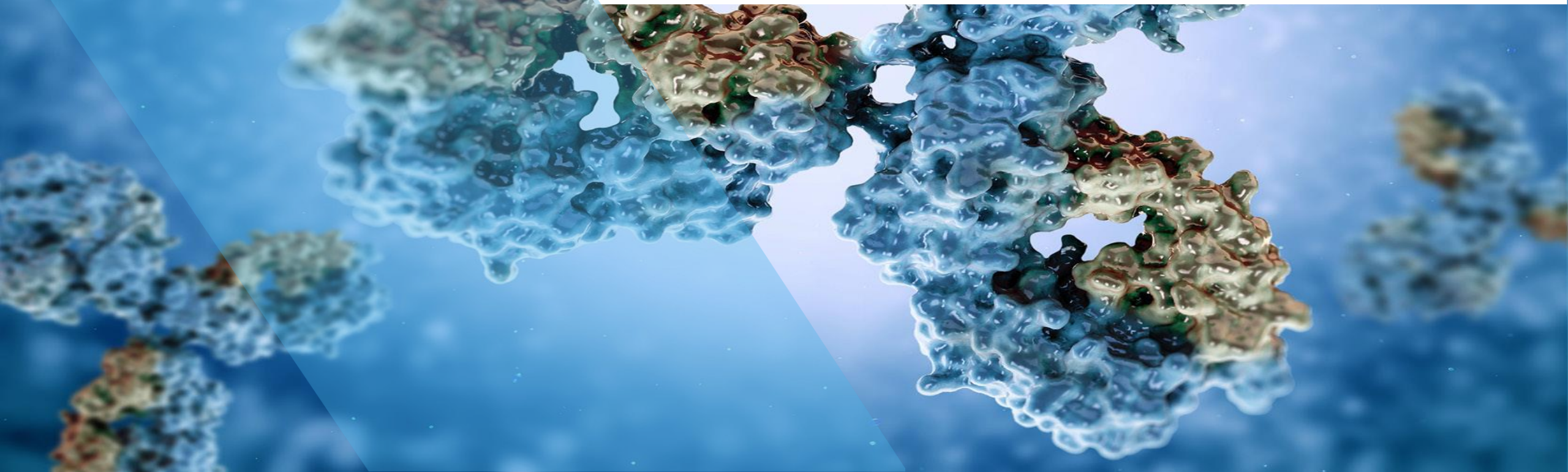
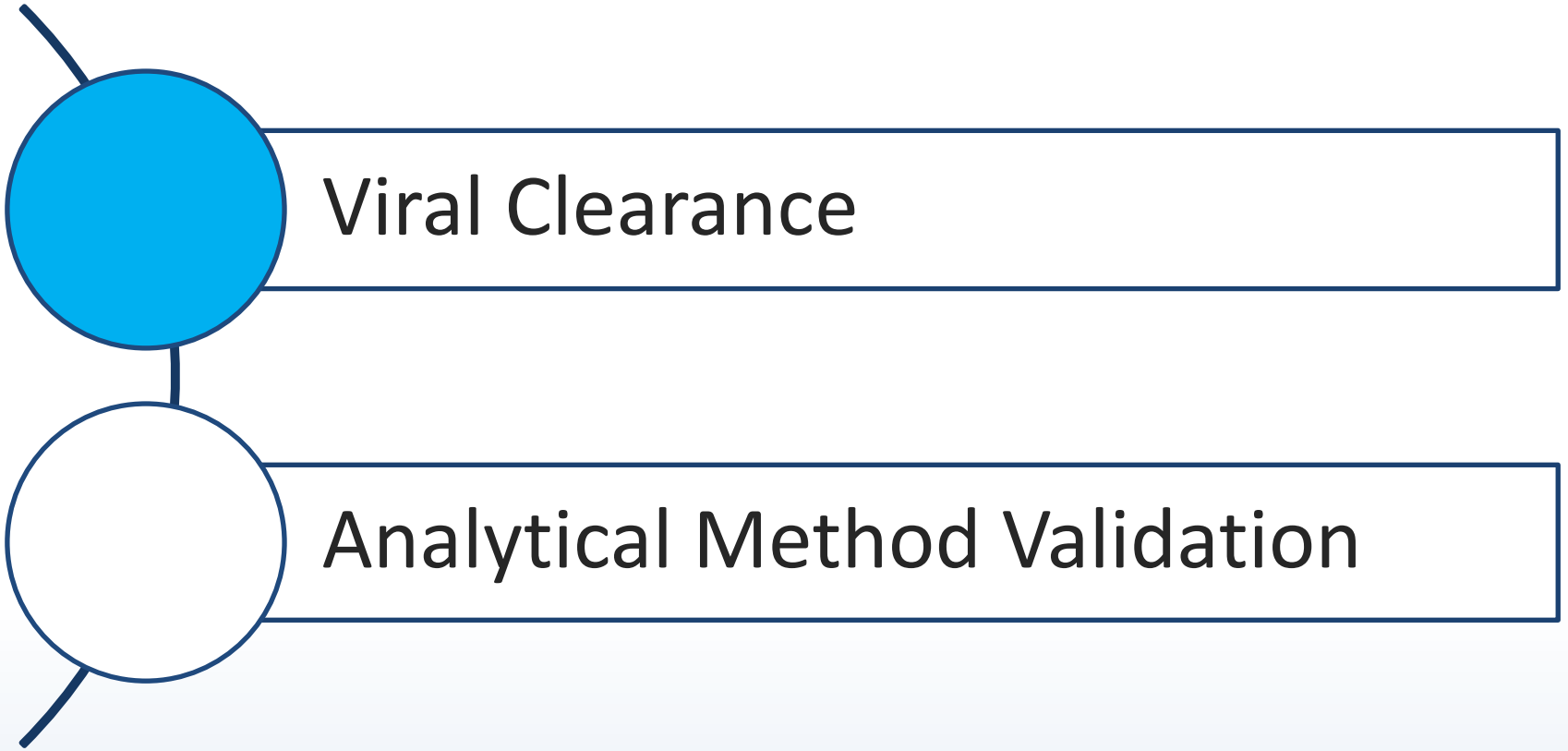
Lecture 7: Viral Clearance & Analytical Method Validation



Topics



Viral Reduction Methodologies

- Recognition of the potential for contamination of biologic drugs by real and supposed viruses during manufacturing has resulted in:
  1. Rigorous and effective process controls, and;
  2. The incorporation of multiple barriers to viruses to provide overlapping and complementary levels of protection.

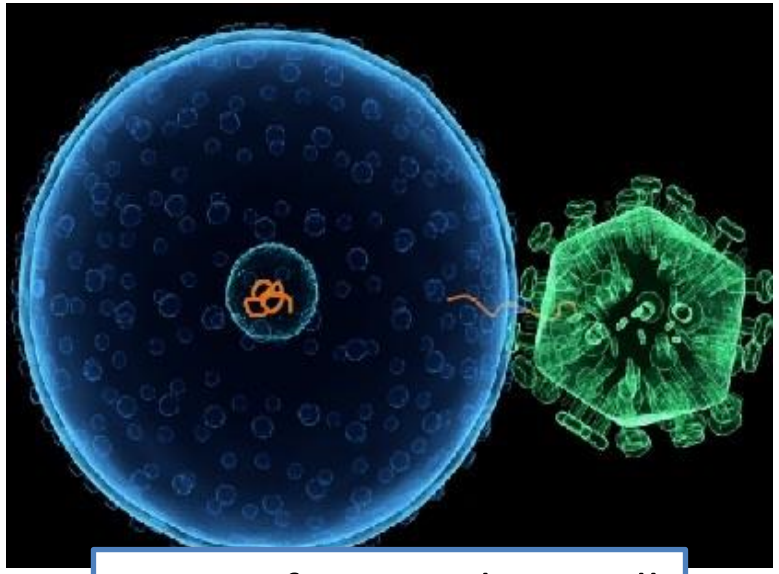
- The most common viral clearance steps found in bioprocessing are:
  1. Viral Inactivation
  2. Viral Filtration

- Viral Clearance occurs in the downstream process and is a regulatory requirement.

Viral Contamination of Biologics

The **risk** of viral contamination is a feature common to all biotechnology products derived from cell lines.

Viral contamination, in contrast to contamination by microbes and mycoplasma, presents a serious threat because of the **difficulty in detecting some viruses**



Virus infecting a host cell

Biotechnology products derived from cell lines have **not** been implicated in the transmission of infectious agents, to-date.

Viral Contamination Cases

Virus	Cell line	Year	Company
EHDV	CHO	1988	Bioferon GmbH
MVM	CHO	1993	Genentech
MVM	CHO	1994	Genentech
Reovirus	Homo 1 Kidney	1999	Abbott
Cache Valley	CHO	1999	Amgen/CMO
Vesivirus 2117	CHO	2003	Boehringer-Ingelheim
Cache Valley	CHO	2003	Not disclosed
Cache Valley	CHO	2004	Not disclosed
MVM	CHO	2006	Amgen
Vesivirus 2117	CHO	2008	Genzyme, Belgium
Vesivirus 2117	CHO	2008	Genzyme, USA
MVM	CHO	2009	Merrimack
PCV-1	Vero	2010	GSK/Merck

https://www.sigmaaldrich.com/technical-documents/articles/biology/viral-invaders.html

Viral Contamination Cases

Genzyme's Massachusetts facility in 2009 was temporarily shut down due to a bioreactor contamination with **Vesivirus 2117.**

It does not cause human infections, but impairs growth of the biologics-producing Chinese hamster ovary (CHO) cells.

The source was never officially identified, but it also was present in their Belgian plant so the likely source was raw material.

Closure of the facility resulted in approximately **$300 million in lost sales, stock price fell, Sanofi takeover**

It resulted in drug rationing of two drugs (Cerezyme, Fabrazyme) – legal action taken from patients

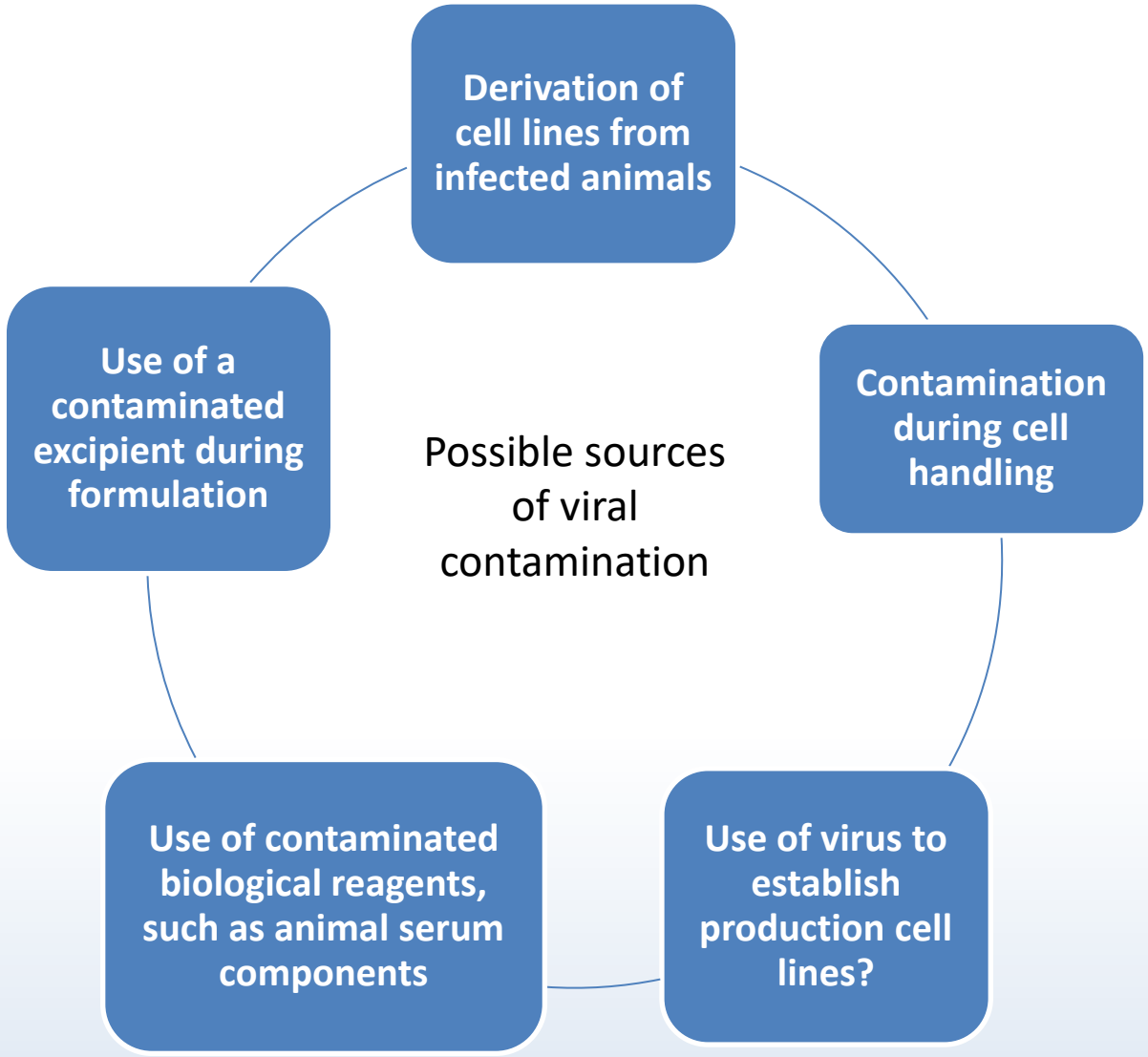
By April 2010, Genzyme had restarted operation at diminished capacity

A worker scrubbed pipes in June during decontamination at Genzyme's Allston plant after a virus infected a bioreactor. (Wendy Maeda/ Globe Staff/ File)

Potential Sources Of Viral Contamination

Viral contamination of biotechnology products may arise from:

1. Cell lines (endogenous)
2. During production processes (adventitious)



Control Strategy

ICH harmonisation for better health

VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN
Q5A(R1)

1. Risk Assessment:

   Where and what is likely to occur

2. Validation:

   Assessing the capacity of the production processes to remove or inactivate viruses

3. Testing/Detection:

   Selecting and testing source material for the absence of detectable viruses

   Testing the product at appropriate stages of production for freedom from detectable viruses

1. Risk Assessment

Cell Line

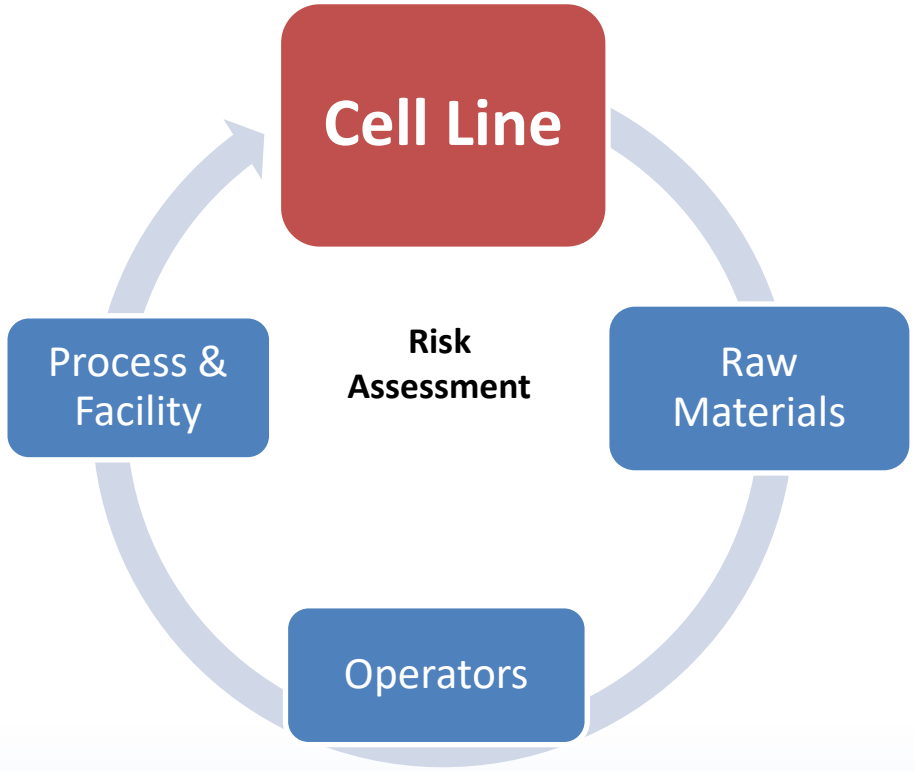
1. Know the risk for your cell line. E.g. CHO cells:
   - Not susceptible to:

   Adenovirus, Coronavirus, Picornavirus, Herpes, Orthomyxo, Togavirus,Retrovirus (although they are prone to RVLPs!)
   - Are susceptible to:

   Reovirus, Parmyxo, Bunya, MVM

2. Assess the risk of viral contamination in:
   - Master Cell Bank
   - Working Cell Bank

3. Assess the risk for:
   - Endogenous viruses
   - Adventitious viruses



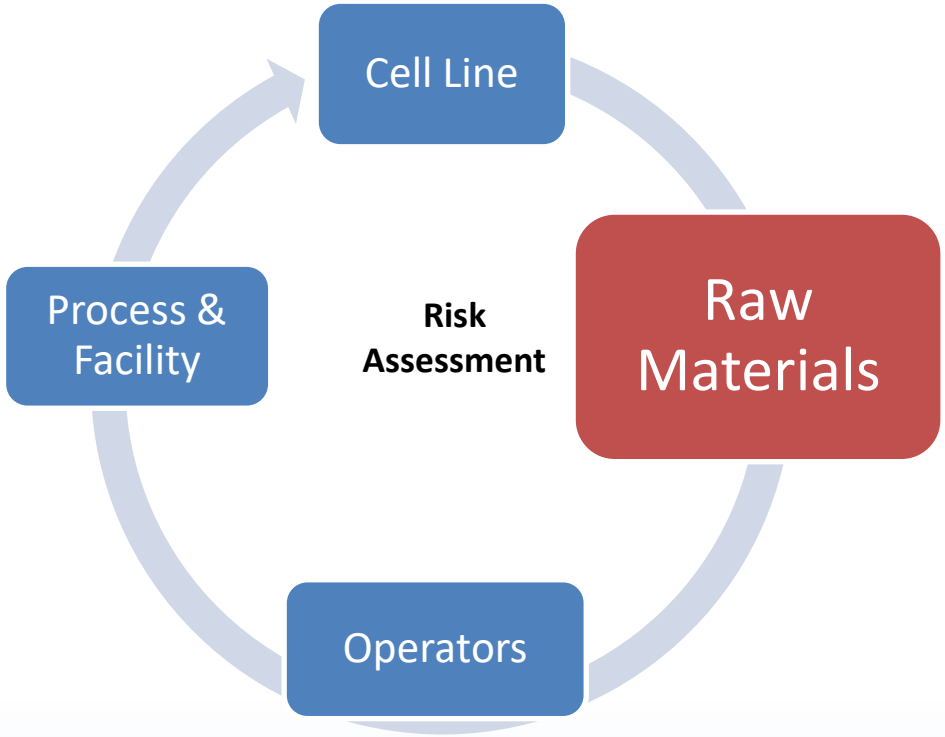
1. Risk Assessment

Raw Materials

1. Media/Buffer components:
   - Are any media/buffer components derived from biological sources e.g. bovine serum.
   - Is media purchased in powder form and prepared using WFI onsite, or pre-prepared by supplier.

2. Supplier:
   - What are the risks of viral contamination from supplier materials
   - How are raw materials developed by supplier
   - How are they shipped and maintained

3. Storage and usage:
   - How are raw materials stored and used onsite



1. Risk Assessment

Operators

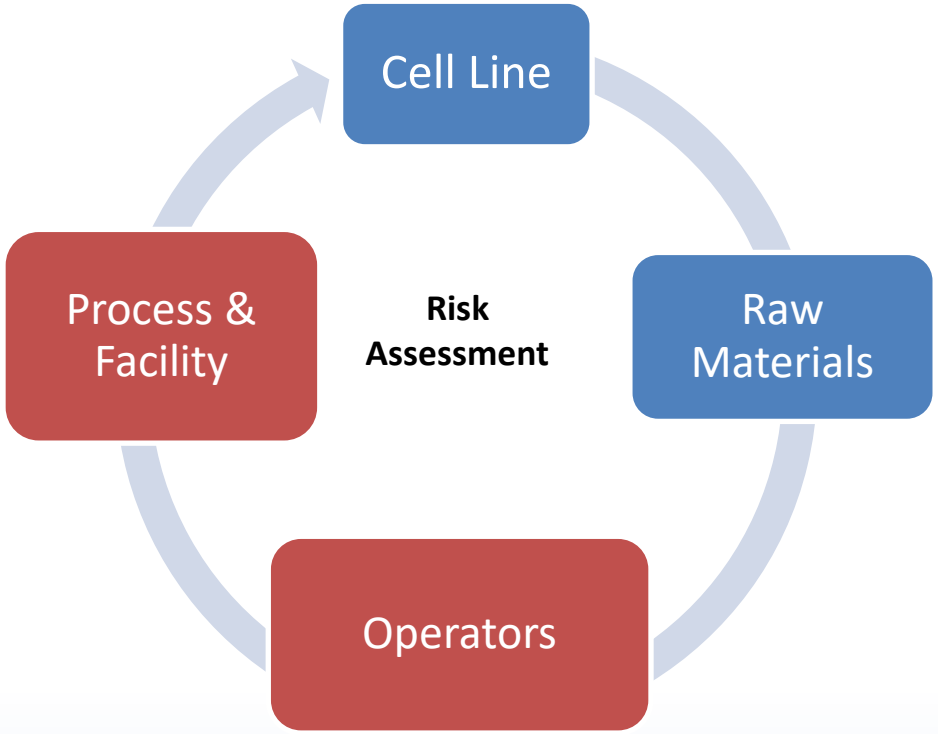
1. What are the risks of viral contamination posed by operators at different parts of the process.

Process & Facility

1. What are the risks of viral contamination in:
   - Upstream processing
   - Harvest
   - Downstream processing
   - Fill Finish
   - Ancillary operations (e.g. buffer prep)

2. What are the risks of viral contamination based on the overall facility design and operation.

3. What are the risks of viral contamination making its way into the final product.



2. Validation - Viral Clearance Studies

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VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN
Q5A(R1)

Definition (CPMP/ICH/295/95)

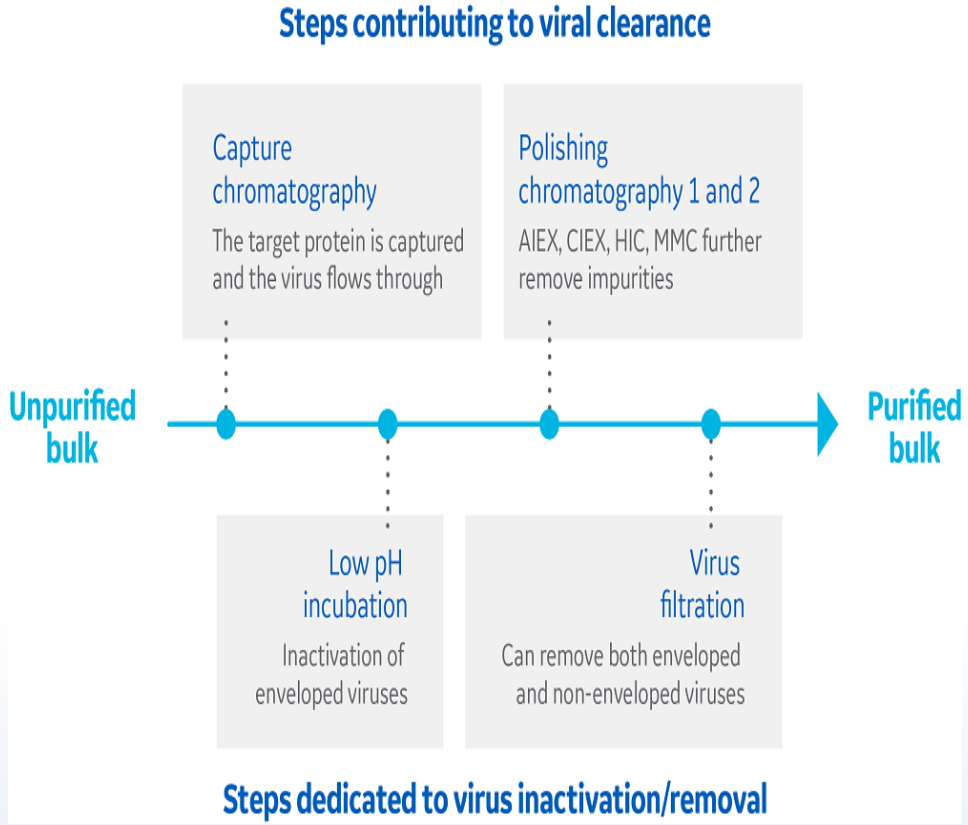
"The objective of viral clearance studies is to **assess process step(s)** that can be considered to be **effective** in **inactivating/removing viruses** and to estimate **quantitatively** the overall level of virus reduction obtained by the process."

Aim of Viral Validation:
Provide evidence that the production process will effectively inactivate/remove viruses which could potentially be transmitted with the product to the patient

2. Validation - Viral Clearance Studies

Not performed in a manufacturing setting due to the risk of viral infection

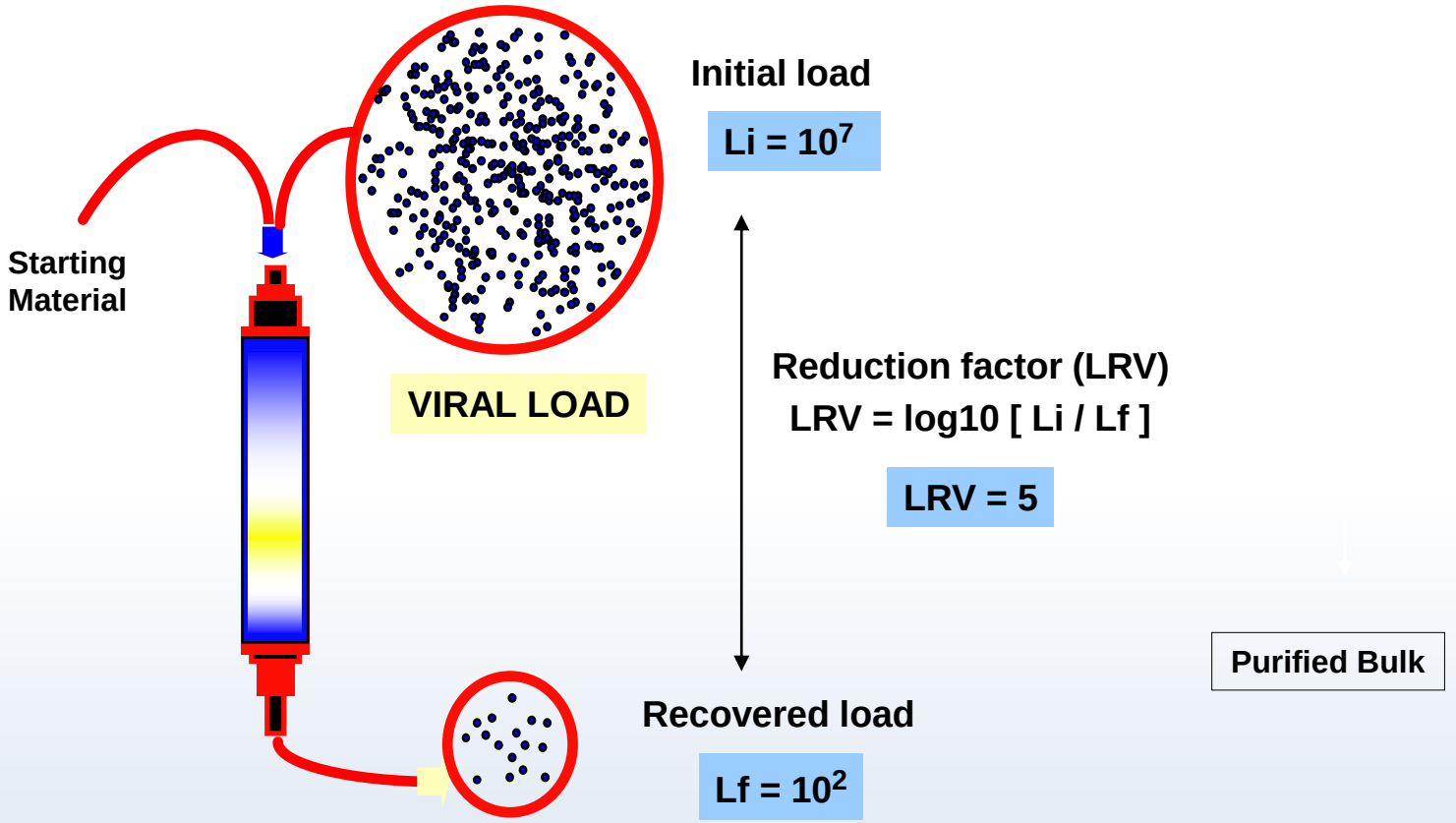
- Small scale representative studies in lab
- Selection of suitable virus strains
- Need to grow model viruses
- Spiking appropriate steps with high titer of infectious virus
- Can only estimate likely reduction in virus load/infectivity
- Determining virus reduction factors for each step
- Summing reduction factors to give a total log reduction value (LRV)



https://www.gelifesciences.com/en/us/solutions/bioprocessing/knowledge-center/viral-clearance-study-basics

2. Validation - Virus Spiking

Deliberately adding known amounts of virus to various production steps



2. Validation - Virus Selection

Choose viruses with a wide range of properties to evaluate robustness of the process

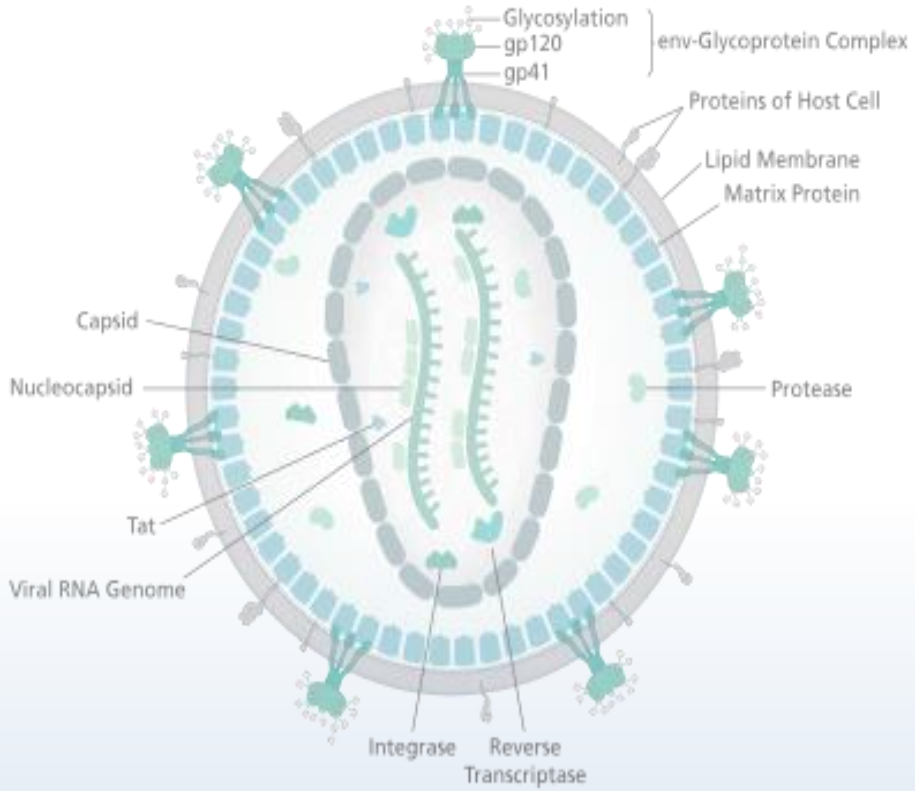
DNA and RNA genome (single and double-stranded)

Lipid-enveloped and non-enveloped

Large, intermediate, and small size

From very highly resistant to inactivation to very easily inactivated

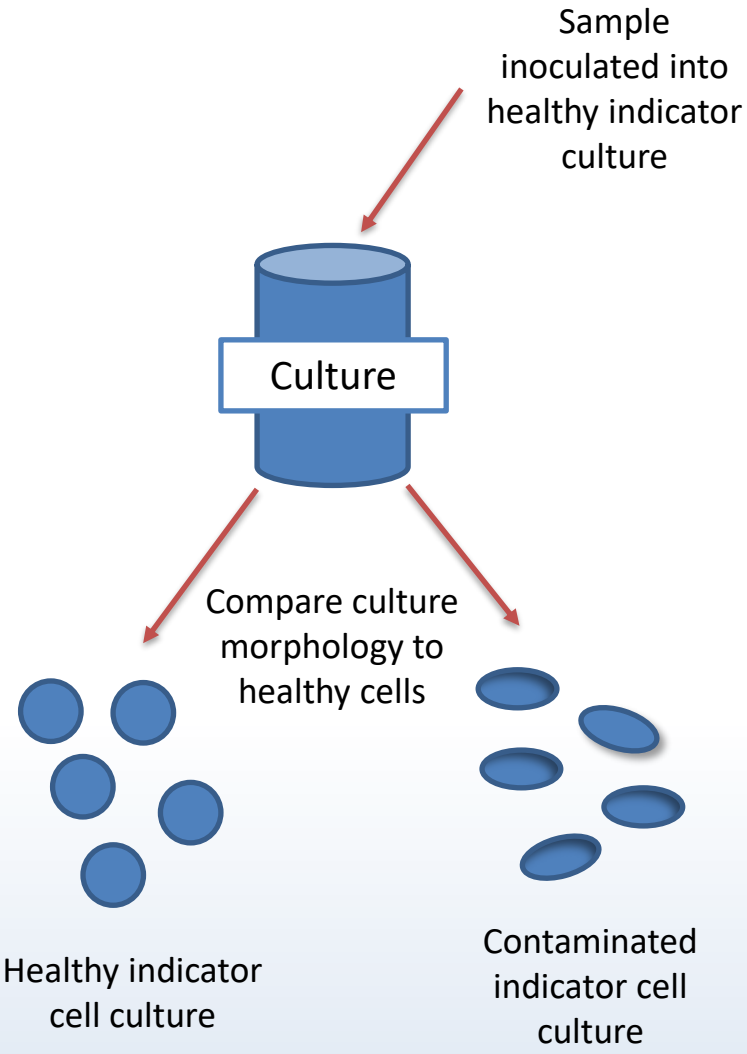
Viruses that can potentially be transmitted by the product



3. Virus Testing/Detection - Physiological

Use cell lines that are susceptible to infection by the viruses in question

- Samples of serum, cell lysates, enzymes, raw materials, product solution etc are inoculated into indicator cell lines
- Cultures are then maintained and monitored for cytopathic effects (structural changes to the indicator cell line)
- If changes are seen this indicates there are contaminating viruses in the cell line

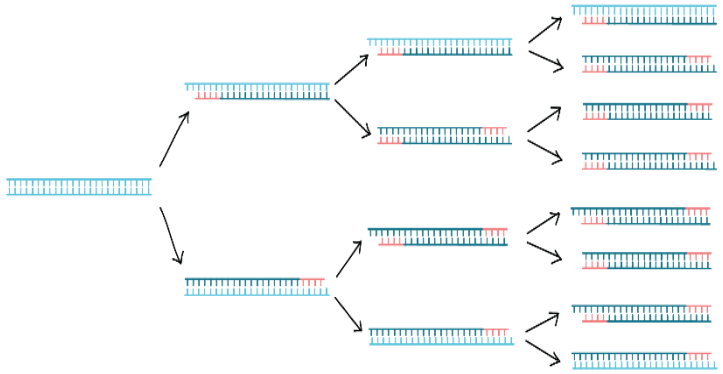


Viruses that do not display cytopathic effects can be tested by inoculating samples into mice or guinea pigs and testing for an immune response

3. Virus Detection - Assays

1. Polymerase Chain Reaction

- Amplifies tiny amount of viral DNA/RNA so that it can be detected
- Very quick and sensitive



2. Immunoassays

- Use recombinant antibodies that bind to the virus
- Detection via labelling antibodies with an enzyme that results in a colour change in a substrate

3. Cell Testing and Monitoring

1. Master Cell Bank

2. Working Cell Bank

3. “Cells at the Limit of *In Vitro* Cell Age Used for Production” (CAL) or “End of Production Cells” (EOPC)
   - CAL: Cells at the limit of *in vitro* cell age used for production are cells at the highest population doubling level that will be claimed in a Marketing Authorisation
   - EOPC: Cells at the end of the production process at the particular scale

4. Bulk Harvest

   Pre-processed, post-culture bioreactor liquid

Effective Viral Clearance

Achieves significant viral clearance

Reproducible and controllable at process scale and model-able at the laboratory scale

Should have minimal impact on product yield and activity

Should not leave toxic residues

Virus Removal vs. Inactivation

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VIRAL SAFETY EVALUATION OF BIOTECHNOLOGY PRODUCTS DERIVED FROM CELL LINES OF HUMAN OR ANIMAL ORIGIN

Q5A(R1)

Reduction in virus infectivity may be achieved by the removal or inactivation of a virus.

Virus Removal = The mechanical removal of viral particles

Viral Inactivation = The irreversible loss of viral infectivity

Ref: ICH - Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin q5a(r1)

Virus Inactivation Methods

Viral Inactivation = The irreversible loss of viral infectivity

Physical:

Pasteurisation

Chemical:

Low/high pH treatment

Solvent/detergent

UV irradiation

Commonly used virus inactivation methods

Heat:	Disruption of envelope / capsid
Low pH:	ionic disruption of envelope and destructive conformational changes of structural proteins
Solvent/Detergent:	S/D-mediated disruption of envelope
Caprylate:	partitioning of non-ionized molecule into envelope and disruption of envelope

Virus Safety of Intravenous Immunoglobulin: Future Challenges
Article· Literature Review in Clinical Reviews in Allergy & Immunology 29(3):333-44 · January 2006 Nicola Boschetti, Martin Stucki, Spaeth Peter, Christoph Kempf.

Virus Removal Technologies

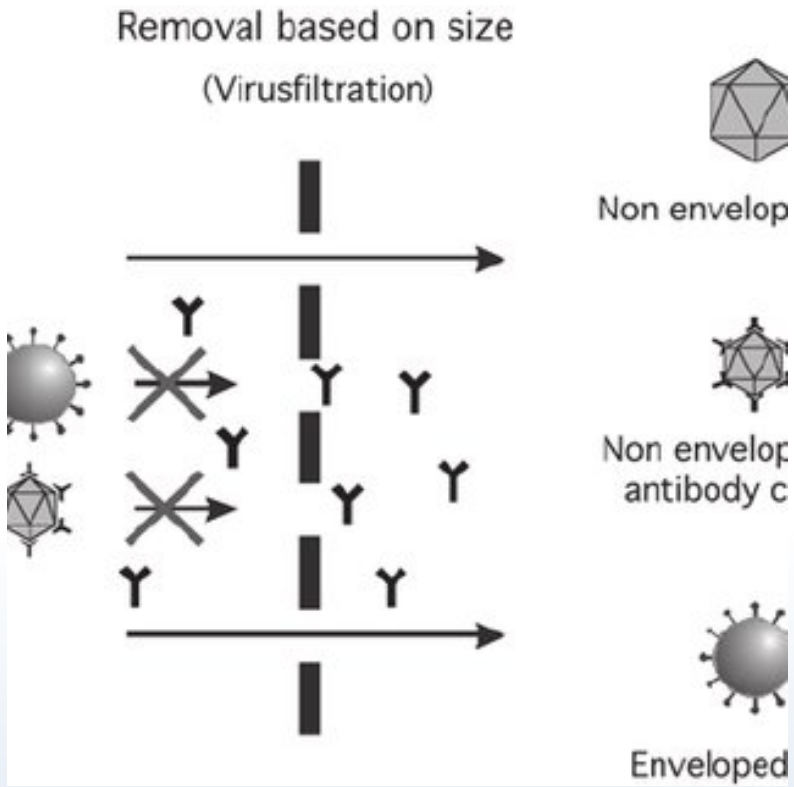
Virus <u>Removal</u> = The mechanical removal of viral particles

Chromatography

Precipitation

Centrifugation

Membrane filtration



Virus Safety of Intravenous Immunoglobulin: Future Challenges
Article· Literature Review in Clinical Reviews in Allergy & Immunology 29(3):333-44 · January 2006 Nicola Boschetti, Martin Stucki, Spaeth Peter, Christoph Kempf.

Virus Filtration Considerations

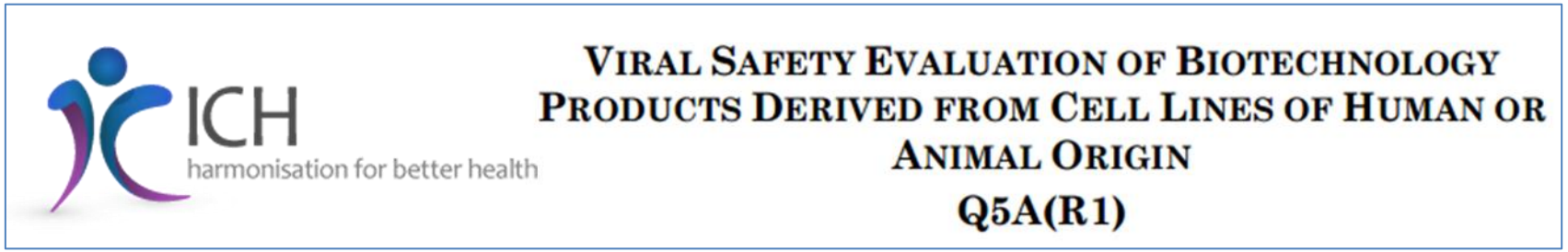
To implement virus retentive filtration successfully within a process, several points should be considered:

1. Regulatory considerations
   - Before marketing authorization, manufacturers **must assess clearance of multiple model and relevant viruses** in their manufacturing processes

2. Process considerations
   - It is important to ensure that virus filtration is implemented at the **best location** in the process

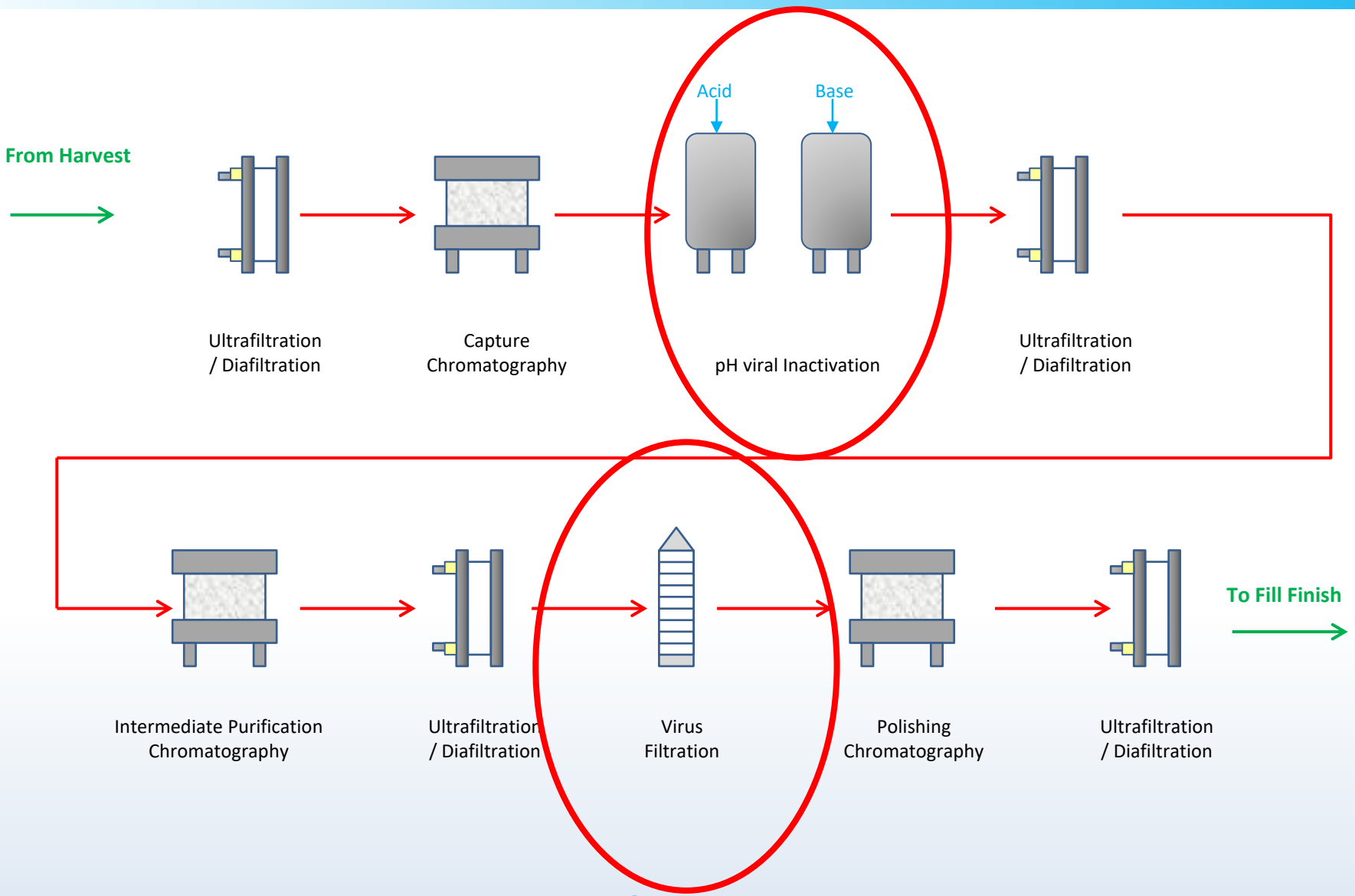
3. Virus filter-related considerations
   - Virus filters very sensitive to **fouling**, **pre-filtration** may be necessary

Deliberate Viral Clearance



The process should include at least **two orthogonal methods** of viral clearance

Deliberate Viral Clearance



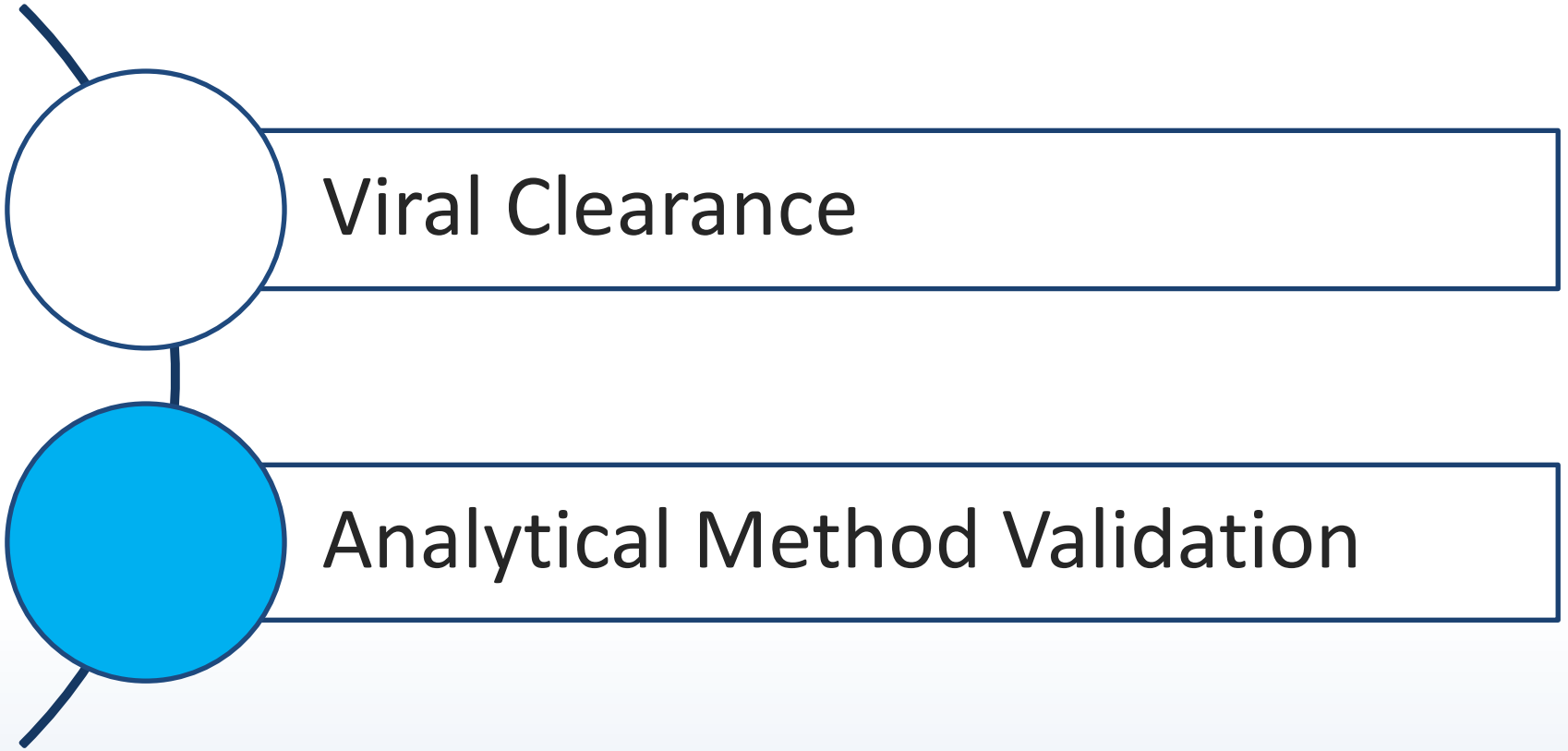
Segregation

European GMP Guidance, Annex 2:

"In cases where a virus inactivation or removal process is performed during manufacture, **measures should be taken to avoid the risk of recontamination** of treated products by non-treated products."

"Appropriate precautions should be taken to prevent potential viral contamination from pre-viral to post-viral removal/inactivation steps. Therefore, open processing should be performed in areas that are separate from other processing activities and have separate air handling units"

Topics



What is Method Validation?

Method validation is the validation of an analytical procedure. It is used to demonstrate that the procedure is suitable for its intended use

The purpose of validation is to:

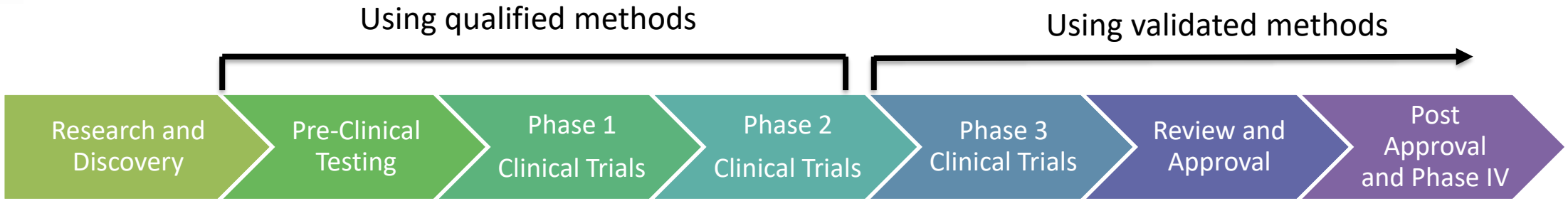
- Gather documented evidence of method performance
- Demonstrate that a method is fit for purpose and is reliable
- Evaluate the measurement uncertainty
- Mandatory for accreditation as per ISO 1025
- Is a GMP requirement - ICH Q7A

"Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference." – ICH Q7A

Intended use:

- CQAs of DS/DP
- Stability information

Drug Development Process: Qualification vs Validation



You cannot validate at this early stage because:

- There are still too many unknowns
- Methods might change based on the new information that is being generated

Method qualification is used to inform method development, prepare for validation and put acceptance criteria in place

Full validation is required for phase 3 clinical trials and onwards

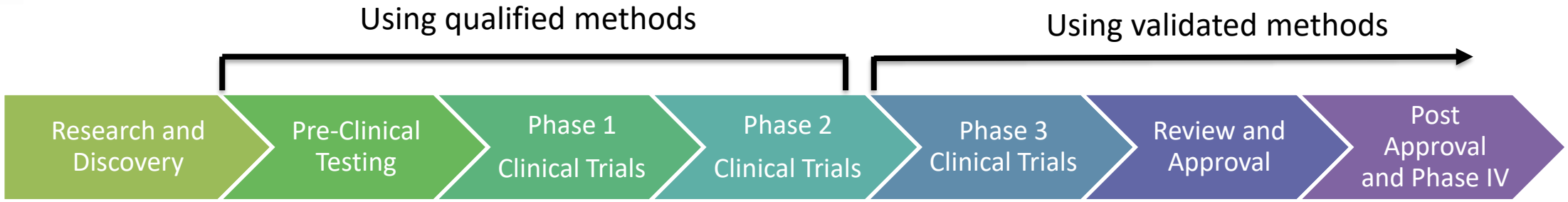
- GLP studies
- Lot release assays
- Raw material testing
- In process testing
- Stability testing

Each test result must meet a pre-determined acceptance criteria

FYI: some assays may need to be validated prior to phase 1 e.g. safety assays such as immunogenicity

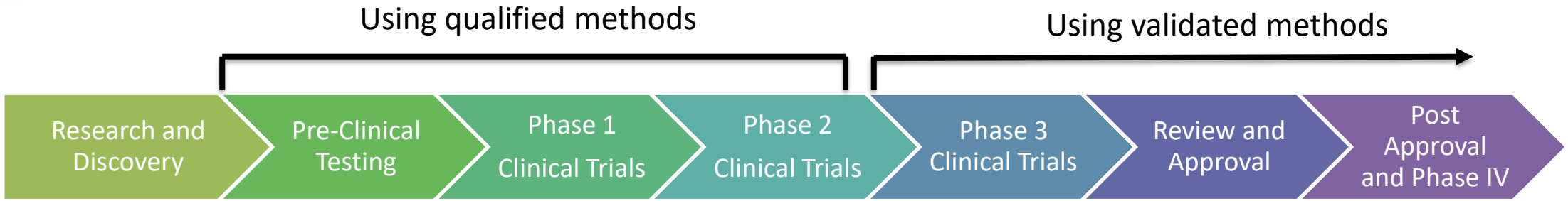
- Bridwell H., et al. Perspectives on Method Validation: Importance of Adequate Method Validation
- Secada, J. Understanding Analytical Method Validation. Pharmaceutical Online
- Ritter, N. et al. (2004) What is Test Method Qualification? Bioprocess International

Drug Development Process: Qualification vs Validation



-CQAs are identified
-Purpose of analytical methods are identified
-appropriate methods are chosen based on CQAs

Drug Development Process: Qualification vs Validation



-start to qualify method for release and stability
-methods chosen are based on process and product knowledge, and risk assessment
-set preliminary release acceptance
-qualified methods should be scientifically sound and provide results that are reliable. Therefore, performance parameters need to be determined

Guidelines:

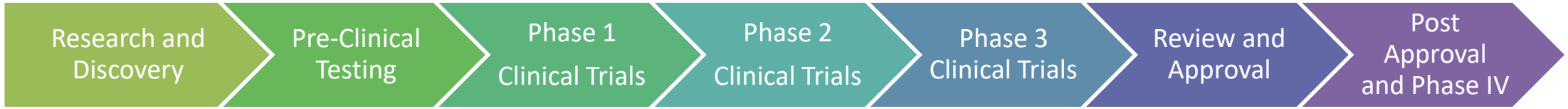
1. FDA Process Validation Guidance (2011):

"Validated analytical methods are not necessarily required during product- and process-development activities or when used in characterization studies. Nevertheless, analytical methods should be scientifically sound (e.g., specific, sensitive, and accurate) and provide results that are reliable"

2. FDA Content and Format of Investigational New Drug Applications for Phase 1 Studies (1995):

"Validation data and established specifications ordinarily need not be submitted at the initial stage of drug development. However, for some well characterized, therapeutic biotechnology-derived products, preliminary specifications and additional validation data may be needed in certain circumstances to ensure safety in Phase 1"

Drug Development Process: Qualification vs Validation



-optimization of qualified methods
-refine release criteria
-set validation acceptance criteria
-define validation parameters

Guidelines:

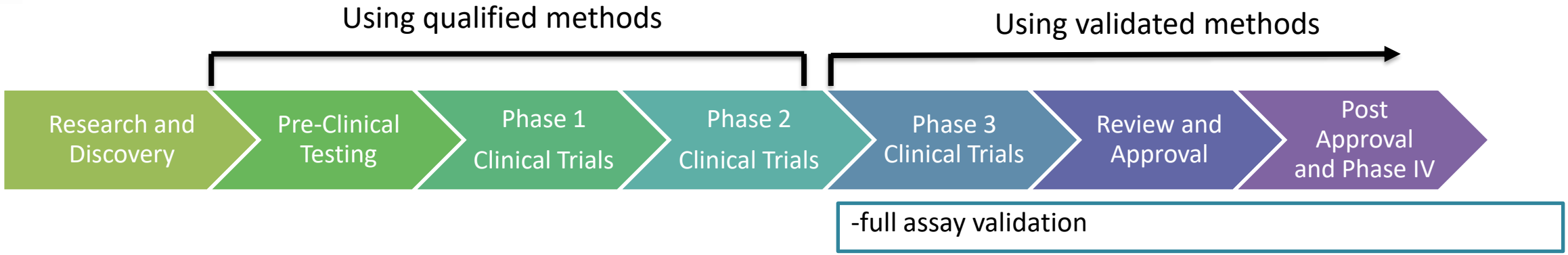
1. Guidance for Industry: INDs for Phase 2 and Phase 3 Studies (2003)

"During the clinical investigation process, the sponsor would establish tentative acceptance criteria that are continually refined based on data obtained from analysis of batches of drug substance and new information that becomes available"

"The analytical procedure used to perform a test and to support the tentative acceptance criteria should be briefly described and changes reported when the changes are such that an update of the brief description is warranted"

"A complete description of analytical procedures and appropriate validation data should be available for the analytical procedures that are not from an FDA recognized standard reference, and this information should be submitted upon request"

Drug Development Process: Qualification vs Validation

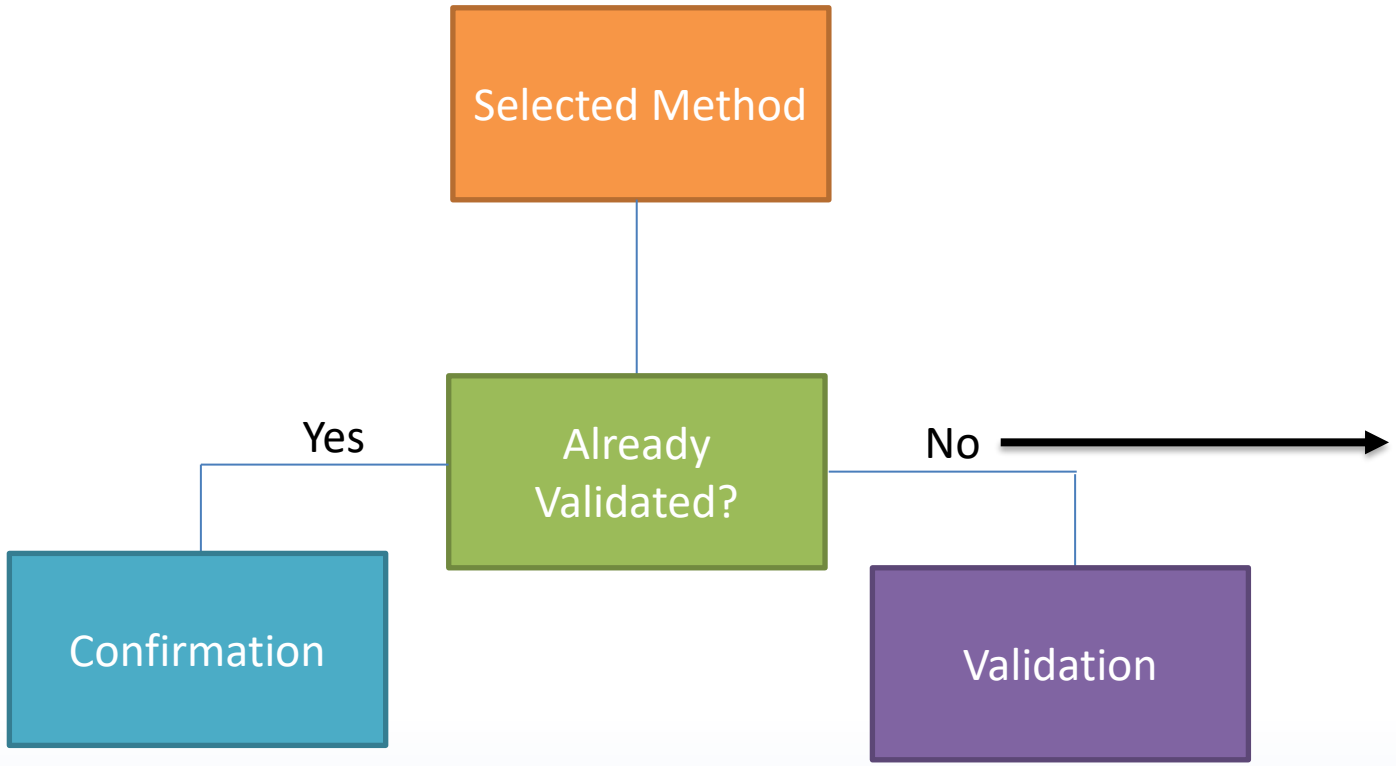


Guidelines:

1. Guidance for Industry: INDs for Phase 2 and Phase 3 Studies (2003)

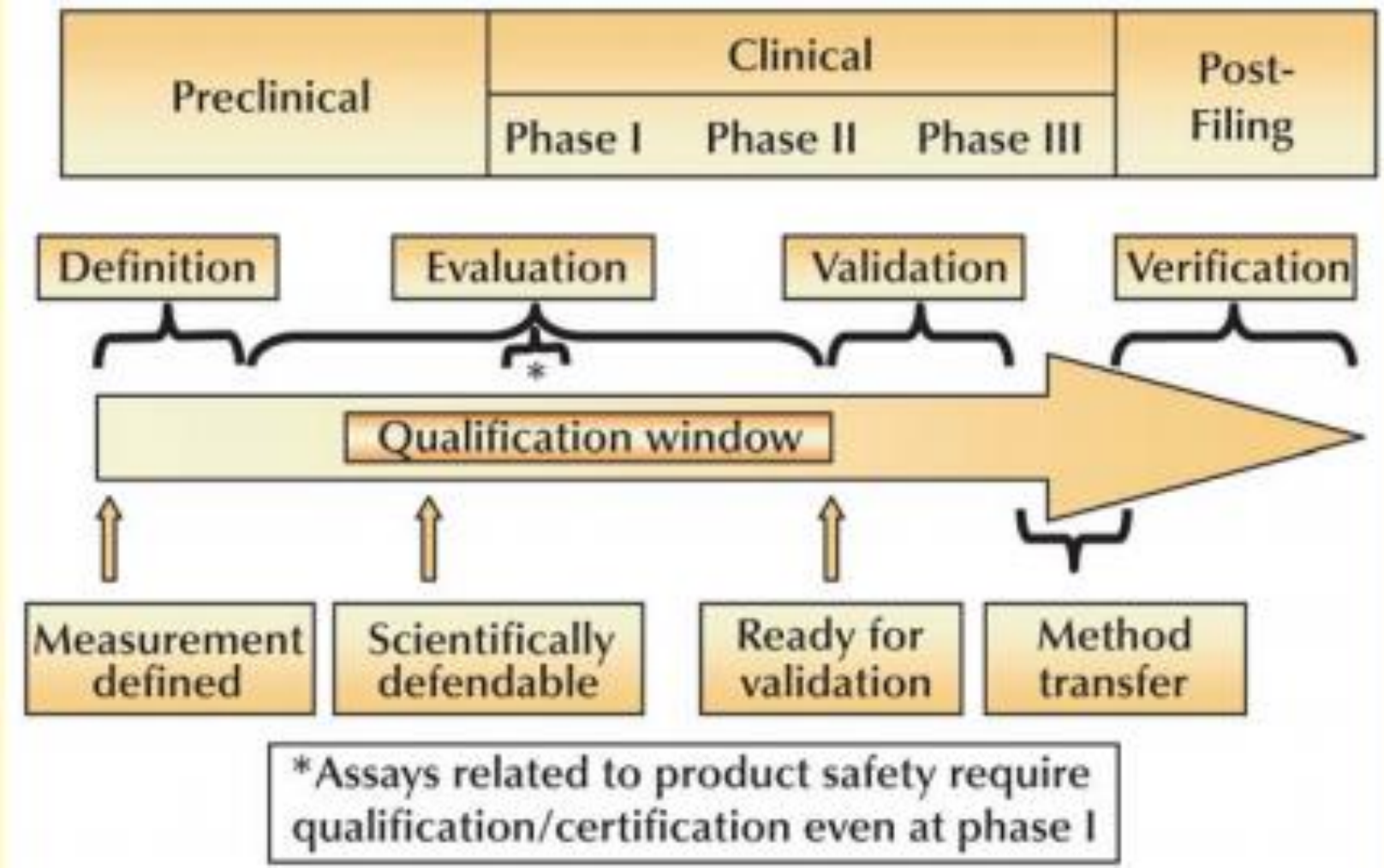
"A complete description of analytical procedures and appropriate validation data should be available for the analytical procedures that are not from an FDA recognized standard reference, and this information should be submitted upon request" – The FDA strongly recommends that assays are validated by phase 3 but they can request validation data at any stage in development. However, validation data is not typically required to be submitted until BLA.

Which Methods Do You Need to Validate?



1. A non standard method (a new method that has been developed “in house”)
2. Standard method used outside its scope
3. Stability assays (if there exists no compendial method)
4. Revision or improvement of a previously validated method
5. Regulatory Authority has requested supplementary validation

Timing for Assay Qualifications



- Ritter, N. et al. (2004) What is Test Method Qualification? Bioprocess International

Types of Analytical Methods to be Validated (ICH Q2 (R1))

Overall, any method used to produce data for a regulatory filing must be validated. This includes methods for analysing:

- Bioavailability
- Bioequivalence
- Pharmacokinetic
- Toxicity
- Clinical studies
- DS/DP

According to ICH, there are four types of methods that require validation

1. Identification tests	2. Quantitative tests for impurities content 3. Limit tests for the control of impurities	4. Quantitative tests of the active moiety in samples of DS/DP or other selected components of the drug product
• Ensure the identity of the analyte • Achieved via comparison of a known property to a reference standard	• Either of these tests are used to establish the purity characteristics of the sample • Both of these tests differ in their validation characteristics	• Measure the analyte present in the sample

The validity of a method must be demonstrated using samples and standards that are similar to the samples and standards that will be used in the routine

What Does Method Validation Require?

Method validation involves carrying out a variety of experiments that focus on the performance elements of the method

Validation Parameters (Performance Elements)

Method: Specificity
Accuracy
Precision
Linearity over a certain concentration range
Robustness
Detection limit
Quantification limit
System Suitability

Stability of the: solutions
controls
samples

These parameters will vary from method to method, depending on the purpose of the method and the type of analyte being assessed

Matrix for Qualification and Validation Activities

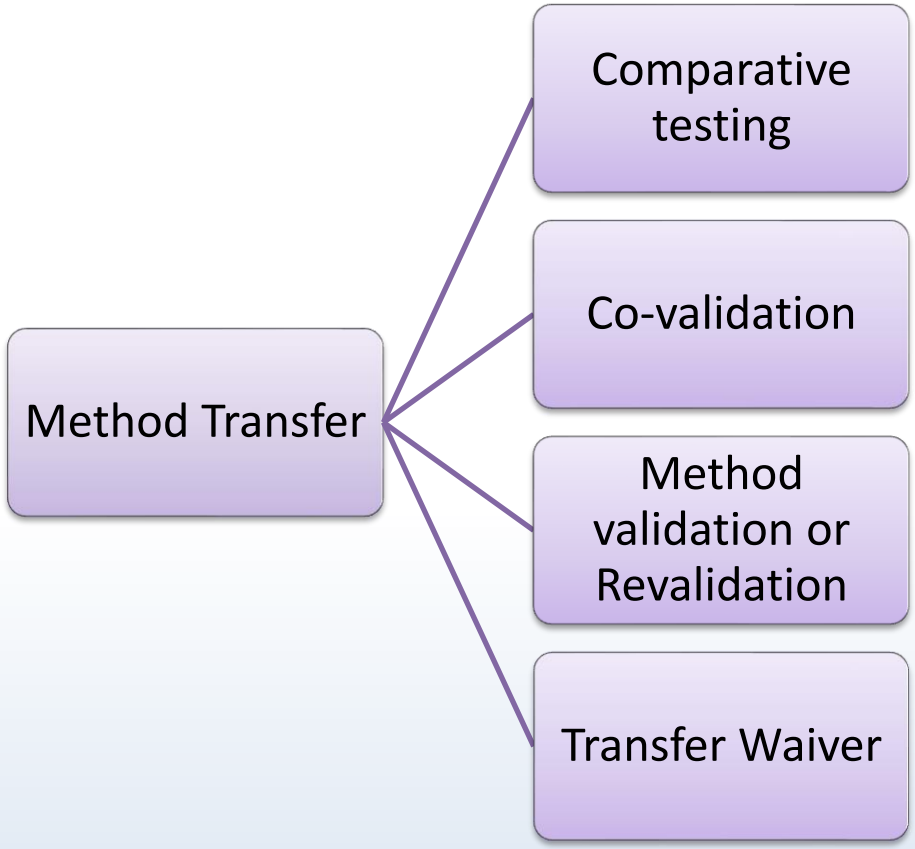
Table 1: Matrix example for qualification activities; (X) = typically included; others may be needed in some cases

Requirements	Qualify New Analyst Training	Qualify New Lab Transfer	Qualify Method (New) Qualification	Qualify Method (Commercial) Validation	Qualify Compendial Method (New Sample Type) Verification
System suitability Assay acceptance	X	X	X	X	X
Specificity/ carryover			X	X	X
Linearity/ range			X	X	
Precision		X	X	X	X
Accuracy/ recovery		(X)	(X)	X	
LOD/LOQ		(X)	(X)	(X)	
Standards/samples stability			X	X	X
Robustness				X	
Equivalence comparability of results	X	X	X		

- Ritter, N. et al. (2004) What is Test Method Qualification? Bioprocess International

Method Transfer

Evidence that a previously validated method has been verified for use in a lab that is not the lab where it was originally validated



Both labs analyse equivalent samples. Results are compared to pre-defined transfer acceptance criteria

Both labs are involved in the initial validation

Complete/partial validation is carried out by the receiving lab

No verification is needed. The systems and environment of both labs are similar

Analytical Method Transfer (AMT)

- The Analytical methods will later be transferred to the large-scale production facility, typically in conjunction with the scale-up/transfer of the manufacturing process.

- However, the analytical control methods should be transferred before the manufacturing process to ensure proper testing of the products.

- Analytical method transfers (AMTs) are typically performed as a precursor to a critical step in the drug-development timeline, such as bringing a new manufacturing facility online, release testing of clinical or commercial material, or initiating stability studies at a quality-control laboratory.

Obstacles for AMT

- The following can lead to preventable AMT failures:
  - Differences in instrumentation between sending site and receiving site
  - Differences in techniques between sending and receiving operators
  - Communication issues
  - Interpretations of an analytical procedure

- To ensure successful AMTs and avoid preventable failures, it is critical to develop a well-defined transfer plan with open lines of communication.

Thank You

Validation Guidelines

ICH	• ICH Q2 (R1): Validation of Analytical Procedure: Text and Methodology
FDA	• Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics, 2015 • Guidance for Industry: Bioanalytical Method Validation, 2018 • Guidance for Industry: Process Validation: General Principles and Practices
EMA	• Guideline on Bioanalytical Method Validation, 2012
PDA	• Technical Report 57: Analytical Method Validation and Transfer of Biotechnological Products • Technical Report 57-2: Analytical Method Development and Qualification for Biotechnology Products
USP	• USP <1225> • USP<1032> • USP<1033> • USP<1034)
EP	• 92 Section 53