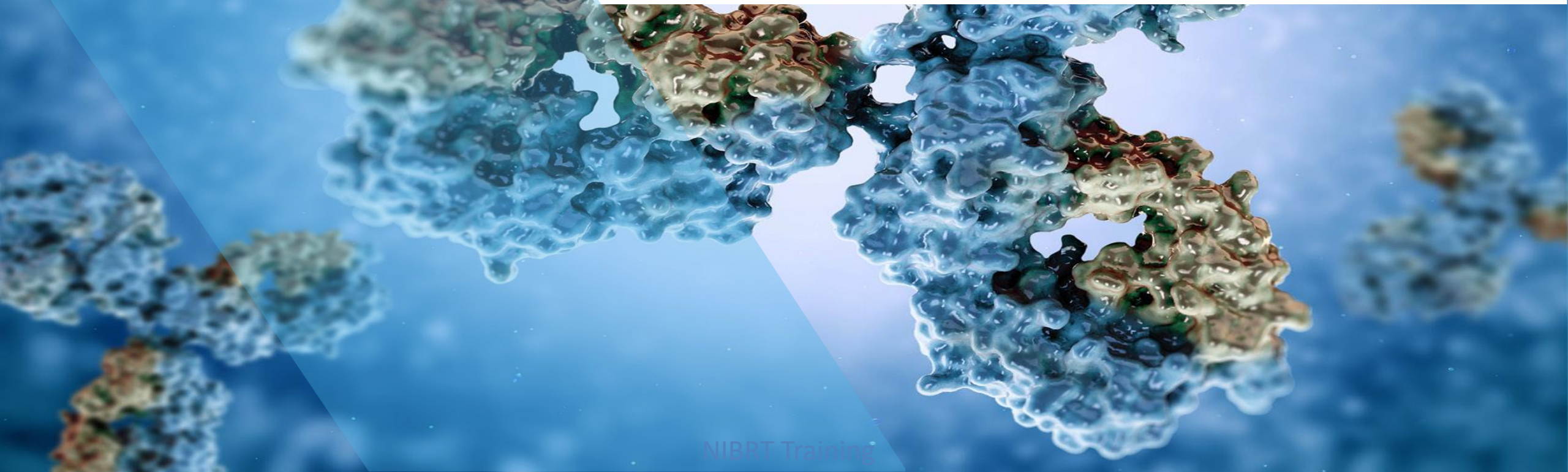
Viral Clearance



Learning Objectives

- Outline the basic properties of viruses
- Discuss why viruses are a critical class of contaminants in mammalian cell culture based processes
- Identify common sources of viral contamination
- Explain why and how viral clearance studies are performed
- Discuss viral clearance technologies

Topics

- Overview of Viruses
- Viral Contamination of Biologics
- Virus Clearance Studies
- Virus Clearance Technology
- Viral Segregation

What is a Virus?

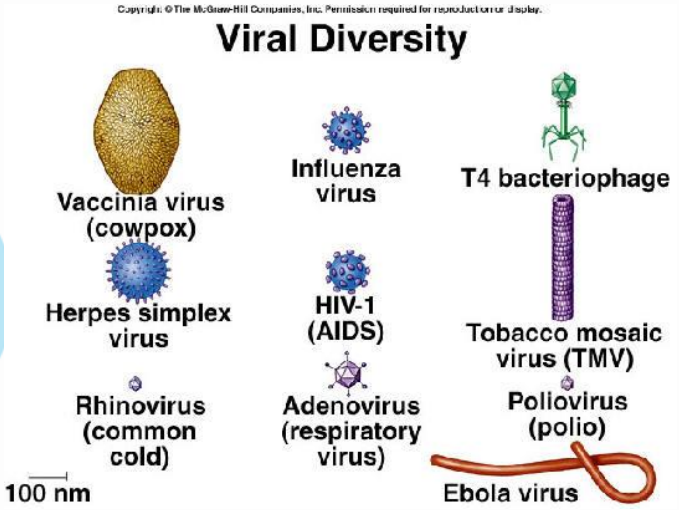
A virus is an ultramicroscopic infectious agent

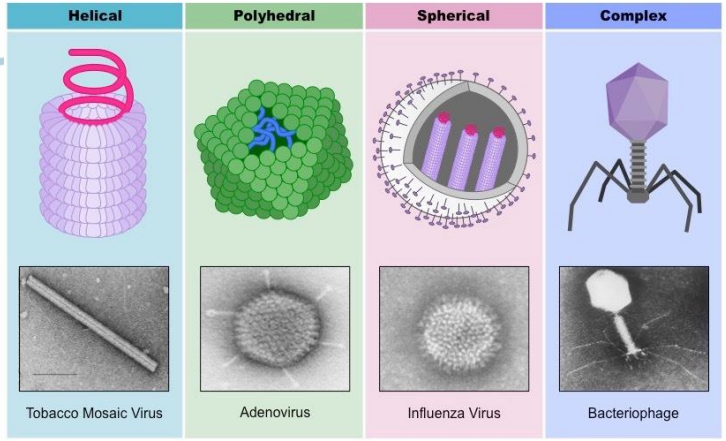
Viruses are **non-living** – use a host cell's internal machinery to replicate

An infected cell will produce viral particles that assemble into new viruses

It is estimated that there is >150,000 viruses, they are constantly evolving so it is impossible to test for them all

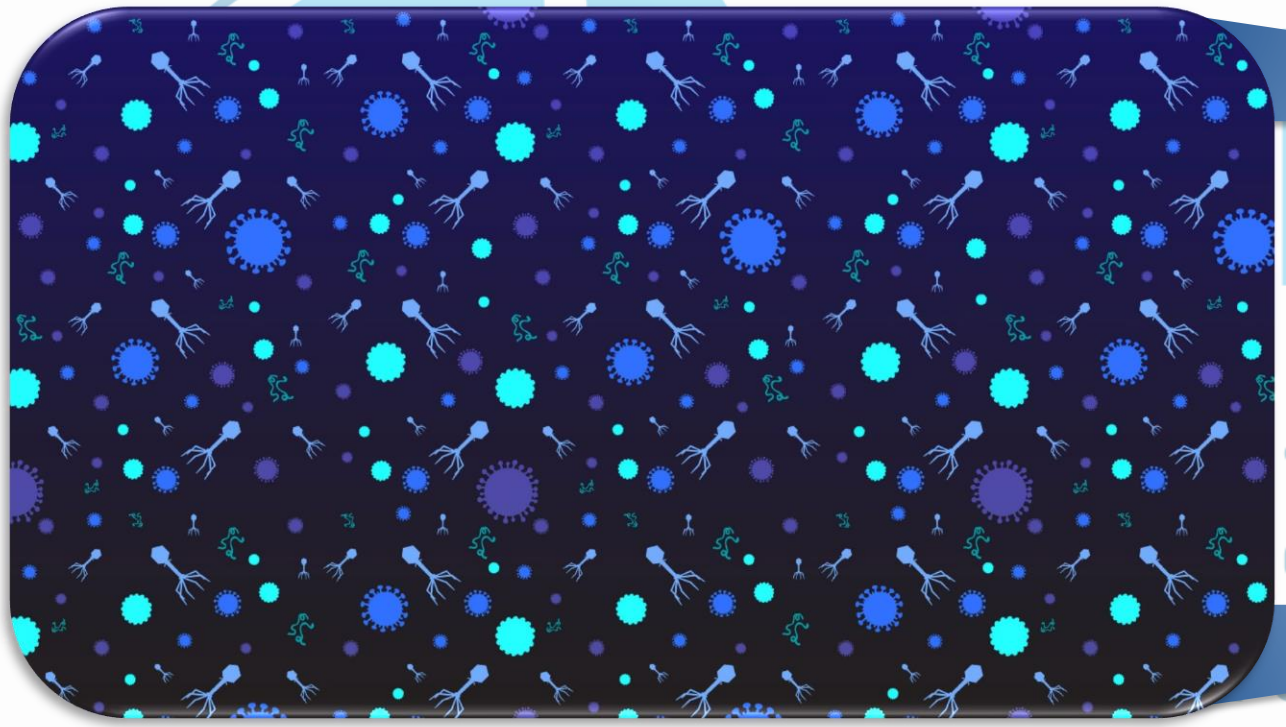
Viruses can be very targeted i.e. infecting a certain host or certain cells within a host





Viruses

Viruses are everywhere!

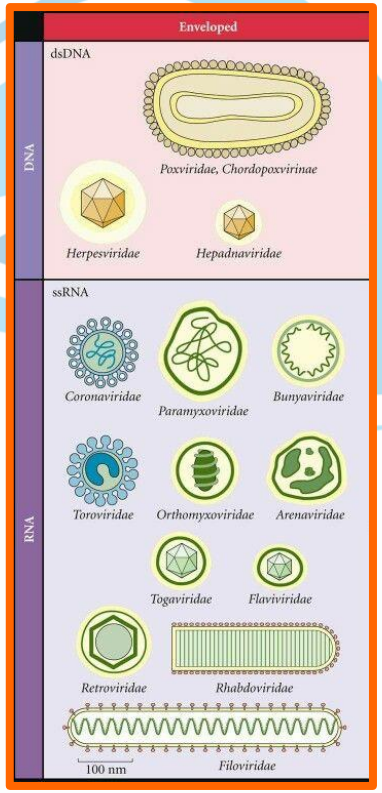


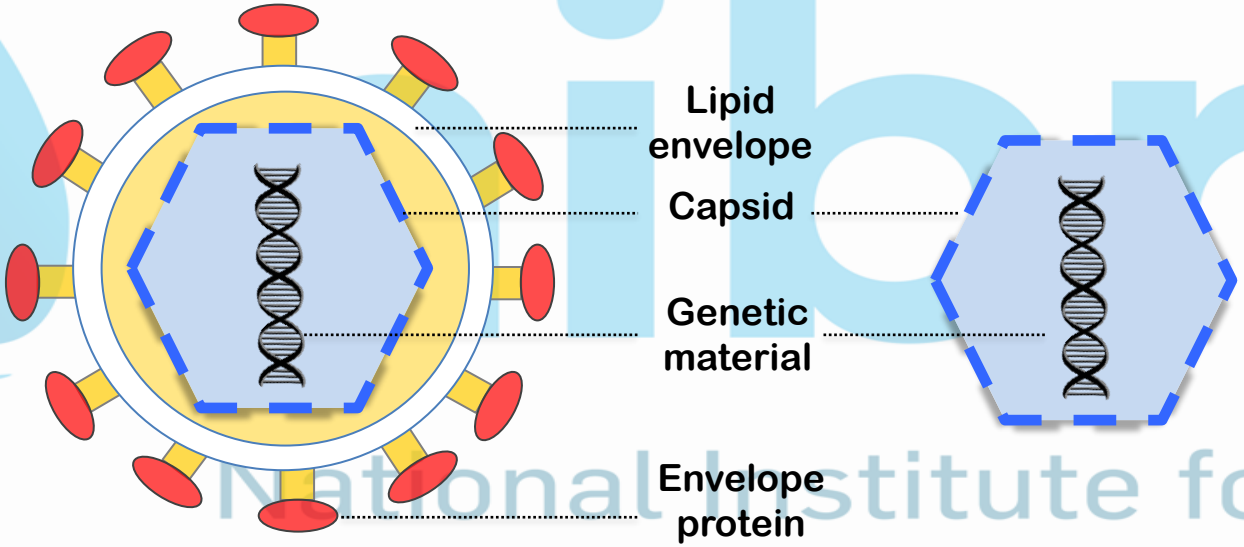
They are the most abundant biological entities on earth

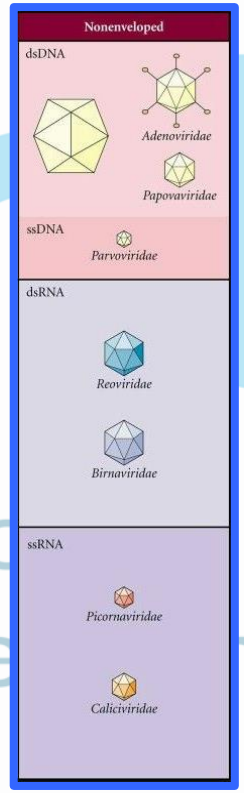
What about their structure?

Viruses

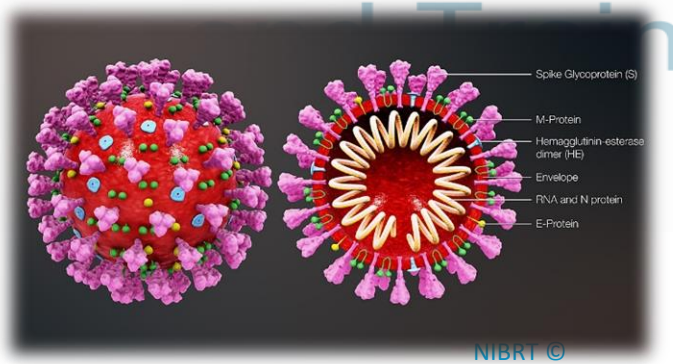
There are Enveloped and non-Enveloped viruses







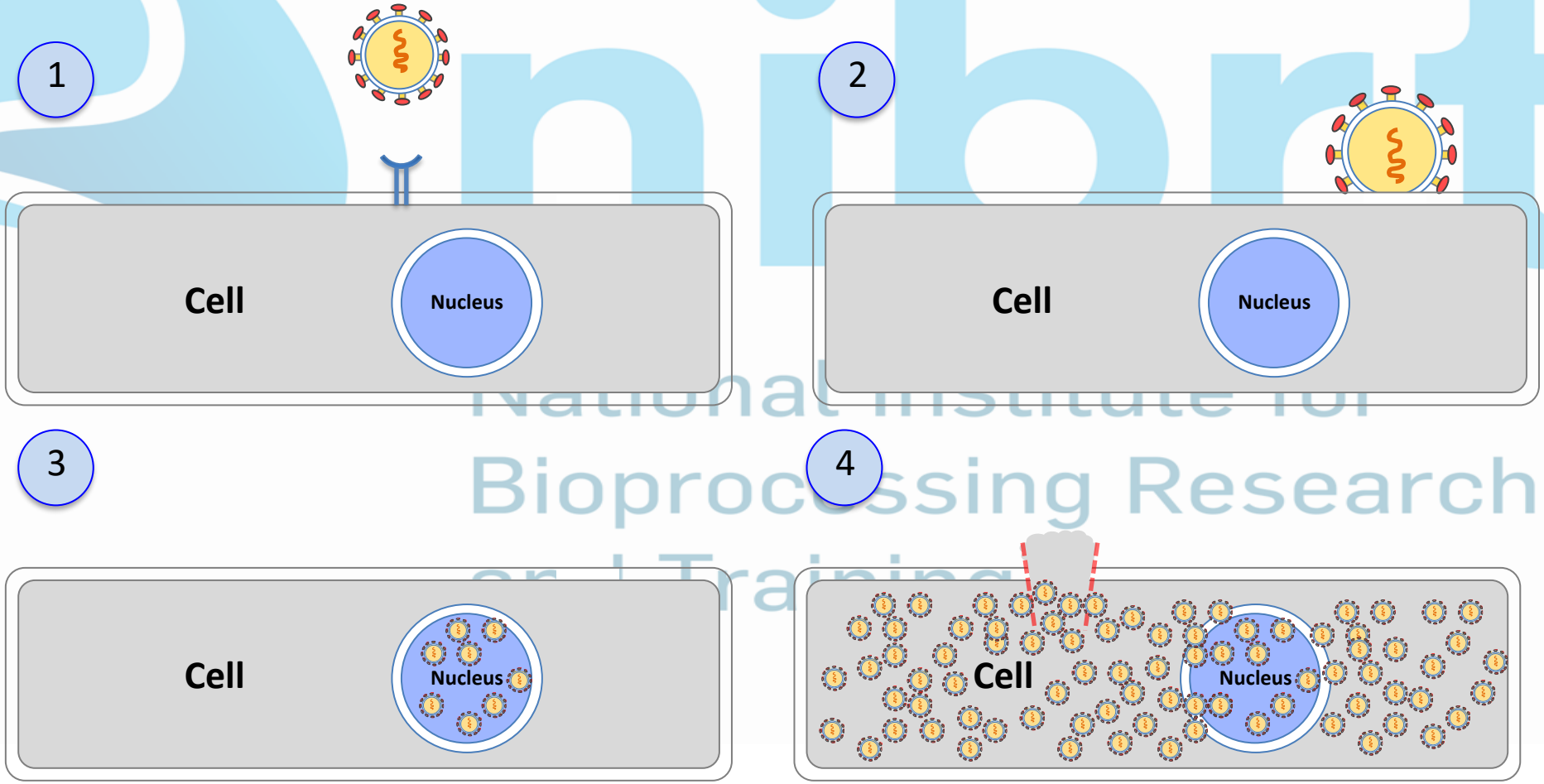
SARS-CoV-2 is an example of enveloped viruses



How do viruses infect cells?

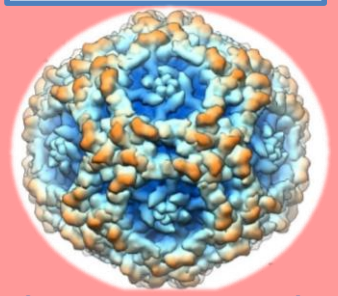
Viruses

The main function of the virus is to deliver its genetic material into the host cell so that the genome can be expressed by the host cell.



Examples of Viral Contaminants

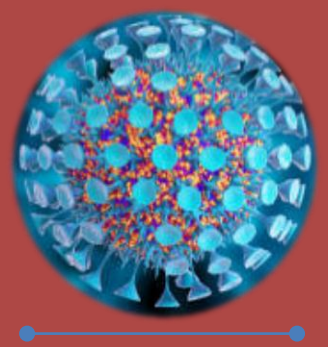
Parvovirus



18 – 25 nm

- DNA virus
- **Non-enveloped**
- Can be found in medium raw materials
- Example: MVM (minute virus of mouse)

Retrovirus



80 – 110 nm

- Contain RNA and an enzyme called reverse transcriptase
- **Enveloped**
- RNA is reverse transcribed to DNA
- Very high mutation rate
- Example of retrovirus: HIV

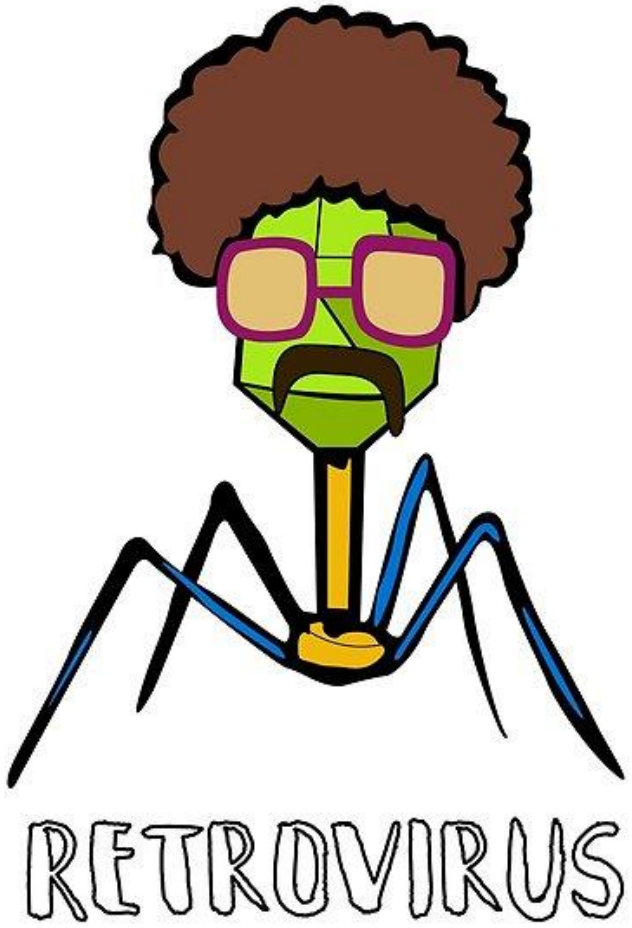
RVLPs

Retrovirus that have encoded themselves into the host cell genome “Fossil viruses”

They can produce retroviral-like particles (RVLPs) which can be immunogenic but none are known to be pathogenic

They should be characterised

Rodent cell lines are known expressers of RVLPs



Topics

- Overview of Viruses
- Viral Contamination of Biologics
- Virus Clearance Studies
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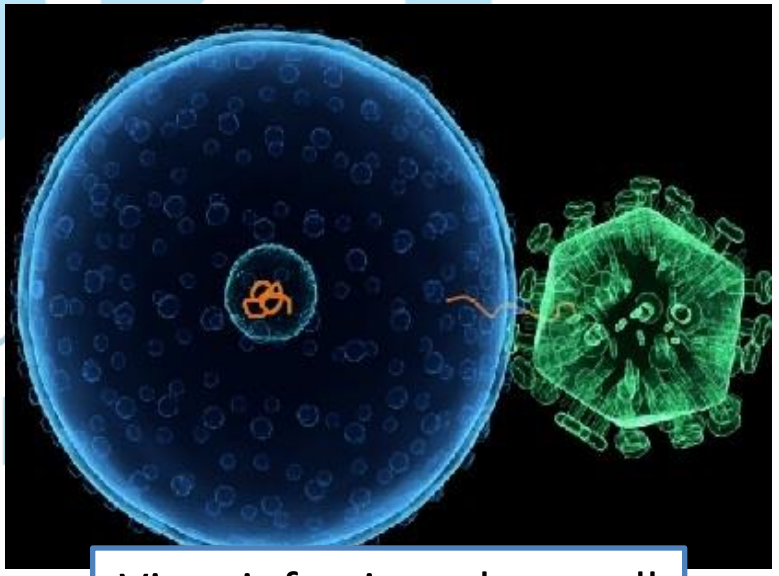
Viral Contamination of Biologics

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

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

Viral Contamination Cost

PATIENT SAFETY NUMBER 1

Internal (product doesn't leave facility)
Lost Raw Materials
Investigation
Cleaning
Production Shut Down
Re-Validation
Additional Regulatory Audits

External (product leaves facility)
Product Recall
Lost Market Share
Legal Liabilities
Brand Damage

Viral Contamination Cases

GSK and Merck- 2010

Porcine circovirus DNA identified in human Rotavirus vaccine in **GSK**

PCV-1 is not known to cause disease in humans or other animals

FDA recommended suspension of use of Rotarix (GSK) vaccine

Found in working and master cell banks

Subsequently found in **Merck** Rotateq vaccine

Likely to have always been present; new test lead to its discovery

3 months later FDA declared the vaccine safe for use

Rotavirus kills 500,000 a year so **benefit far outweighs risk**

Hypothesized it may have entered cells from using Trypsin in early development

Viral Contamination Cases

Genzyme's Massachusetts facility 2009 was temporarily shut down owing 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 identified but it also was present in it 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

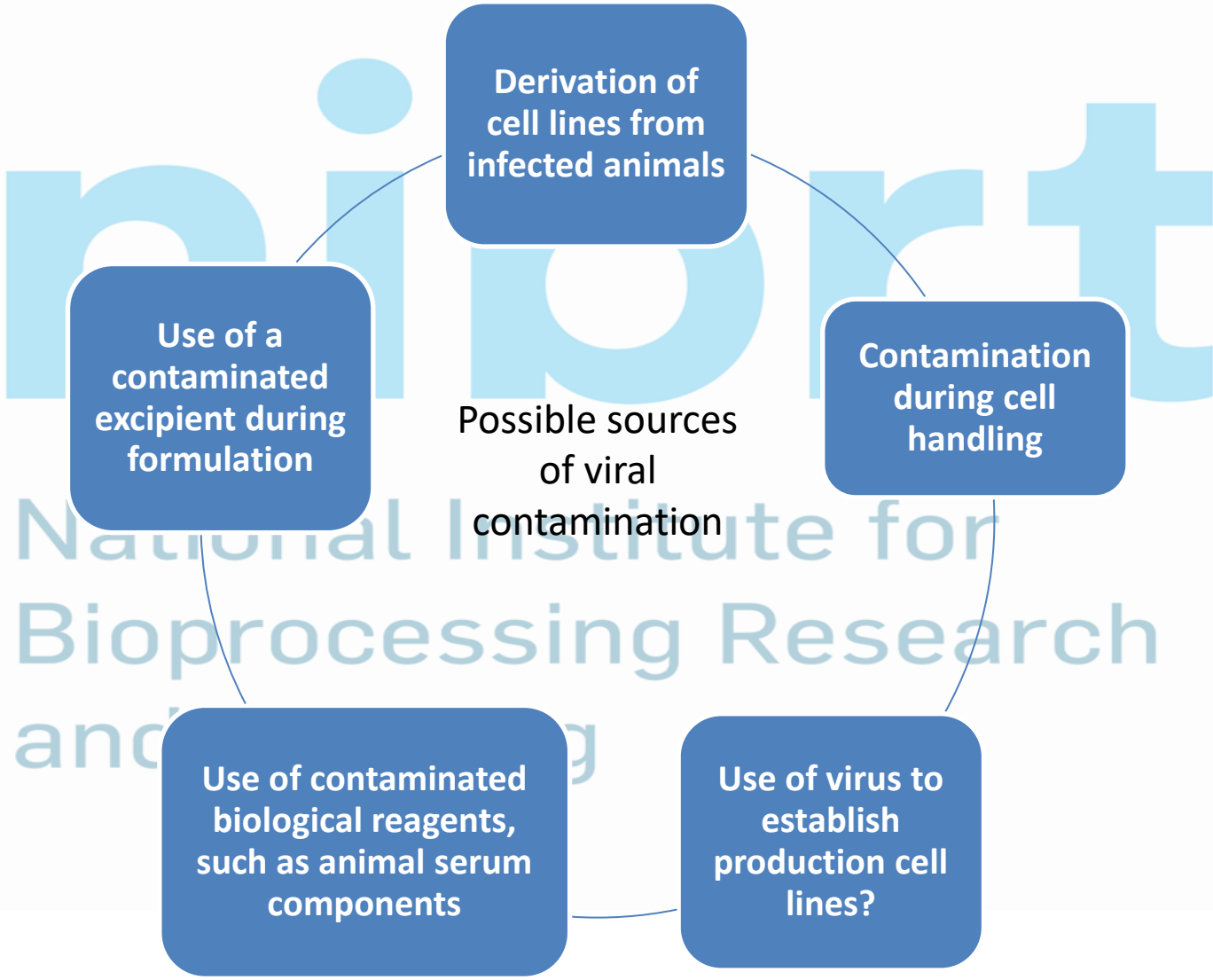
By April 2010, it had restarted operation at diminished capacity

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)

Risk Assessment:

Where and what is likely to occur

Validation:

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

Testing/Detection:

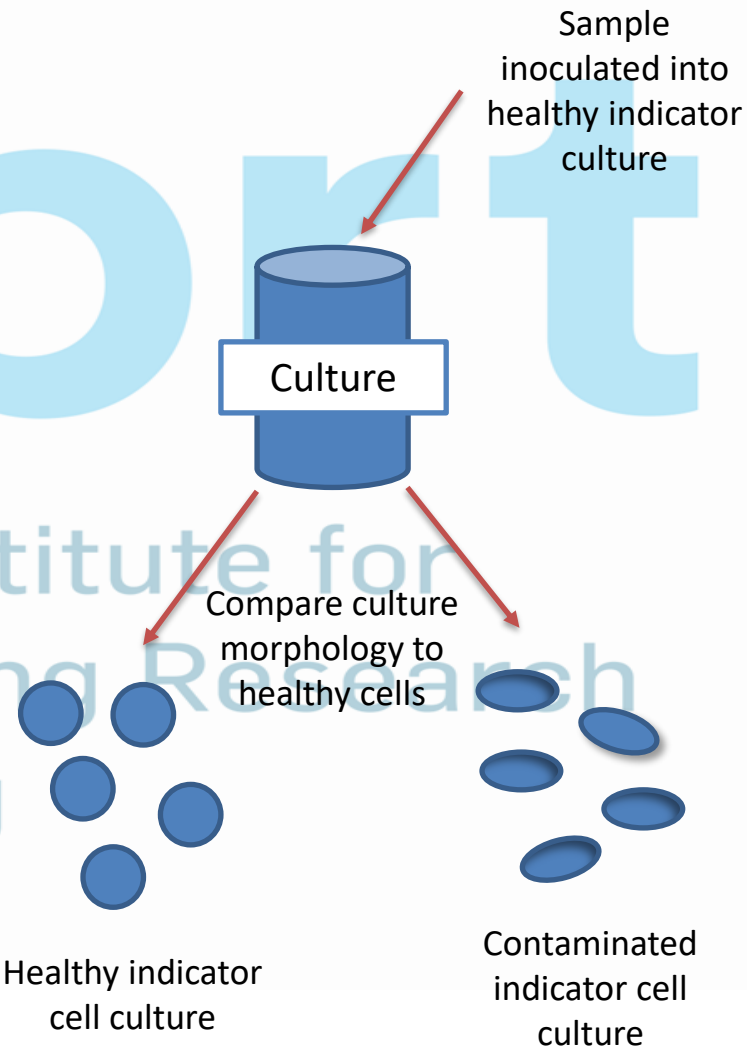
Selecting and testing source material for the absence of detectable viruses

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

Virus Testing/Detection

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

- Samples of serum, cell lysates, enzymes, raw materials 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 is contaminating viruses in the cell line

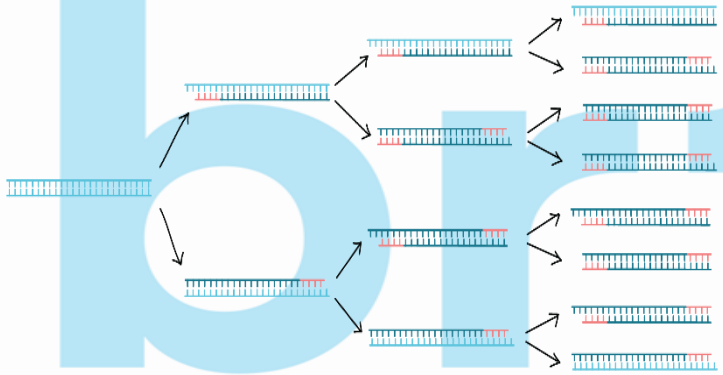


Viruses that do not display cytopathic effects can be tested by inoculating samples into mice or guinea pigs and testing for an immune response

Virus Detection - Assays

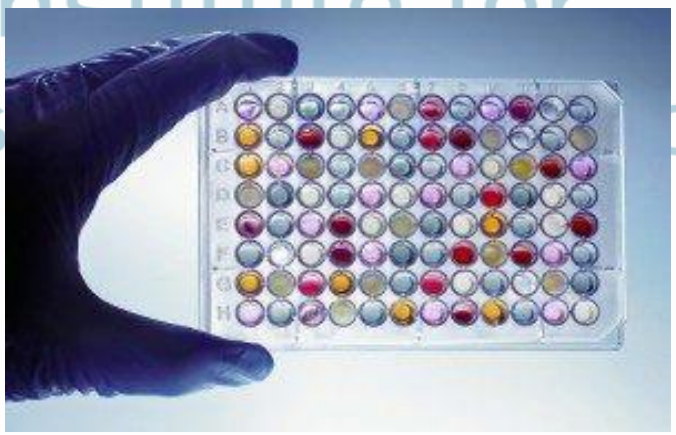
Polymerase Chain Reaction

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



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



Cell Purity Testing and Monitoring

Master Cell Bank

Working Cell Bank

"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

Bulk Harvest

Pre-processed, post-culture bioreactor liquid

Risk Assess

Cells

Know the risk for your cell line e.g. CHO:

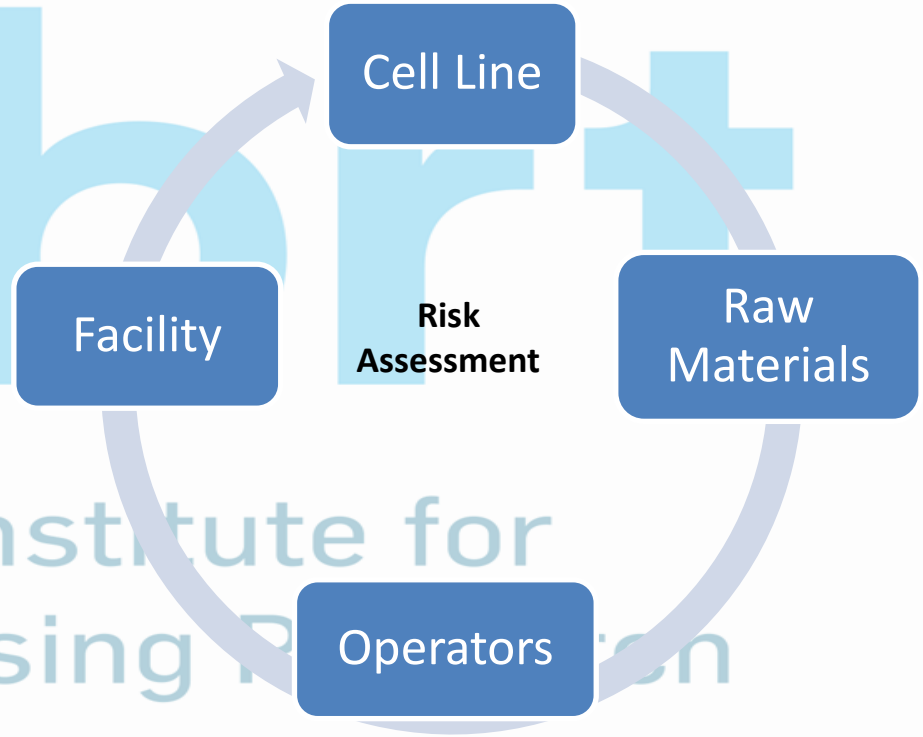
Not susceptible to:

Adenovirus, Coronavirus, Picornavirus, Herpes, Orthomyxo, Togavirus

Retrovirus (although they are prone to RVLPs!)

Are susceptible to:

Reovirus, Parmyxo, Bunya, MVM



Media

There is a trend to filtering cell culture solutions to reduce the chance of viral contamination

Topics

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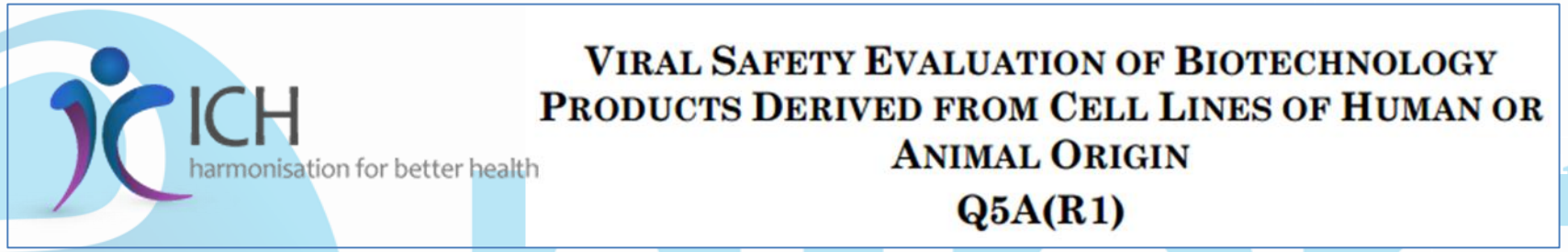
- Virus Clearance Studies
- Virus Clearance Technology
- Viral Segregation

Viral Clearance Studies



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 by the product

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 strains

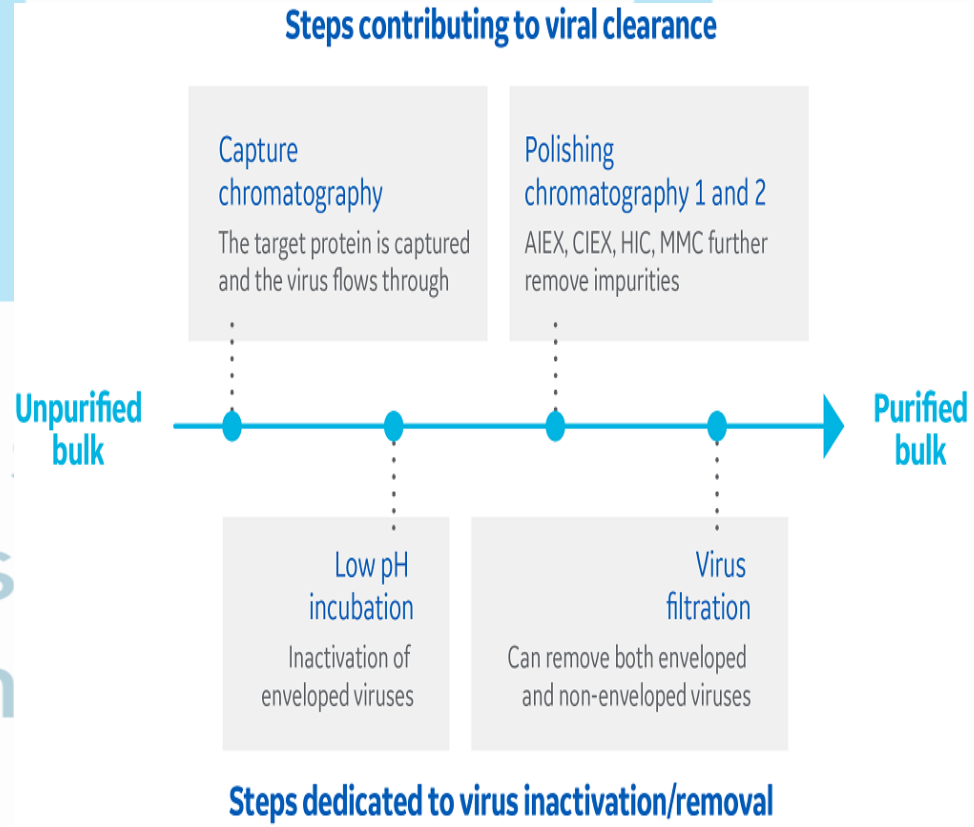
Need to grow model viruses

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

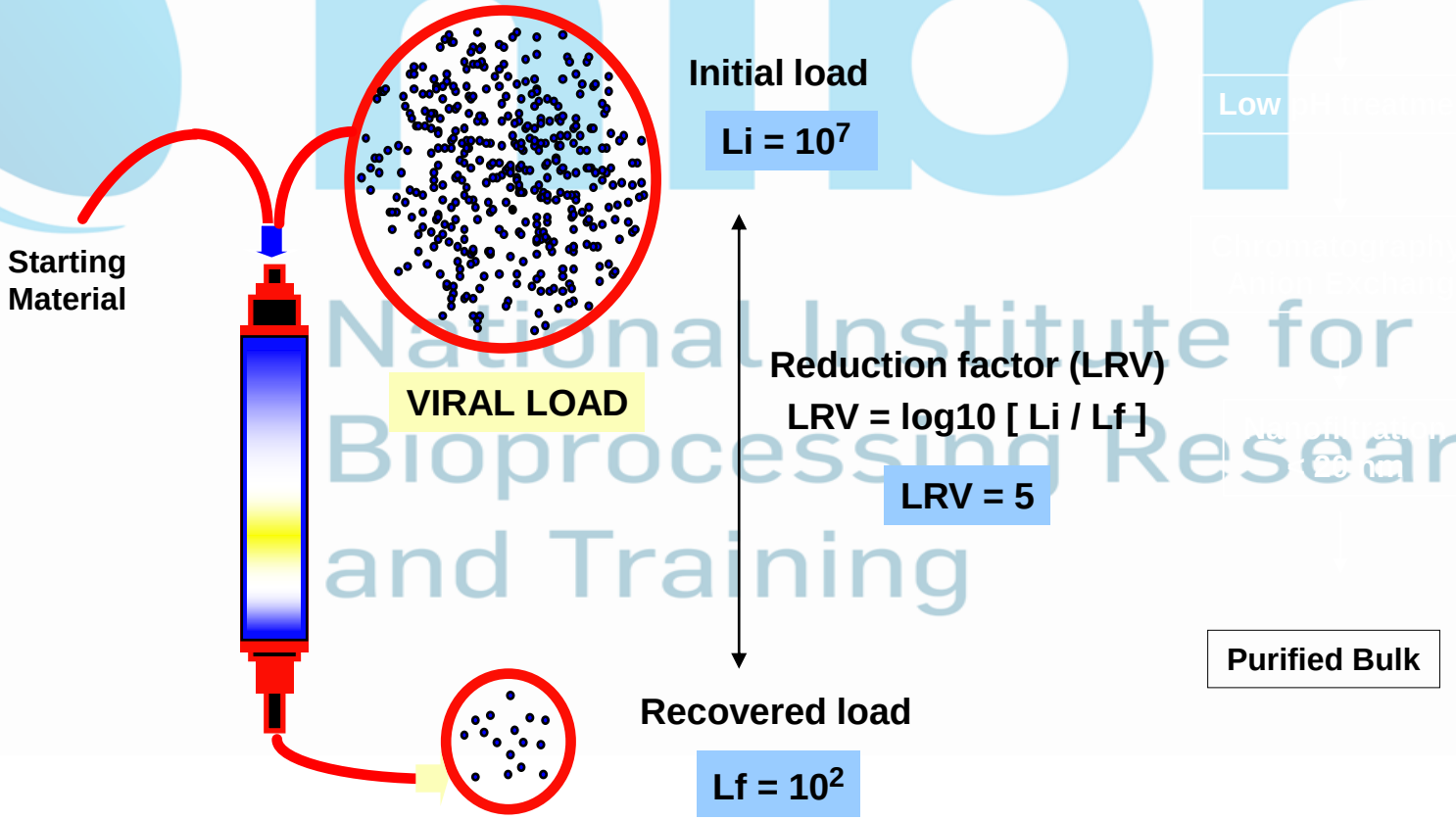
Summing reduction factors to give a total log reduction value (LRV)



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

Virus Spiking

Deliberately adding known amounts of virus to various production steps



Topics

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Effective Viral Clearance

Achieve 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

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

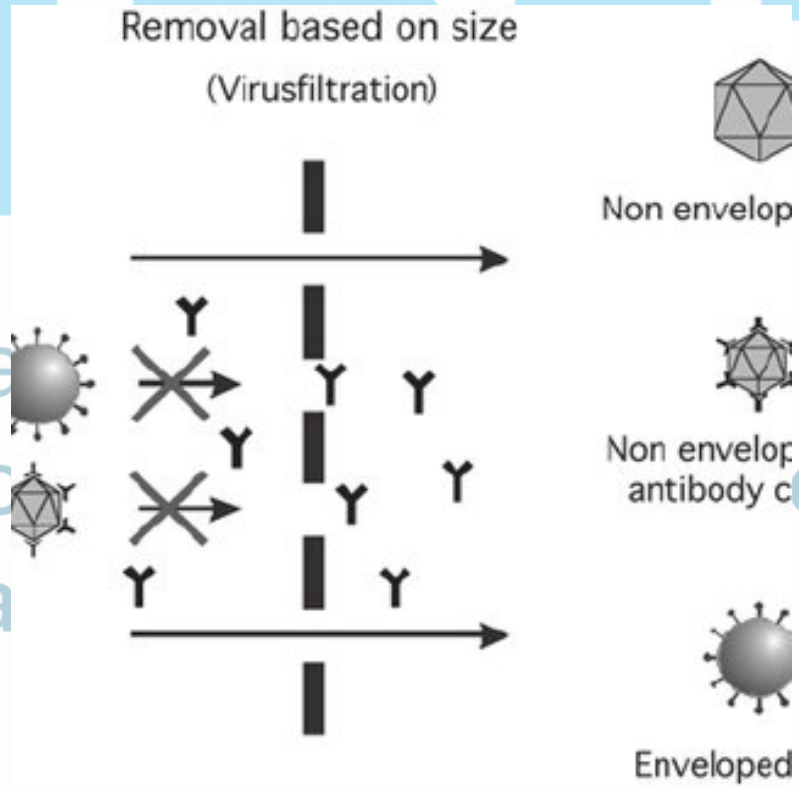
Virus Removal = The mechanical removal of viral particles

Chromatography

Precipitation

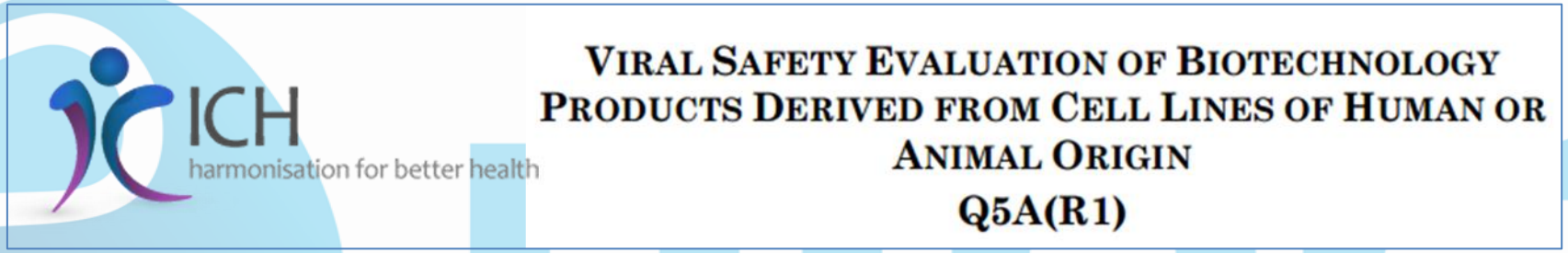
Centrifugation

Membrane filtration



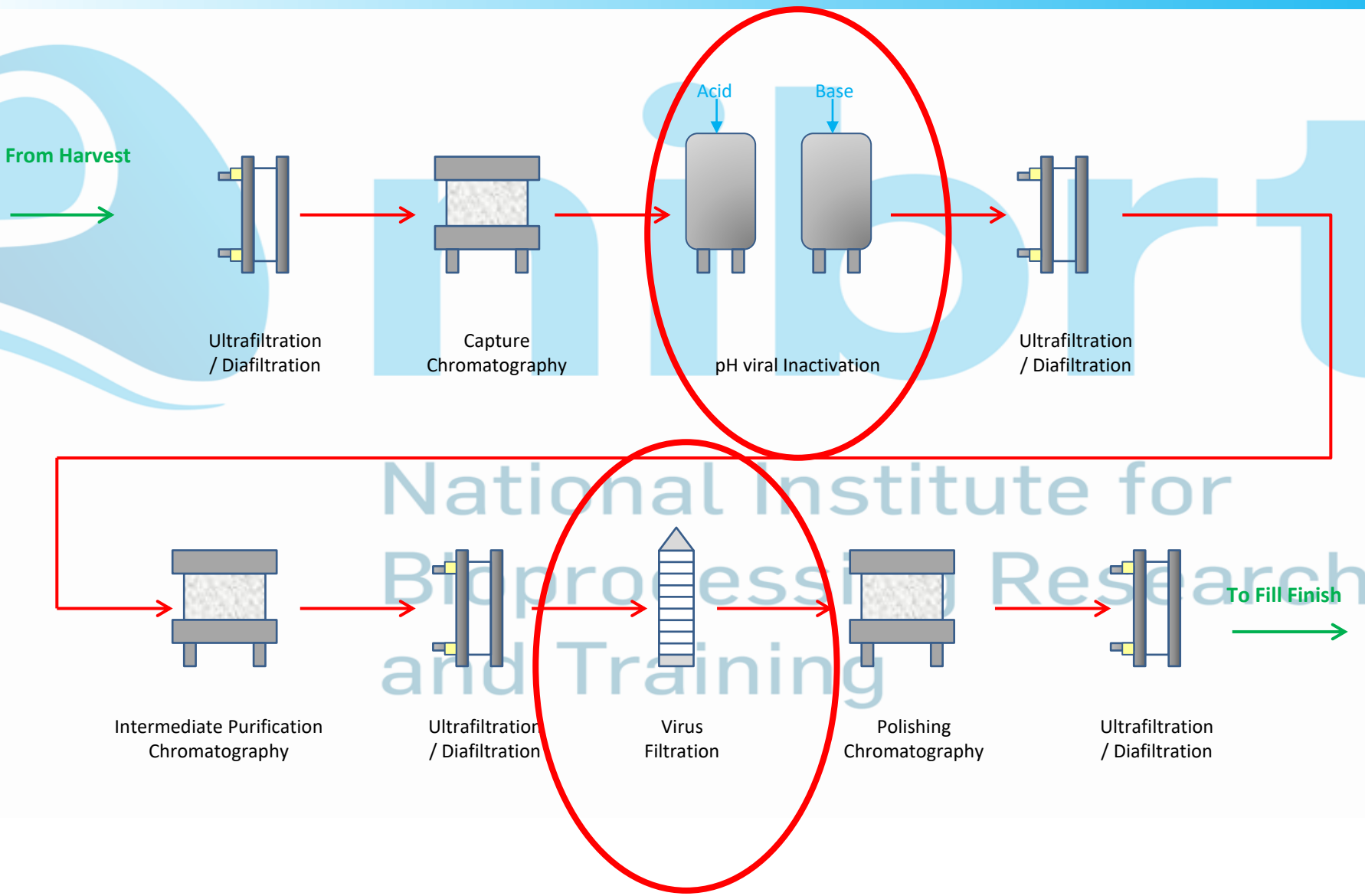
Virus Safety of Intravenous Immunoglobulin: Future Challenges
Article· Literature Review in Clinical Reviews in Allergy & Immunology 29(3):333-44 · January 2006 Nicola Boschetti, Martin Stucki, Spaeth Peter, Christoph Kempf.

Deliberate Viral Clearance



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

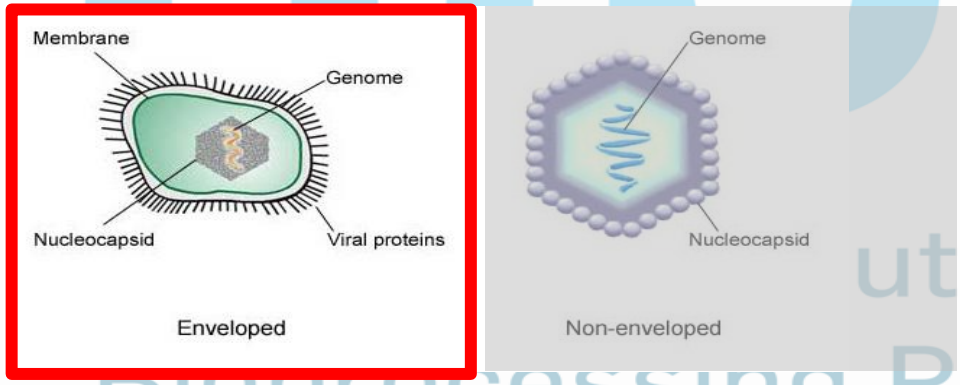
Deliberate Viral Clearance



Low pH Viral Inactivation

Some viruses, when exposed to a low pH, will denature spontaneously.

This technique is effective against enveloped viruses.



The equipment typically used is simple and easy to operate.

The target protein **must be resistant** to low pH conditions.

Low pH Viral Inactivation

Some viruses, when exposed to a low pH, will denature spontaneously.

The equipment typically used is simple and easy to operate.

Can lead to protein aggregation.

Detergent Viral Inactivation

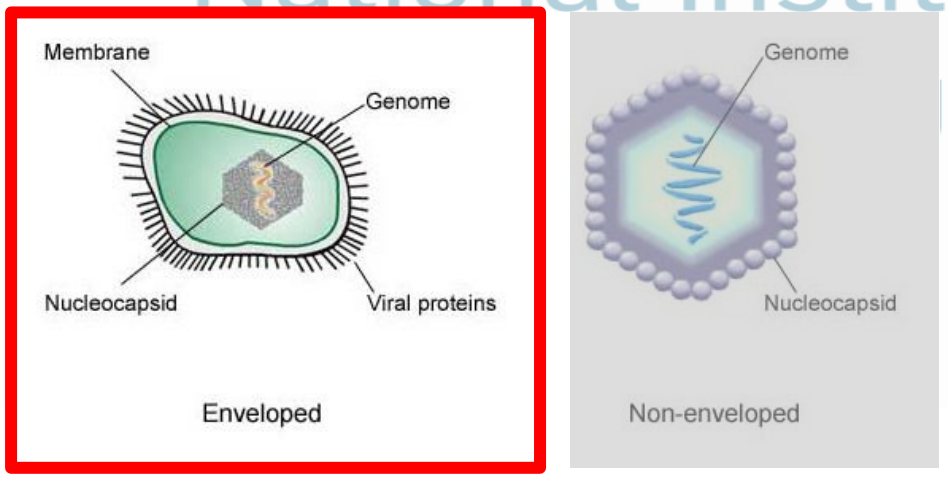
Used for products that are sensitive to low pH

Detergents interrupt the interactions between the molecules in the virus's **lipid coating**.

Similar to detergent action during cleaning – help dissolve oily substances

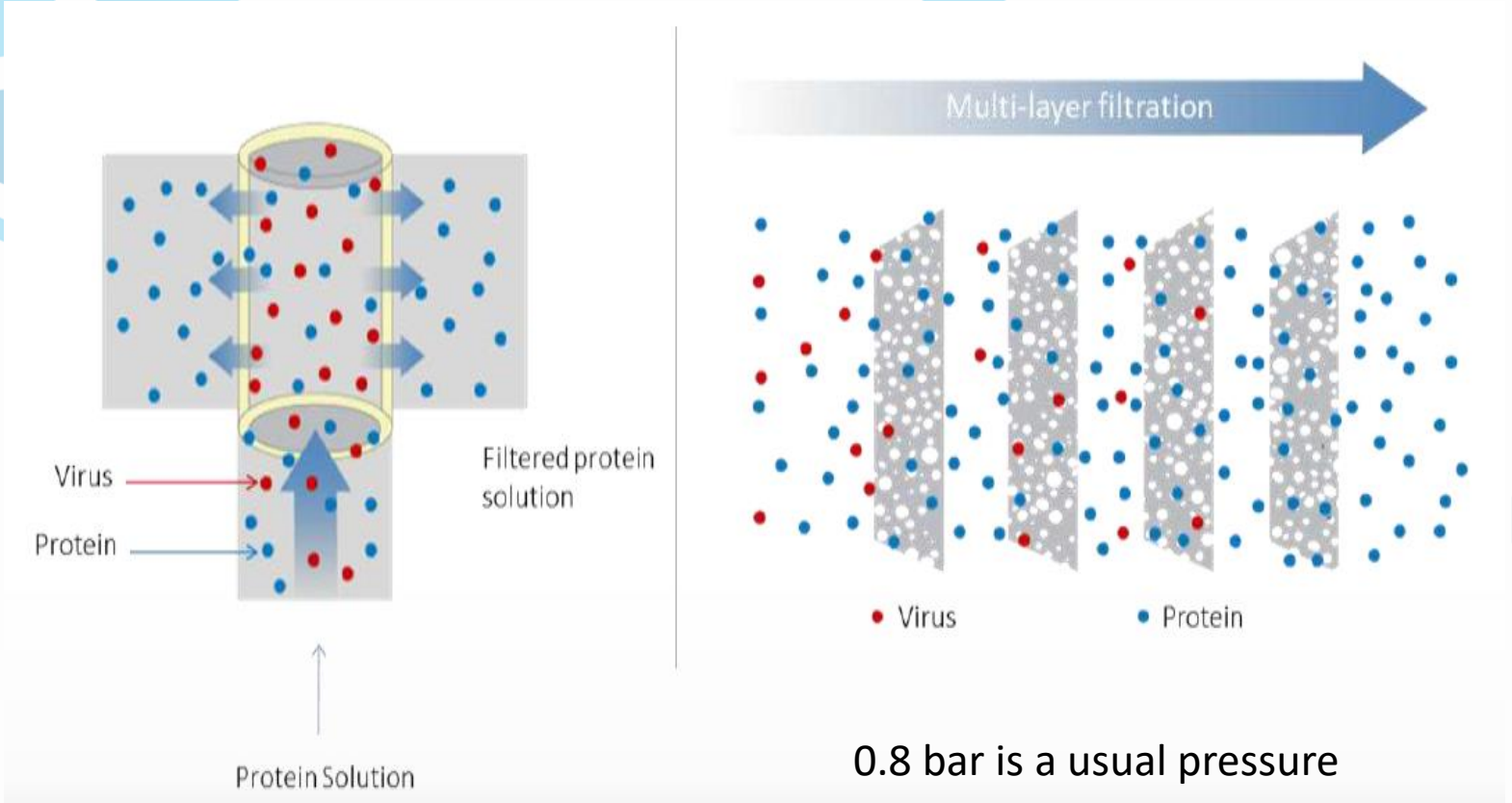
Generally not effective against non-enveloped viruses.

Example detergents include Triton-X 100 and Tween.



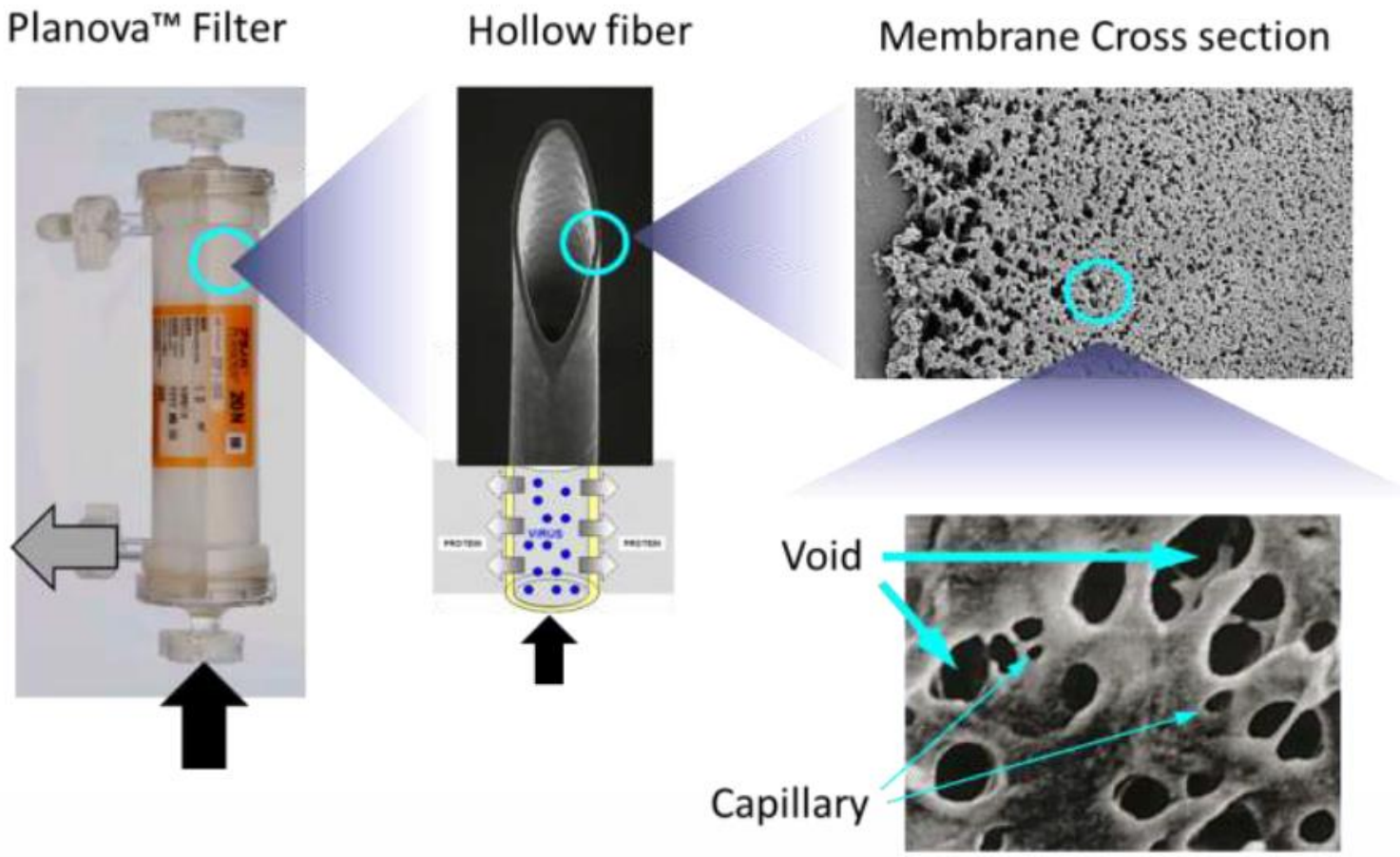
Virus Removal – Filtration

Virus filtration removes viruses by size exclusion



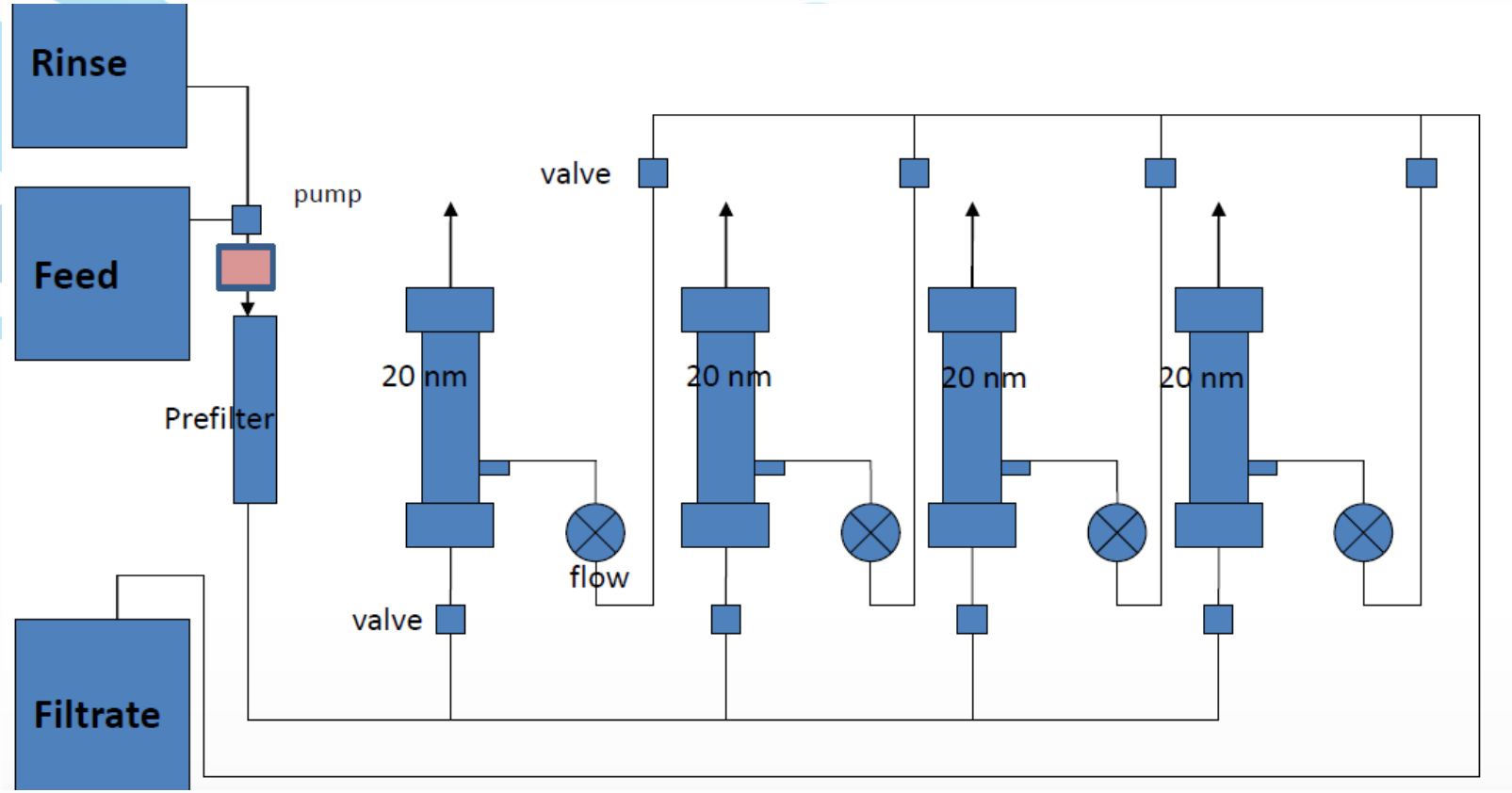
0.8 bar is a usual pressure

Virus Removal – Filtration



Membranes can be up to 50µm thick

Typical VF Process



VF Operating Sequence

Assemble	• Install • Set pressure or flow rate
Pre-Use Integrity Test	• Diffusion testing • Bubble point cannot be performed
Pre-Wash	• With water and/or buffer • Removes residues and air
Filtration	• Protein solution passed through filter • Constant pressure or flow rate (usually FR for manufacture)
Post-Use Integrity Test	• Expectation not mandatory • Diffusion or destructive gold particle test

No consensus to whether you need to clean or steam a Viral Filter

Virus Filter Considerations

Integrity Testing

The integrity test can confirm:

- the virus removal filter is properly installed
- the filter is free from gross defects and damage
- the filter removes viruses consistent with both manufacturer's specifications and end-user virus retention studies

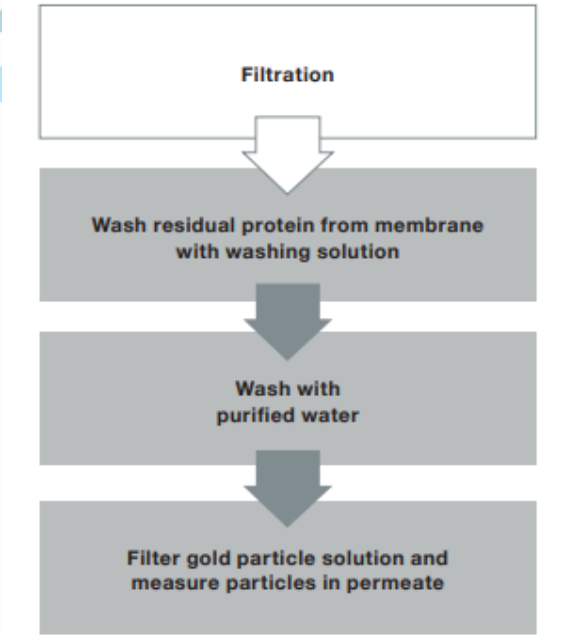
It is recommended that filter integrity be checked both pre- and post-use

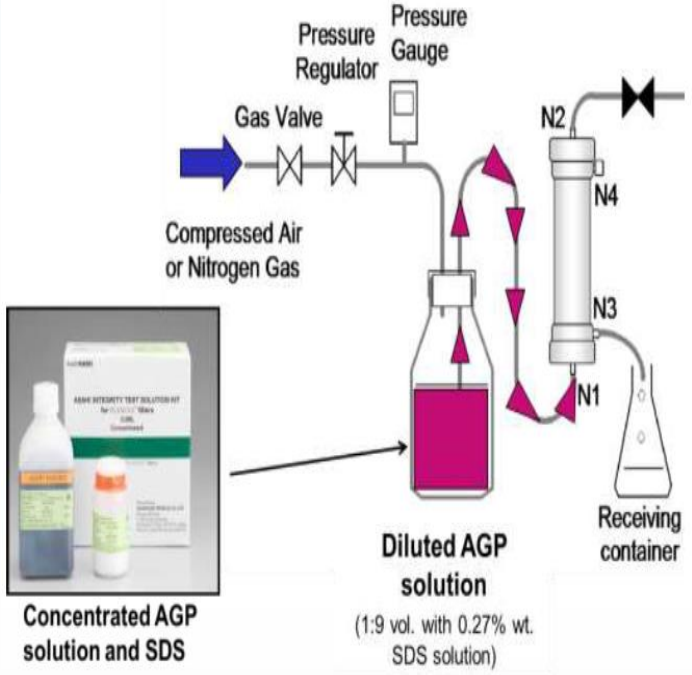
- Typically end-user tests are used to confirming installation and the absence of gross defects
- The filter manufacturer performs tests for detecting subtle changes in filter pore size distribution

Gold-Particle Test

Destructive test, only done post-use

Only for Planova Cellulose based filters





$$\Phi i = \log_{10} \frac{Amax}{(A - Apvp - Awm)}$$

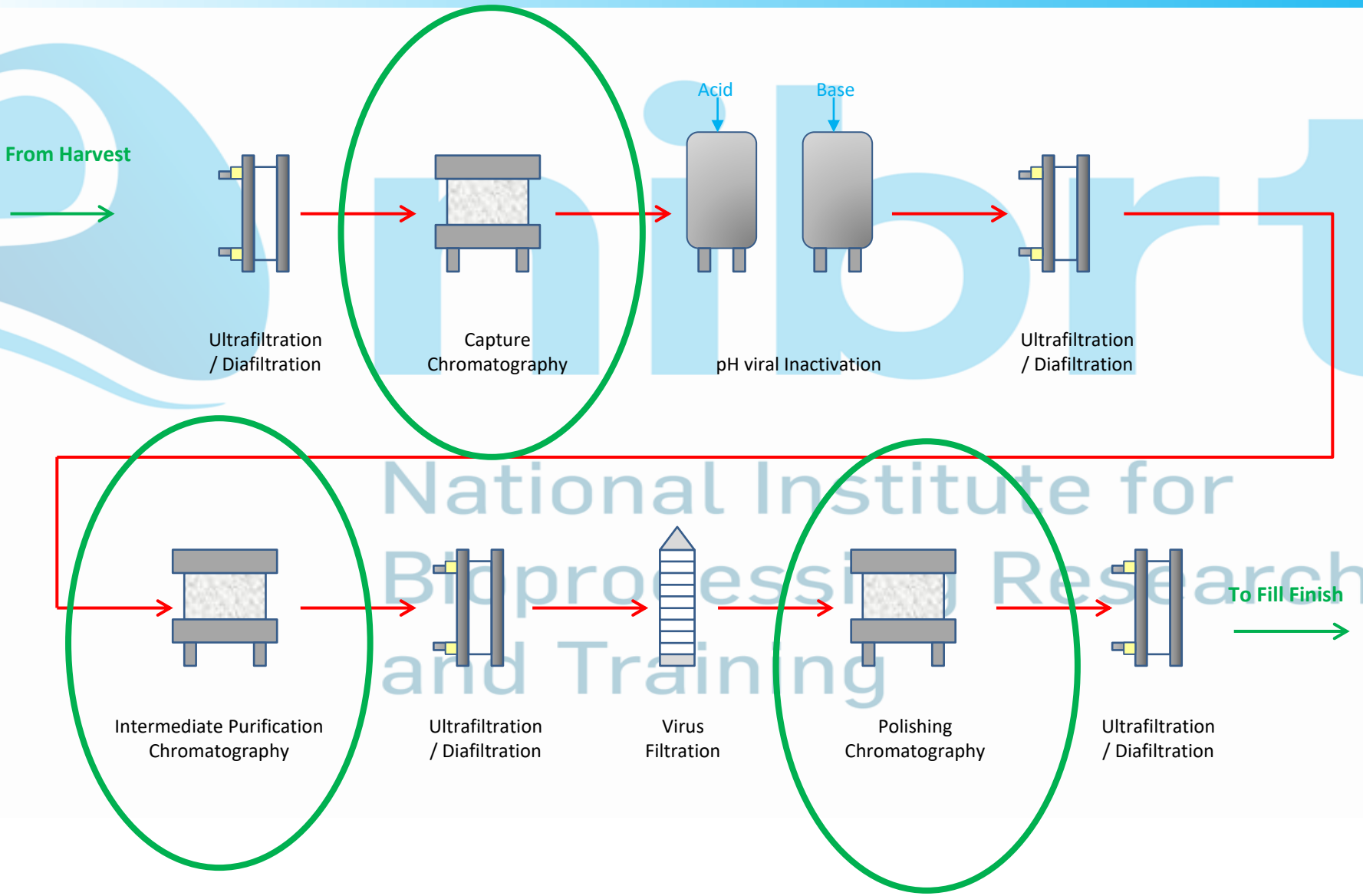
Amax: absorbance of diluted AGP (feed)

A: absorbance of the filtrate

Apvp: absorbance of PVP contained in the AGP solution (value given in AGP's COA)

Awm: mean absorbance of water

Fortuitous Viral Clearance



Topics

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- Virus Clearance Technology
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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

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Example questions

Viral Clearance

a) Explain what is meant by effective, moderately effective and ineffective viral clearance methods.

b) Regulatory bodies expect at least two effective and technically 'orthogonal' steps be included for viral clearance. Provide two examples of such methods and briefly explain each of them with respect to mode of action, target viruses, and log reduction values.

Thank You

Further Reading

- ICH Q5A - Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin
- Annex 2 - Manufacture of Biological active substances and Medicinal Products for Human Use
- Eudralex volume 4 part II GMP guideline (Refers to ICH Q5A)
- PDA website TR 41 (Virus Filtration), TR 47 (Virus Spiking) and 83 (Risk Management)
- EMEA/CHMP/BWP/398498/2005 (Clinical Trials)
- EMEA/CHMP/BWP/398498/2005 (Validation Studies)