Centre for Biopharmaceutical Excellence

Virus Safety Strategies for Biopharmaceuticals

Module Outcomes

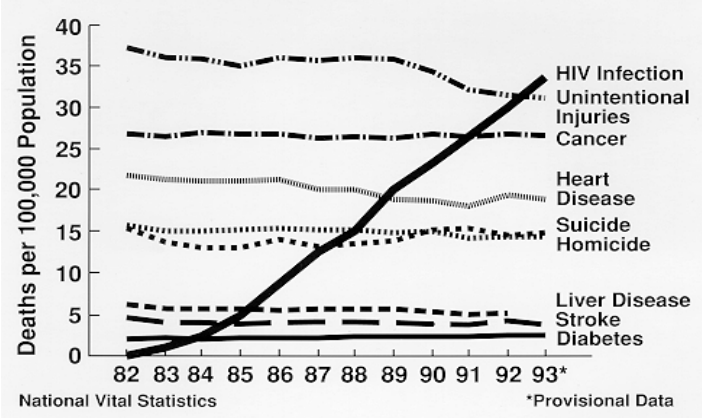
On completion of this module participants should be able to define:

- Regulatory framework for demonstrating virus safety of biopharmaceuticals
- The basic virus safety strategies
- The basic prion safety strategies

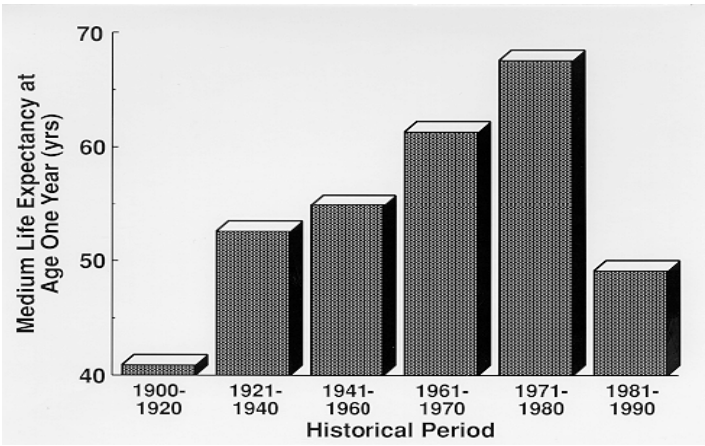
Module Topics

- Validation of virus elimination steps. Why?
- Virus Safety – Regulatory Guidelines
- Material Sourcing
- Validation of virus elimination steps

HIV infection rapidly became a major cause of death in the USA…

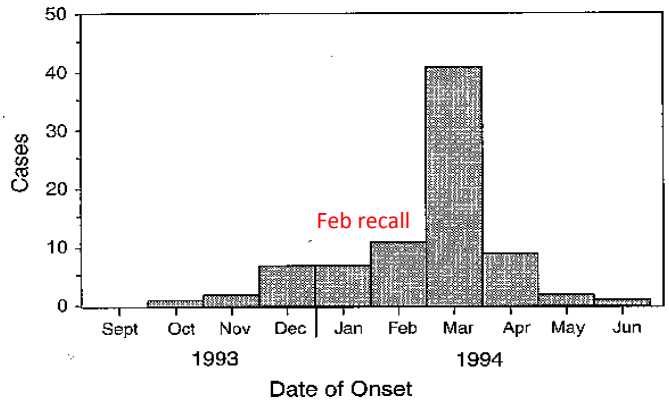


...with devastating impact on people with haemophilia many of who were later found to also be infected with HCV..



IVIG was thought to be relatively safe due to the manufacturing processes until….

FIGURE 1. Possible cases* of hepatitis C virus infection reported among persons receiving Gammagard® or Polygam® — United States, October 1993–June 1994



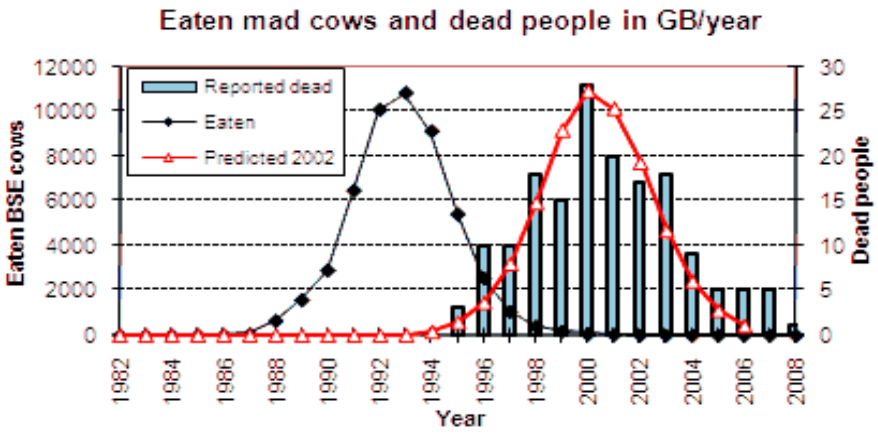
*Of 112 reported possible cases, the date of illness onset or date of first abnormal alanine aminotransferase level was available for 81 cases.

Emergence of comprehensive guidelines in Europe driven by the Paul Erlich Instutite

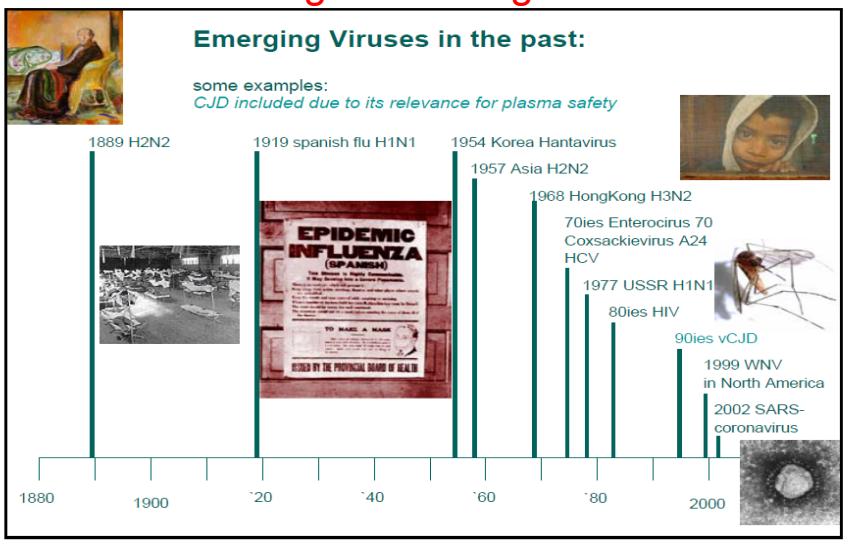
Required:

- processes assessed for potential to clear virus
- two dedicated VI steps with >4 log reduction and a total of 10 log reduction for enveloped viruses (HIV/HCV/HBV)
- one dedicated VI step with > 4 log reduction and a total of > 6 log reduction for non-enveloped viruses (HAV, B19)

...and then BSE and variant CJD came along..



Viruses change and new infectious agents emerge



Some Important Regulatory Requirements

- ICH Q5 A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin (also FDA Guidance)
- WHO - Annex 4 Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products
- EMA/CHMP/BWP/706271/2010 Committee for medicinal products for human use (CHMP) Guideline on plasma-derived medicinal products
- EMA CPMP BWP 328/99 Guidance on Developing Biologics
- EMA CPMP BWP 268/95 Guidance Viral Validation Studies
- EMEA/CHMP/BWP/3794/03 (2006) Guideline on the Scientific data requirements for a plasma master file (PMF)

Adventitious Contamination and GMPs (ICHQ7 – Section 18.5)

- 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.
- Appropriate precautions should be taken to prevent potential virus carry-over (e.g. through equipment or environment) from previous steps.
- If the same equipment is to be used, the equipment should be appropriately cleaned and sanitized before reuse.

EU Directives

2001/83/EC, Annex I (3.2.1.2. manufacturing process of the active substance(s)), amended by Directive 2003/63/EC,

- The conditions for manufacture of active substances for biological medicinal products are applicable …..
- *"If the presence of potentially pathogenic adventitious agents is inevitable, the corresponding material shall be used only when further processing ensures their elimination and/or inactivation, and this shall be validated."*

Application (per ICH Q5A)

Viral Safety of Biotechnology Products Derived from Cell Line of Human or Animal Origin

- biotechnology products derived from characterized cell lines of human or animal origin (i.e., mammalian, avian, insect) including:
  - Antibodies (monoclonal antibodies / Interferons)
  - Recombinant Proteins and Glycoproteins, including recombinant subunit vaccines
  - tissue and blood-derived products
- term virus excludes nonconventional transmissible agents like those associated with bovine spongiform encephalopathy (BSE) and scrapies
- Required as early as Phase 1 / (IND) studies

Some Important Definitions

- **Inactivation**: Reduction of virus infectivity caused by chemical or physical modification. A process of enhancing viral safety in which virus is intentionally “killed”.
- **Viral removal** A process of enhancing viral safety by physically removing or separating the virus from the protein(s) of interest.
- **Viral Clearance**: Elimination of target virus by removal of viral particles or inactivation of viral infectivity.

Some Important Definitions

- **Adventitious Virus**: Unintentionally introduced contaminant virus.
- **Endogenous Virus**: Viral entity whose genome is part of the germ line of the species of origin of the cell line
- **Relevant Virus**: Virus used in process evaluation studies which is either the identified virus, or of the same species as the virus that is known.
- **Specific Model Virus:** Virus which is closely related to the known or suspected virus (same genus or family)
- **Nonspecific Model Virus:** A virus used to characterize the robustness of the purification process.

Main Principles for Viral Control

Sourcing

- Select and test cell lines and other raw materials, including media components, for the absence of undesirable viruses.

Viral Clearance Target

- Assess potential for adventitious contamination during production
- Assess capacity of the production process(es) to clear infectious viruses
- Validate CPPs and CQAs at scale down and Verify scale up integrity
- Typically > 6 $\log_{10}$ reduction (> 6 LRF)

Testing

- Test the product at appropriate steps of production for absence of contaminating infectious viruses, where possible.

Limitations of Laboratory Testing

- Initial characterisation/testing of cell lines or plasma is essential to understand viral species present.
- No single test can detect the presence of all known viruses
- Tests require a minimum viral load to detect presence
- Statistical limitations for sampling eg tests for Abs to HCV in human plasma
- Sero-conversion windows in plasma may mean virus is not detected. Pre-screening of plasma donors is a key component
- Many instances historically where test has been negative yet viral contamination has manifested

Potential Sources of Contamination

- **Existing in the Master Cell Bank (MCB)** and be vertically transmitted through cell lines to the WCBs.
- **Present in starting material (eg plasma)** e.g window period donation or potentially an unknown virus that is not tested for.
- **Introduced During Production (contamination)**
  - use of contaminated biological reagents such as animal serum components;
  - use of a contaminated reagent, such as a monoclonal antibody affinity column;
  - use of a contaminated excipient during formulation;
  - contamination during cell and medium handling.
  - cross contamination via residue from a **"Pre-VI**" to a **"Post VI"** area

Aims of Validation

- To provide documented evidence that a manufacturing process will effectively inactivate or remove viruses which may be a contaminant of the start material
- To provide documented evidence for the removal and or inactivation of unknown viruses, novel virus infections or unpredictable contaminants
- Scaled down systems are used to assess the 3 mechanisms of clearance ;
  - Partitioning
  - Inactivation
  - Elimination by size

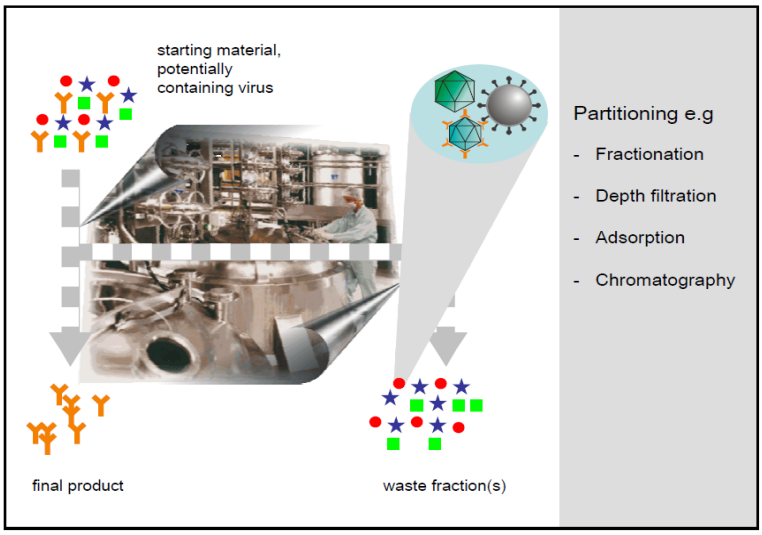
Example Viral Inactivation Steps

Treatment	Advantages	Important CPPs
Pasteurisation (60oC / 10 hours)	Inactivates enveloped and some non-enveloped viruses (HAV) Relatively simple process	Uniform Temp. 60oC 10 hours, stir rates
Terminal Dry Heat (80oC / 72 hours)	As above Conducted in sealed final container	Lyophiliser cycle conditions Uniform temperature Residual moisture
Vapour Heat	As above	Lyophiliser cycle conditions Uniform temperature Moisture pre /post heating
Solvent / Detergent	Effective against enveloped viruses, limited against non-enveloped	Temperature, duration & solvent concentration
Low pH High pH	As above	Pre-clean, pH, temperature and duration/contact time

Example Virus Removal Steps

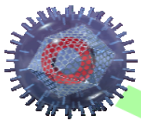
Treatment	Advantages	Some Important CPPs
Precipitation	Effective against enveloped and non-enveloped viruses (but difficult to model).	Concentration of agents Protein concentration, pH, and possibly ionic strength, temperature, time for addition etc.
Chromatography	Effective against enveloped and non-enveloped viruses (easier to model).	Resin packing by e.g. HETP measurements. Protein elution profile. Flow rate. Buffer volumes. Number of resin cycles.
Nano-Filtration	Effective against enveloped viruses, maybe also HAV and B19.	Filtration Pressure Flow rate Filter integrity Protein concentration Ratio of product volume to filter surface area

Partitioning



Inactivation

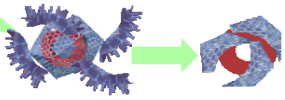
Virus envelope - target of inactivation



Solvent/Detergent: S/D-mediated *disruption of envelope*

Caprylate: partitioning of non-ionized molecule into envelope and ev. *disruption of envelope*

Pasteurization (heat): *disruption of envelope* (before capsid)

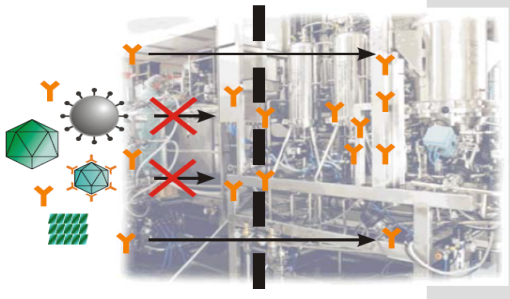


Low pH: ionic *disruption of envelope* and destructive conformational changes of structural proteins

Elimination by size (nanofiltration)

Principle of virus filtration

(nanofiltration; exclusion based on size)



Non enveloped virus

Non enveloped virus antibody complex

Enveloped virus

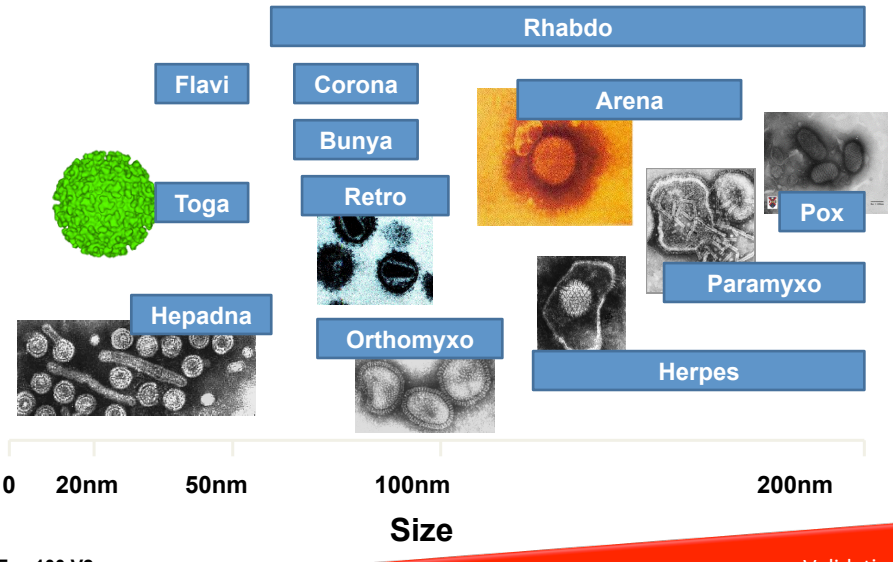
Protein aggregates (e.g. PrP^{sc})

Choice of viruses for validation studies

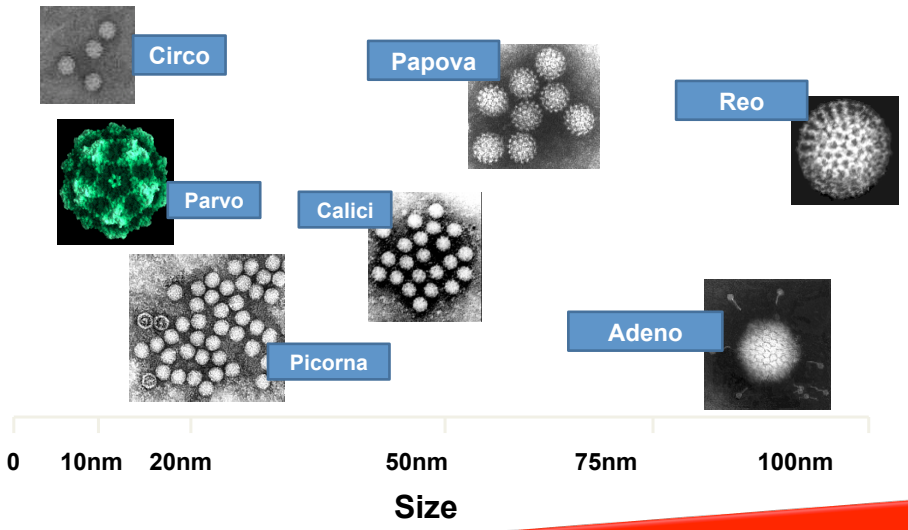
"Viruses for validation should be chosen firstly to resemble viruses which may contaminate the product as closely as possible and secondly to represent as wide a range of physico-chemical properties as possible in order to test the ability of the system to eliminate viruses in general"

(CPMP/BWP/268/95, Section 4.1)

Physico-Chemical Properties: Enveloped Viruses



Physico-Chemical Properties: Un-enveloped Viruses



ICH Q5A / FDA Guidance
(Virus Selection Parameters)

Case	Viral Profile of Unprocessed Bulk	Suggested Viruses for Studies
A	No virus, virus-like particle, or retrovirus-like particle has been demonstrated.	Nonspecific model viruses
B	Only a rodent retrovirus (or a retrovirus-like particle that is non-pathogenic) is present.	Specific model virus, such as a murine leukemia virus.
C	Known to contain a virus, other than a rodent retrovirus, for which there is no evidence of capacity for infecting humans.	Use the identified virus, where possible.
D	Known human pathogen is identified, the product may be acceptable only under exceptional circumstances.	Use the identified virus be used plus very specific and sensitive testing methods.
E	Virus cannot be classified regarding pathogenicity	The product is usually considered unacceptable

Specific Tests for Bovine Viruses

FDA 9CFR

- For bovine sourced serum or where cell lines have been exposed to bovine derived components, the following viruses must be tested for:
  - Bluetongue and related orbiviruses
  - Bovine adenovirus
  - Bovine parvovirus
  - Bovine respiratory syncytial virus
  - Bovine viral diarrhoea virus
  - Rabies virus
  - Reovirus 3

Recombinant Products: Choice of Virus

- Choice of virus is dependent on origin of cell line /primary seed virus / transgenic animal and use of process reagents of animal origin (bovine serum, porcine Trypsin etc)

Target	Model (Target)	Genome	Size [nm]	Envelope	Resistance
Retroviruses	MuLV	2x ssRNA	80-110	yes	low
Pestiviruses	BVDV	ssRNA	50-70	yes	low
Paramyxoviruses	PI-3	ssRNA	100-200+	yes	low
Herpesviruses	PRV, BHV	dsDNA	120-200	yes	low - med
Reoviruses	REO 3	dsRNA	60-80	no	medium
Polyomaviruses	SV40	dsDNA	40-50	no	very high
Parvoviruses	MMV, PPV, CPV	ssDNA	18-22	no	very high

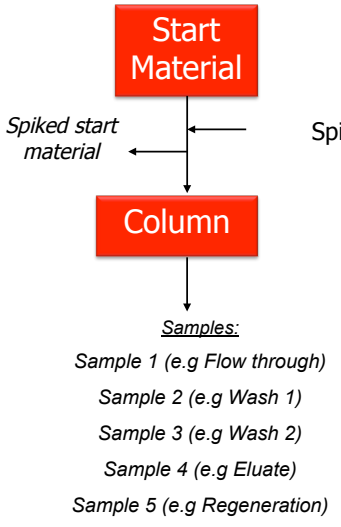
Highlighted virus families now almost universally used

Characteristics of a Robust VI Process Stream

- Remove or inactivate typically 4 logs or more**;
- Be easy to model convincingly and be relatively insensitive to changes in process conditions.
- A production process should include two robust VI steps particularly if the steps act by different mechanisms (e.g. inactivation by a chemical treatment followed by a robust physical removal step)
- At least one of the 2 steps should be effective against non-enveloped viruses.

** Steps removing 1 log of virus or less cannot be regarded as significant

Processes are scaled down for clearance studies



- Perform infectivity assays on collected samples to determine log reduction values (LRV)
- All data is only an approx. of the virus inactivation or removal capacity
  - Not all viruses behave the same!
- Steps to be evaluated/not evaluated must be justified.
  - Not all steps need to be validated
  - Not all products have the same level of risk!

Validation Challenges

- Justification for the extent of validation will depend on a number of factors, notably the process itself and the source materials.
- Difficult to assay titres below 10^3 . Starting titre > 10^7 - 10^9
- Tests can be difficult to validate – interference, virus killed
- Spiking studies have to occur in a scaled down situation
- Comparability of the model to the full scale process is crucial – so validity of the scaling is pivotal to validation.
- Viruses may aggregate causing abnormal behaviour eg. in filtration studies.
- Inactivation kinetics cannot be assumed to be linear so time based inactivation curves are required.

Scaled-Down Production System and Typical Critical Process Parameters (CPPs)

- The level of purification of the scaled-down version should represent as closely as possible the production procedure and be representative of commercial-scale manufacturing. Requires QA oversight and verification of integrity
- For chromatographic equipment typical CPPs include:
  - column bed-height
  - linear flow-rate and pressure
  - flow-rate-to-bed-volume ratio (i.e., contact time)
  - buffer and gel types
  - pH
  - temperature
  - concentration and purity of protein
  - conductivity
  - column age/ re-use
- A similar elution profile (CQA) to the scale up is expected.

Example – Recombinant Process VI

- Cell line – Chinese Hamster Ovary (CHO) – Case B
- Viruses selected – specific model virus
  - Murine Leukaemia Virus (MuLV)
  - Minute Virus of Mice (MVM)
- Process Steps Evaluated
  - Anion Exchange chromatography
  - 20N nanofiltration
  - Solvent Detergent (SD)
  - Capto Adhere (mixed mode) chromatography
- Scale down studies in R&D virology facility under OECD GLPs

Example Selected Virus Panel

Virus	MuLV	MVM
Family	Retroviridae	Parvoviridae
Size (nm)	80 - 110	18 - 24
Shape	Spherical	Icosahedral
Nucleic Acid type	ssRNA	ssDNA
Envelope	Yes	No
Model Type	Specific model virus	Non-specific model virus

Example Anion Chromatography "CPPs" Scale Down Comparison

CPP	Manufacturing Scale	Scale Down	Comment
Bed Height (cm)	15	15	Held Constant
Diameter (cm)	30	1.1	Scale Down
Column Volume (mL)	11,000	15	Scale Down
Target Product Load	1500 IU/mL	2000 IU/mL	Worst Case Load
Elution Pool Volume	0.2	2.0	Worst Case Load

Example Total Process Viral Clearance (Viral Log10 Reduction Factor)

Method	MuLV (envelope)	MVM (non envelope)
Anion Exchange Chromatography	> 5.5 +/- 0.2	> 4.5 +/- 0.3
CA Chromatography	N/A	> 4.9 +/- 0.5
Solvent Detergent (SD) Inactivation	> 5.6 +/- 0.4	N/A
20N Nano-filtration	> 4.8 +/- 0.6	> 3.3 +/- 0.1
Cumulative Log10 Reduction Factor	**> 15.9 +/- 1.2**	**>12.7 +/- 0.9**

Regeneration of Chromatographic Columns

- Column performance may vary over time or with repeated usage
- Age / stability of a column is a CPP candidate
- Assurance should be provided that any virus potentially retained by the production system would be adequately destroyed or removed prior to reuse of the system.
- For example, such evidence may be provided by demonstrating that the cleaning and regeneration procedures do inactivate or remove virus.

Equipment Cleaning and Sanitation (CIP, COP, SIP and Manual)

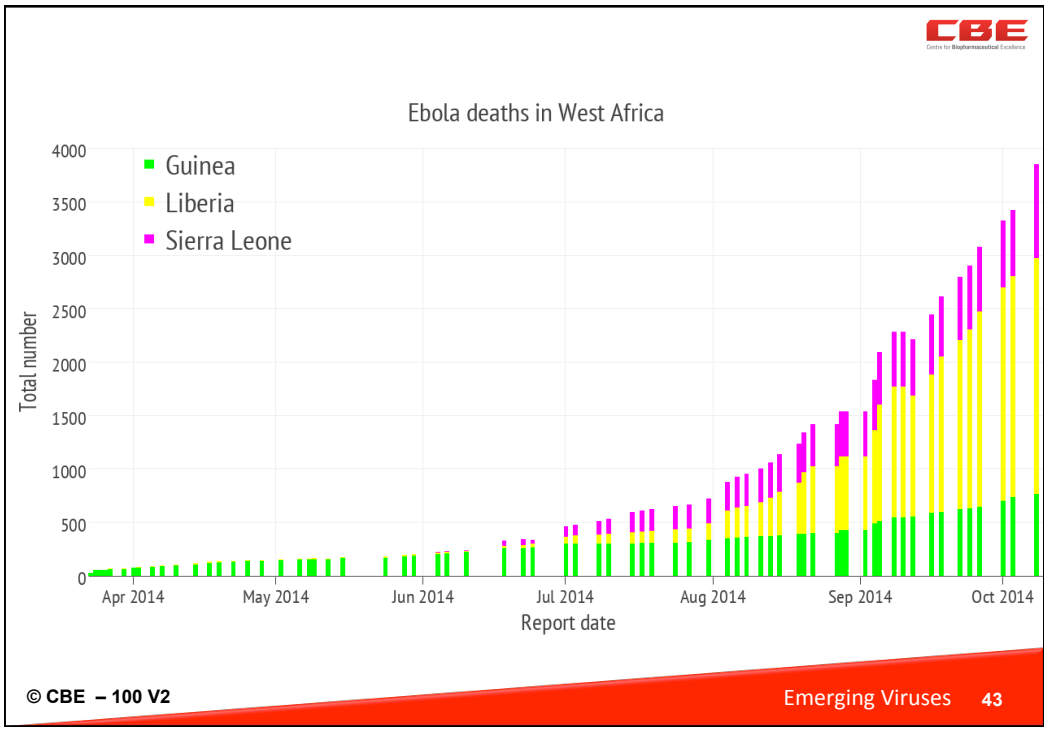
- SIP is effective against viruses but less so against prions
- CIP can be demonstrated to be VI up to 4 logs with pre-cleaning then 0.1M NaOH, > 2 minutes contact time at >72^{o}C.
- Manual Cleaning up to 4 logs with pre-cleaning then >0.25M NaOH with 30 minutes contact time at room temperature.

Limitations of Viral Clearance Studies

- The “safety factor” is dependent on the incoming titre – important to estimate starting titres;
- Pilot-plant scale processing may differ from commercial-scale
- The behavior of a tissue culture virus in a production step may be different from that of the native virus (purity, aggregation);
- It is possible that virus escaping a first inactivation step may be more resistant to subsequent steps eg. by aggregation;
- Additive log reduction approach may overestimate the true potential for virus elimination. Repetitions of the same step and < 1 Log reduction should be excluded;
- Titre estimates below 1000 are unreliable;
- logarithmic reductions in titre implies that it will never be reduced to zero.

Re-evaluation of Viral Clearance

- Results have shown that even small modifications in manufacturing procedures or small changes in virus strains can significantly alter the established LFR.
- Whenever significant changes in the production or purification process are made, the effect of that change, both direct and indirect, on viral clearance should be considered and the system re-evaluated as needed.
- For example, changes in production processes may cause significant changes in the amount of virus produced by the cell line; changes in process steps may change the extent of viral clearance.
- Refer back to the original CPPs and CQAs when assessing change impact



Acknowledgements

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VIRUSURE
VIRUS & PRION TESTING

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