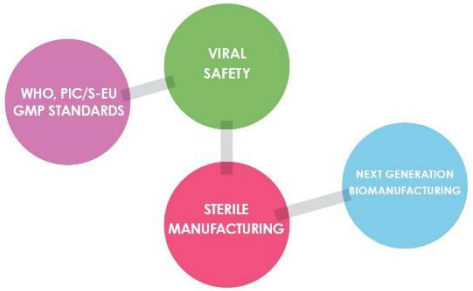


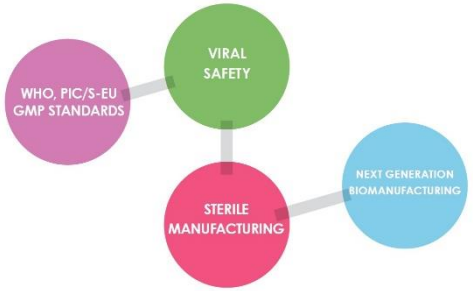
Dinesh Khokal, PhD, Singapore

- Director, External Affairs, JAPAC and LATAM, Amgen
- Certified Quality Auditor and Biomedical Auditor of American Society for Quality
- President of Parenteral Drug Association Singapore Chapter

GaBI Educational Workshops

18 December 2019, Hotel Gran Mahakam, Jakarta, Indonesia

2nd ASEAN Educational Workshop on
GMP FOR BIOLOGICALS/BIOSIMILARS



Validation of viral removal and inactivation

Dinesh Khokal, PhD
18 December 2019

GaBi
GENERICS AND BIOSIMILARS INITIATIVE
Building trust in cost-effective treatments

PPCI
Pro Pharma Communications International

asean

VALIDATION OF VIRAL REMOVAL AND INACTIVATION

2nd ASEAN Educational Workshop on GMP for Biologicals/Biosimilars
Generics and Biosimilars Initiative (GaBI)
18 December 2019, Jakarta, Indonesia

DINESH KHOKAL, PH.D.
DIRECTOR, EXTERNAL AFFAIRS, JAPAC & LATAM

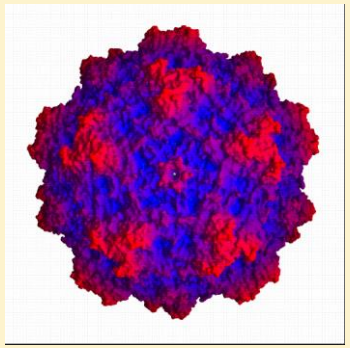
SYNOPSIS

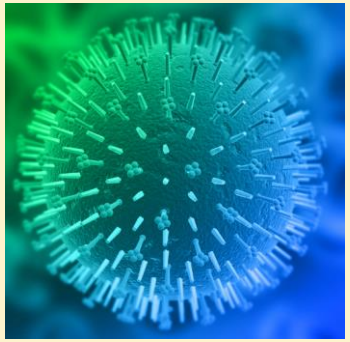
- Introduction
- Viral clearance methods
- Validation of viral removal

VIRUS CONTAMINATION IN BIOPROCESSING INDUSTRY

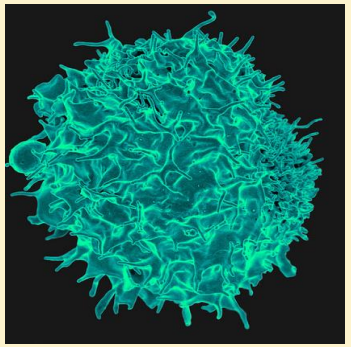
- **There have been several reported cases of viral contamination in large scale cell culture processes**
  - MMV (mouse minute virus)
  - Reo-3
  - Calicivirus
  - Porcine circovirus (PCV)
- **Although rare, these events can potentially have significant consequences**
  - Product impact
  - Long term facility shutdowns for decontamination/reboot
  - Disruption of medicine supply to patients
  - Business impact

Minute virus of mice (MMV);Retrovirus type 3 (Reo-3)





Calicivirus (Feline virus); Circovirus (Porcine virus)



VIRAL RISK MITIGATION STRATEGY IN MAMALLIAN CELL BIOMANUFACTURING

1. Control of Raw Materials
2. Cell Line Development
3. Virus testing for cell bank & In-process products
4. Plant Design & contamination control
5. Viral Clearance Process

Validation of viral removal → Focus of this presentation

No single approach provides a sufficient level of assurance alone

WHY VIRUS CLEARANCE/ INACTIVATION VALIDATION?

- No single test is able to demonstrate the presence of all known viruses
- All test systems require a minimum level of viral contamination to record a positive (sensitivity)
- Tests are also limited by statistical considerations in sampling

WHY VIRUS CLEARANCE/ INACTIVATION VALIDATION?

Establishing the freedom of a biological product from virus will not derive solely from testing but also from a demonstration that the manufacturing process is capable of removing or inactivating them

Validation of the process for viral removal/ inactivation play an essential and important role in establish product safety

SYNOPSIS

- Introduction
- **Viral clearance methods**
- Validation of viral removal

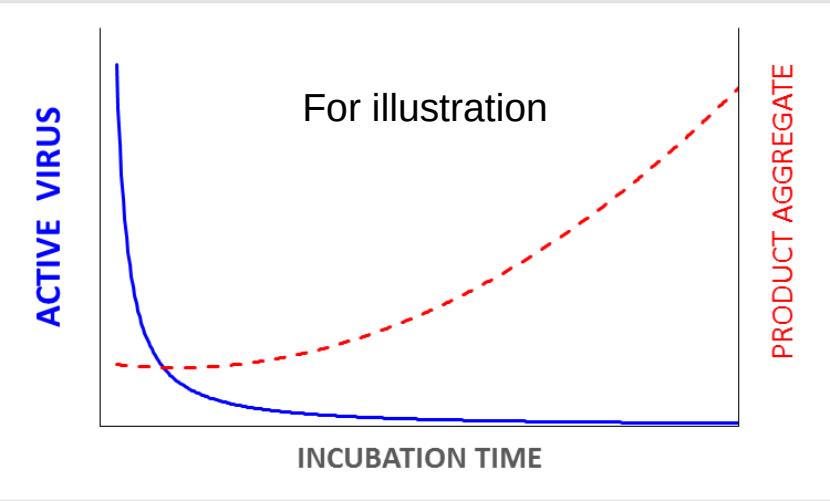
VIRAL CLEARANCE METHODS

- **Inactivation**
  - **Low pH incubation** (most commonly used method)
  - **Surfactant / Detergent** (any additives need to be cleared in later downstream process)
  - **Heat treatment**
  - **UV**
- **Physical removal**
  - **Size** (Nanofiltration)
  - **Charge & hydrophobicity** (chromatography)

LOW PH INCUBATION

Process Design consideration for validation

- pH set point (typical pH 3-4)
- Time
- Temperature
- Protein concentration
- Homogeneity (mixing)
- Type of titrants
- Titrant addition method
- pH probe selection
- pH measurement and calibration techniques

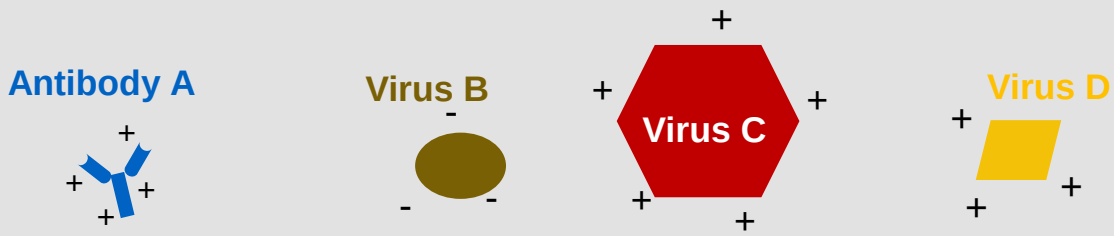


➢ LOW PH INACTIVATION IS THE MOST COMMON METHOD USED IN INDUSTRY

➢ EFFECTIVE TO INACTIVE ENVELOPED VIRUSES

PHYSICAL VIRAL REMOVAL

- Viruses possess surface **charge** and **hydrophobicity**
- Viruses' isoelectric point are typically lower than most recombinant antibodies' isoelectric point. Thus virus removal using ionic exchange chromatography is feasible
- Viruses size are larger than antibodies, thus size separation using nanofiltration or hydrophobic chromatography is feasible



Pictures for illustration only

B. Michen and T. Graule, Journal of Applied Microbiology 109 (2010) 388–397

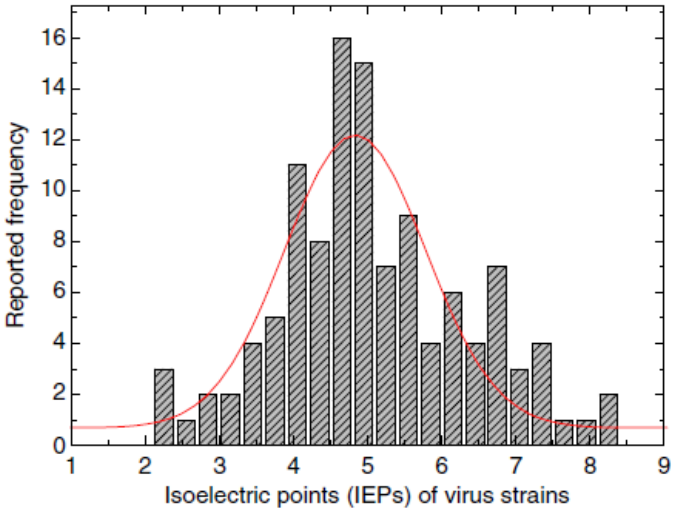
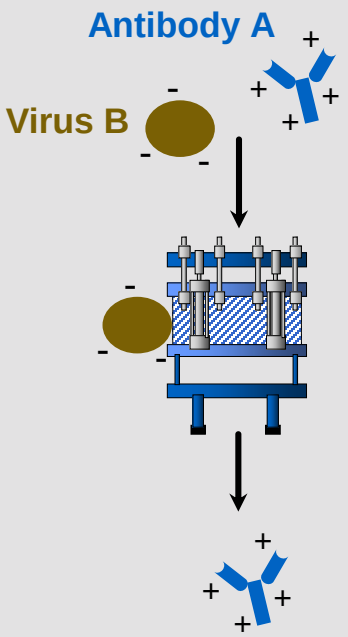


Figure 3 Displays isoelectric points of viruses and their reported frequency in literature. Columns plotted in the range $0 < pH < 10$, increment = 0·3. Line presents a Gaussian fit of data resulting in a mean value of 5·0 ± 1·3, R^2 = 0·81.

AT pH 7, MOST VIRUSES ARE NEGATIVELY CHARGED AND BOUNDABLE TO A POSITIVELY CHARGED CHROMATOGRAPHY RESIN

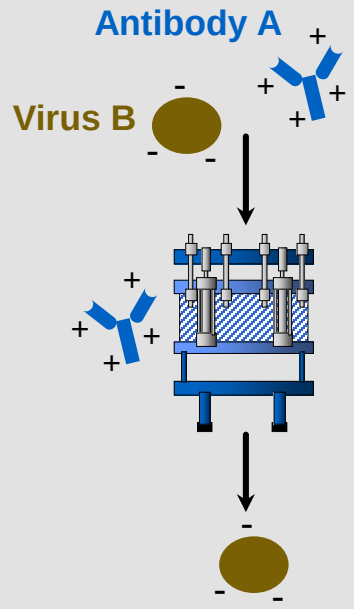
CHROMATOGRAPHY FOR VIRAL REMOVAL

- **Ionic Exchange Chromatography separates molecules by charge (ionic interaction).**
- **Viral removal can be performed using a 'flow through' or 'bind-elute' mode ionic chromatography process**



Flow through mode with AEX (Anionic Exchange Chromatograhy)

Virus (-) binds into **positively charged resin**, and antibody (+) passes through the column resin



Bind-elute mode with CEX (Cationic Exchange Chromatography).

Antibody (+) binds into **negatively charged resin**, and Virus (-) passes through the column resin.

Antibody is recovered later at elution step (using high salt or higher pH buffer)

NANOFILTRATION FOR VIRAL REMOVAL

Process Design consideration for validation:

- Flow rate
- Pressure
- Temperature
- % flow decay
- Pause and hold time
- Integrity test method

Virosart from Sartorius

Viresolve Pro Solution from Merck Millipore

The Viresolve® Pro Magnus Holder's small footprint and unique design fit into any production facility.

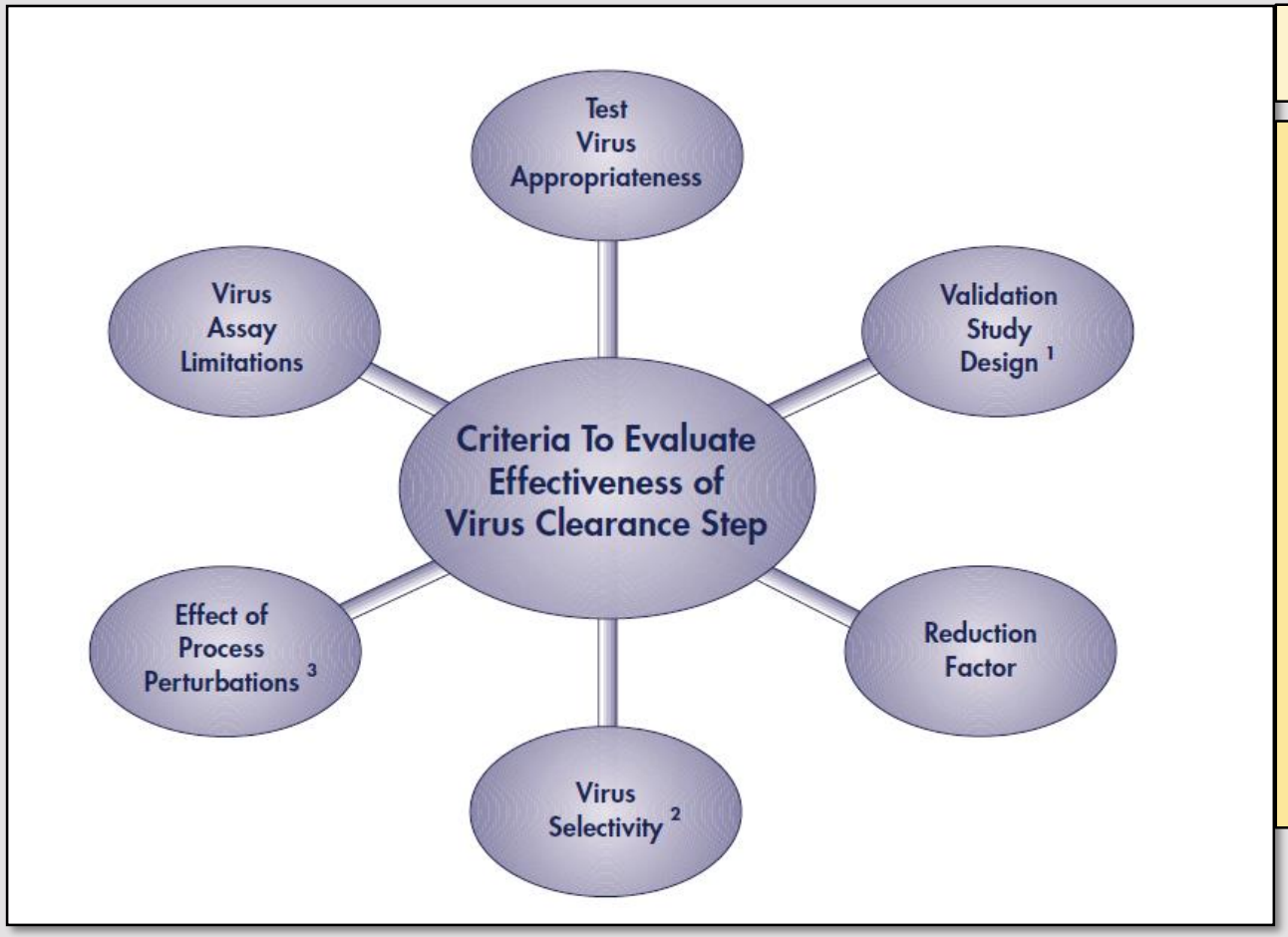
The Mobius® FlexReady Solution for Virus Filtration

Typically using 20nm pore size filter.
Product antibody (~5-10nm) can pass through while larger viral particles will be trapped by filter

SYNOPSIS

- Introduction
- Viral clearance methods
- **Validation of viral removal**

VIRUS STUDY ASSESSMENT CRITERIA

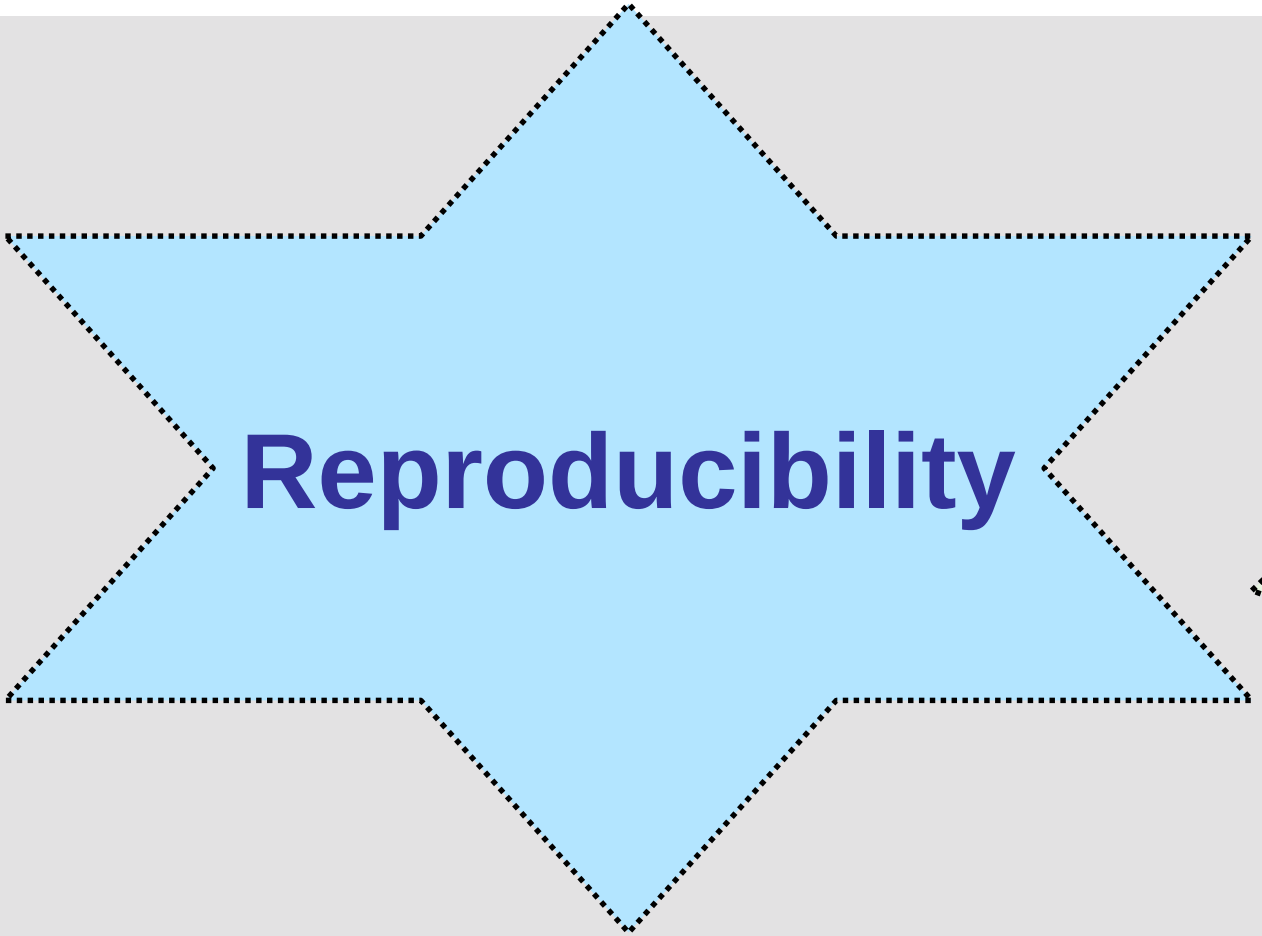


Limitation of Viral validation study

- Viral validation studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved
- But do not by themselves establish safety

Courtesy: Millipore

CRITICAL PARAMETERS FOR VIRAL VALIDATION



Courtesy: Millipore

CRITICAL PARAMETERS FOR VIRAL VALIDATION

Reproducibility is a concern in two areas.

- First, virus assays **"should have adequate sensitivity and reproducibility** and should be performed with sufficient replicates and controls to ensure adequate statistical accuracy of the result.
- Second, in accord with **good scientific practice,** the overall study results should be **reproducible.**
- Accordingly, **"an effective virus removal step should give reproducible** reduction of virus load shown by at least two independent studies.

Courtesy: Millipore

CRITICAL PARAMETERS FOR VIRAL VALIDATION

- **Virus stock preparations** may be characterized for purity, degree of aggregation and titer.
- Because viruses are prepared by **using cell culture methods**, the preparations are inherently susceptible to a **large amount and variety of impurities.**
- **These impurities contribute to filter fouling**
- There is **no specific regulatory guidance or standard industry practice** related to **virus purity characterization or quantitation.**

Courtesy: Millipore

SMALL SCALE VIRAL CLEARANCE VALIDATION STUDY

- Viruses model should be chosen to resemble viruses which may contaminate the product
- To represent a wide range of physico-chemical properties
- In order to test the ability of the system to eliminate viruses

Federal Register Volume 63, Issue 185 (Se 24, 1998)

TABLE A–1.—EXAMPLES OF VIRUSES WHICH HAVE BEEN USED IN VIRAL CLEARANCE STUDIES

Virus	Family	Genus	Natural Host	Genome	Env	Size (nm)	Shape	Resistance[1]
Vesicular Stomatitis Virus	Rhabdo	Vesiculo-virus	Equine Bovine	RNA	yes	70 x 150	Bullet	Low
Parainfluenza Virus	Paramyxo	Paramyxo-virus	Various	RNA	yes	100–200+	Pleo/Spher	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80–110	Spherical	Low
Sindbis Virus	Toga	Alphavirus	Human	RNA	yes	60–70	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50–70	Pleo/Spher	Low
Pseudo-rabies Virus	Herpes		Swine	DNA	yes	120–200	Spherical	Med
Poliovirus Sabin Type 1	Picorna	Entero-virus	Human	RNA	no	25–30	Icosa-hedral	Med
Encephalomyo-carditis Virus (EMC)	Picorna	Cardio-virus	Mouse	RNA	no	25–30	Icosa-hedral	Med
Reovirus 3	Roe	Orthoreo-virus	Various	DNA	no	60–80	Spherical	Med
SV40	Papova	Polyomavirus	Monkey	DNA	no	40–50	Icosa-hedral	Very high
Parvoviruses (canine, porcine)	Parvo	Parvovirus	Canine Porcine	DNA	no	18–24	Icosa-hedral	Very high

[1] Resistance to physico-chemical treatments based on studies of production processes. Resistance is relative to the specific treatment and it is used in the context of the understanding of the biology of the virus and the nature of the manufacturing process. Actual results will vary according to the treatment. These viruses are examples only and their use is not considered mandatory.

ROBUSTNESS OF VIRAL CLEARANCE IS CHARACTERIZED IN QUALIFIED SMALL SCALE STUDIES USING VIRUS MODEL

EXAMPLE OF VIRUS MODEL CHOSEN IN SMALL SCALE STUDY

FOR ILLUSTRATION ONLY

Virus	Genome	Envelope	Family	Approximate Size (nm)	Resistance to Physicochemical Reagents
xMuLV	RNA	Yes	Retroviridae	80 to 110	Low
PrV	DNA	Yes	Herpesviridae	120 to 200	Low
Reo-3	RNA	No	Reoviridae	60 to 80	Medium
MMV	DNA	No	Parvoviridae	18 to 24	High

- Example of a panel comprises 4 representative model viruses having different physicochemical properties, size, and chemical resistance, to demonstrate the robustness of viral clearance capability.
- These model viruses include members from each of the four major classes of virus (enveloped or non-enveloped, containing DNA or RNA)
- Two enveloped viruses, xenotropic murine leukemia virus (xMuLV) and pseudorabies virus (PrV), model the retrovirus-like particles found in CHO cells and herpesvirus, respectively.
- Non-enveloped Reovirus type 3 (Reo-3) has the ability to infect both human and animal cells. Murine minute virus (MMV) is a model rodent virus which can infect CHO cells.

SMALL SCALE VIRAL CLEARANCE STUDY

GE AxiChrom
300 to 1600 mm (Diameter)

SCALE DOWN

GE Hiscale and Tricorn
50 to 5 mm (Diameter)

Virus Spiking Study

- Small scale models need to be qualified to represent process in production scale
- Use of worst case process conditions
- Replicate of testing is required due to inherent variability of viral assay
- Load material are spiked with model viruses and clearance is expressed in Log reduction

$$Log_{10}\ Reduction\ Value = Log_{10}\left(\frac{total\ virus_{start}}{total\ virus_{end}}\right)$$

EXAMPLE OF SMALL SCALE MODEL QUALIFICATION

Maintain critical scale-independent parameters

Unit Operation	Process Parameters	Small scale	Production Scale	Performance Indicators to be evaluated
Chromatography 1	Column diameter (cm)	1	100	Yield, Charge Variants, Monomers, High Molecular Weight species, Fragments, Impurities (DNA, HCP), Chromatogram profile
	Bed height (cm)	25 ± 2	25 ± 2	
	Linear flow rate for load and wash (cm/hr)	≤ 150	≤ 150	
	Product load (g/L resin)	≤ 80	≤ 80	
	Linear flow rate for elution (cm/hr)	≤ 150	≤ 150	
	Start & Stop collection (AU/cm)	0.5	0.5	
	Product recovery wash volume	1 CV	1 CV	
Viral Filtration	Filter Area	0.0003 m2	3.3 m2	Yield, Filtration Profile
	Flow Rate (LMH)	≤170	≤170	
	Target Product Load (L/m2)	≤ 300	≤ 300	
	Product Chase Buffer Volume			

- **Use a scientific-sound statistical method to determine the equivalency of small scale vs production scale**

LOG REDUCTION CALCULATION FROM SMALL SCALE STUDY

PROCESS STEP	xMuLV	PrV	Reo-3	MMV
Viral Inactivation	≥ 6	≥ 6	-	-
Chromatography	≥ 6	≥ 6	≥ 6	≥ 6
Nanofiltration	≥ 4	≥ 4	≥ 4	≥ 4
Total	≥ 16	≥ 16	≥ 10	≥ 10

FOR ILLUSTRATION ONLY (VALUE PROVIDED ARE RANDOMLY GENERATED FOR EDUCATION PURPOSE)

Assuming harvest bulk material (20,000L, 2 g/L protein) containing 1 x 10^8 retro-virus like particle (VLP)/ml. For 50% purification yield and a final dose of 100mg protein, the risk of finding the VLP in final dose:

$$\frac{1 \times 10^8 \text{ VLP/ml} \times 20{,}000\text{L} \times 1{,}000\text{ml/L}}{20{,}000\text{L} \times 2\text{ g/L} \times 50\% \times 1{,}000\text{mg/1g} \times 1\text{ dose/100 mg}} = 1 \times 10^{10} \text{ VLP/dose}$$

Risk WITHOUT viral clearance process

≥ 16 LOG REDUCTION OF VIRAL CLEARANCE STEPS

“Probability to find ≤ 1 Retro-virus particle in 1 million doses”

$\leq 1 \times 10^{-6}$ VLP/dose

Risk POST viral clearance process

FACTORS AFFECTING VIRAL VALIDATION (1)

- Virus preparations for clearance studies are produced in tissue culture.
- The behaviour of a tissue culture virus is different from that of the native virus
- For example, native and cultured viruses differ in purity or degree of aggregation

The Cutter Incident: The worst pharma disasters in US history
Some lots of cutter inactivated-virus polio vaccine container live polio virus – Attributed to incomplete inactivation due to virus aggregation issue

FACTORS AFFECTING VIRAL VALIDATION (2)

- It is possible that virus escaping a first inactivation step may be more resistant to subsequent steps.
  - For example, if the resistant fraction takes the form of virus aggregates, infectivity may be resistant to a range of different chemical treatments and to heating.

FACTORS AFFECTING VIRAL VALIDATION (3)

- The ability of the overall process to remove infectivity is expressed as the sum of the logarithm of the reductions at each step.
- The summation of the reduction factors of multiple steps with little reduction (e.g., below 1 $\log^{10}$), may overestimate the true potential for virus elimination.
- Reduction values achieved by repetition of identical or near identical procedures should not be included unless justified.

FACTORS AFFECTING VIRAL VALIDATION (4)

- The expression of reduction factors as logarithmic reductions in titer implies that, while residual virus infectivity may be greatly reduced, it will never be reduced to zero.
- For example, a reduction in the infectivity of a preparation containing 8 $\log^{10}$ infectious units per ml by a factor of 8 $\log^{10}$ leaves zero $\log^{10}$ per ml or one infectious unit per ml, taking into consideration the limit of detection of the assay.

FACTORS AFFECTING VIRAL VALIDATION (5)

- Pilot-plant scale processing may differ from commercial-scale processing despite care taken to design the scaled-down process.
- Addition of individual virus reduction factors resulting from similar inactivation mechanisms along the manufacturing process may overestimate overall viral clearance.

APPLICATION OF STATISTICS

- The viral clearance studies should include the use of statistical analysis of the data
- The study results should be statistically valid to support the conclusions reached
- The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

TAKE HOME MESSAGE

- Virus titrations suffer the problems of variation
- Reliability of a study depends on
  - Assessment of accuracy of the virus titrations
  - reduction factors derived
  - validity of the assays

FURTHER READINGS

- ICH Q5A (1999)
- Federal Register Volume 63, Issue 185 (Sep 24, 1998)
- EMA MEA/CHMP/BWP/398498/2005 (2006)
- WHO Technical Report, Series No. 924, Annex 4 (2004)
- Pharmaceutical Technology, June, 26-41, 2001
- *Fundamental Strategies for Viral Clearance Part 2: Technical Approaches*, Bioprocess International, 2015
- *https://www.slideshare.net/MilliporeSigma/viral-risk-mitigation-strategies-key-considerations-in-the-prevention-and-detection-of-viral-contamination-79898083*

ACKNOWLEDGMENT

- Dr James Weidner, Amgen Singapore Manufacturing
- Dr Yusdy Pan, Amgen Singapore Manufacturing

THANK YOU FOR YOUR ATTENTION

BACKUP SLIDES

APPLICATION OF STATISTICS (1)

- Assay methods may be either quantal or quantitative.
- Quantal methods include infectivity assays in animals or in tissue-culture-infectious-dose (TCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected.
- In quantitative methods, the infectivity measured varies continuously with the virus input.
- Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit.
- Both quantal and quantitative assays are amenable to statistical evaluation.

APPLICATION OF STATISTICS (2)

- Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control.
- These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

APPLICATION OF STATISTICS (3)

- The 95% confidence limits for results of within-assay variation normally should be on the order of $\pm 0.5 \log^{10}$ of the mean.
- Within-assay variation can be assessed by standard textbook methods.
- Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately $0.5 \log^{10}$ of the mean estimate established in the laboratory for the assay to be acceptable.
- Assays with lower precision may be acceptable with appropriate justification.

APPLICATION OF STATISTICS (4)

- The 95% confidence limits for the reduction factor observed should be calculated wherever possible in studies of clearance of “relevant” and specific “model” viruses.
- If the 95% confidence limits for the viral assays of the starting material are +s, and for the viral assays of the material after the step are +a, the 95% confidence limits for the reduction factor are:

$$\pm\sqrt{S^2 + a^2}\ 1.$$

APPLICATION OF STATISTICS (5)

Probability of Detection of Viruses at Low Concentrations

At low virus concentrations (e.g., in the range of 10 to 1,000 infectious particles per liter) it is evident that a sample of a few milliliters may or may not contain infectious particles. The probability, p, that this sample does not contain infectious viruses is:

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

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

If $V >> v$, this equation can be approximated by the Poisson distribution:

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

where c is the concentration of infectious particles per liter.

APPLICATION OF STATISTICS (6)

or, $c = \ln p / -v$

As an example, if a sample volume of 1 ml is tested, the probabilities p at virus concentrations ranging from 10 to 1,000 infectious particles per liter are:

c	10	100	1000
p	0.99	0.90	0.37

This indicates that for a concentration of 1,000 viruses per liter, in 37% of sampling, 1 ml will not contain a virus particle.

If only a portion of a sample is tested for virus and the test is negative, the amount of virus which would have to be present in the total sample in order to achieve a positive result should be calculated and this value taken into account when calculating a reduction factor. Confidence limits at 95% are desirable. However, in some instances, this may not be practical due to material limitations.

CALCULATION OF REDUCTION FACTORS IN VIRAL VALIDATION

Calculation of Reduction Factors in Studies to Determine Viral Clearance

The virus reduction factor of an individual purification or inactivation step is defined as the $\log_{10}$ of the ratio of the virus load in the pre-purification material and the virus load in the post-purification material which is ready for use in the next step of the process. If the following abbreviations are used:

Starting material:
vol v'; titer $10^{a'}$;
virus load: $(v')(10^{a'})$,

Final material:
vol v''; titer $10^{a''}$;
virus load: $(v'')(10^{a''})$,

the individual reduction factors Ri are calculated according to
$10^{Ri} = (v')(10^{a'}) / (v'')(10^{a''})$

This formula takes into account both the titers and volumes of the materials before and after the purification step.

Because of the inherent imprecision of some virus titrations, an individual reduction factor used for the calculation of an overall reduction factor should be greater than 1.

The overall reduction factor for a complete production process is the sum logarithm of the reduction factors of the individual steps. It represents the logarithm of the ratio of the virus load at the beginning of the first process clearance step and at the end of the last process clearance step. Reduction factors are normally expressed on a logarithmic scale which implies that, while residual virus infectivity will never be reduced to zero, it may be greatly reduced mathematically.