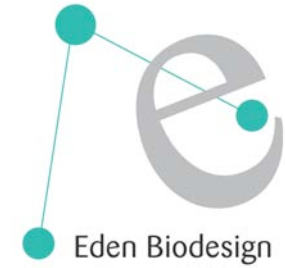


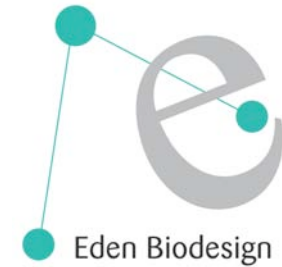
Undertaking Effective and Compliant Validation of Virus Removal

Validation of Virus Safety



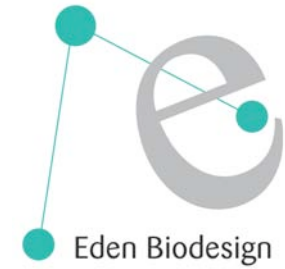
- Technical and Regulatory Implications
- Viral Safety Strategy
- Selection of Methods for Viral Removal/Inactivation
- Validation of Viral Removal/Inactivation

History



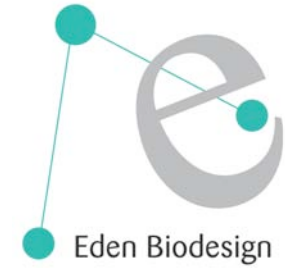
- Historic record of accidents related to viral contamination
 - Incomplete viral inactivation in vaccines
 - Polio (1955), Rabies (1960), Foot and mouth (1981)
 - Endogenous viral contaminants from cell lines
 - Polio infected with SV40 (1975), Yellow fever infected with ALV(1967)
 - Contaminants from Excipients/Stabilisers
 - HBV (1967) and parvovirus B19 (1997) contamination of HAS
 - Infected Source materials or inadequate viral clearance

Regulatory Implications



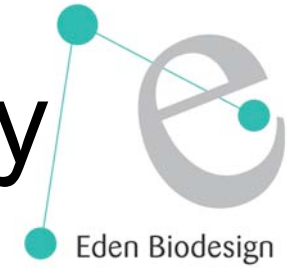
- Delayed Initiation of clinical trials
- Clinical Hold
- Refusal to file
- Long list of questions after filing
- Refusal of license application

Technical Implications



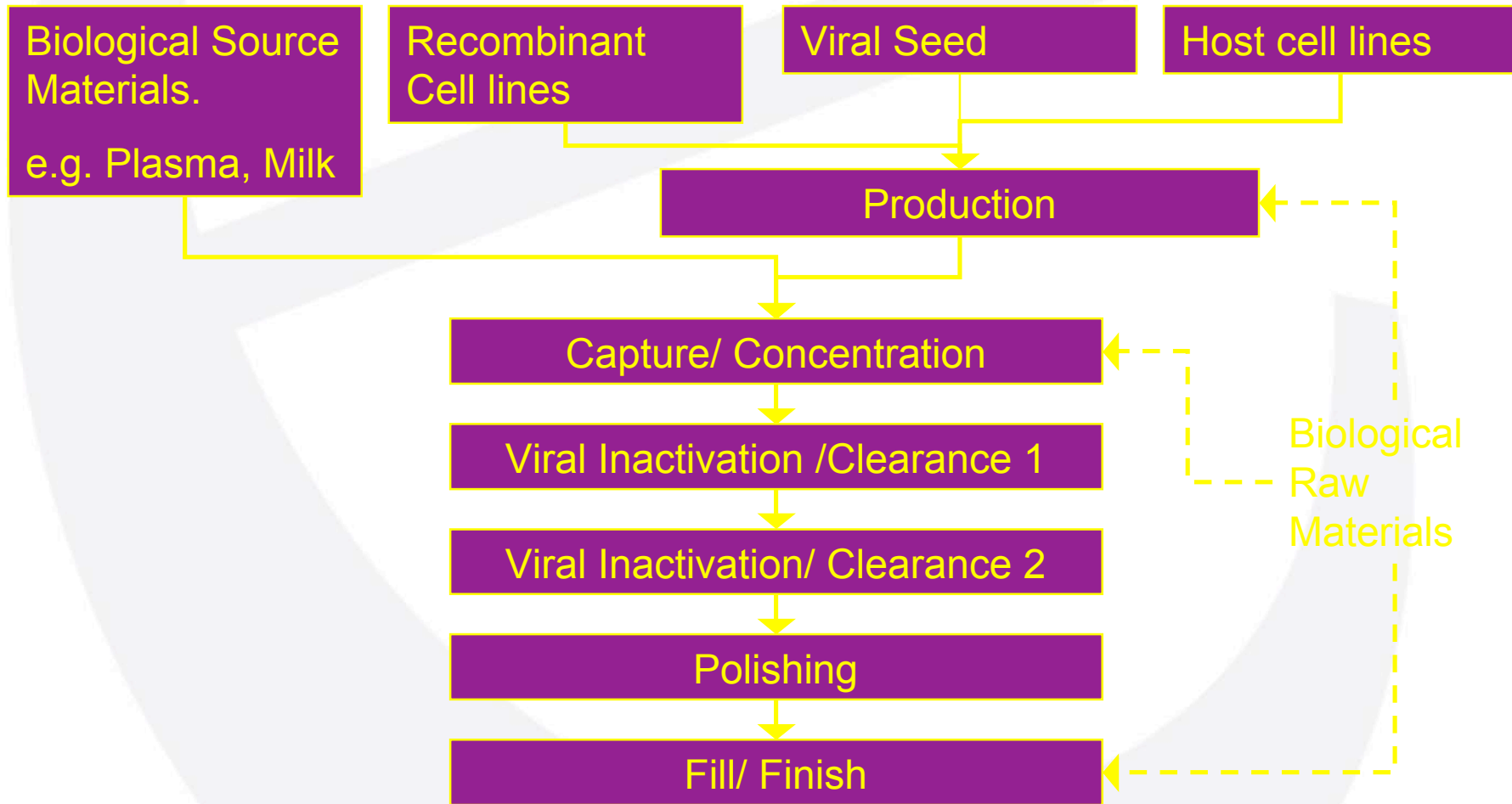
- Time consuming and costly
- Removal processes are product dependent
- Cleaning and sanitisation of equipment
- Where will validation be performed
- May require a number of batches of material.

Validation of Virus Safety



- Technical and Regulatory Implications
 - Viral Safety Strategy
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Strategy - Viral Safety

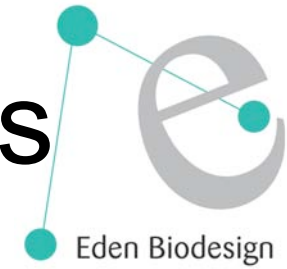


Potential Sources of Viral Contamination



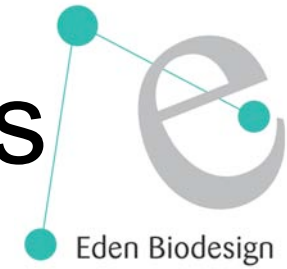
- Source materials of human or animal origin
- Production Cell line
 - Endogenous or adventitious virus
 - Culture using human or animal raw materials
- Process related
 - Fermentation raw materials from human or animal origin (media, serum etc)
 - Purification raw materials from human or animal origin (affinity matrices)
- GMP Failure
 - Operator
 - Equipment

Strategy – Source materials



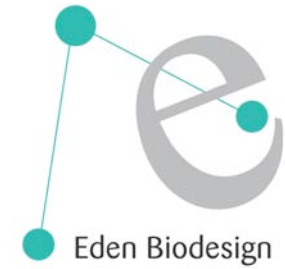
- Full relevant testing should be performed on all starting material.
 - Cell Banks – recombinant and host
 - Human or animal biological – e.g plasma, milk
 - Viral Seeds

Strategy – Raw materials



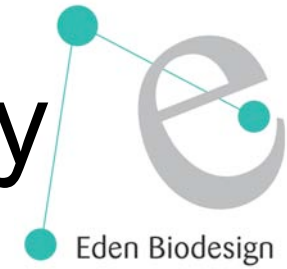
- Avoid using human and animal raw materials where possible.
- Full relevant testing and validation of viral clearance (if appropriate) should be performed on all Biological raw materials in production
 - Serum
 - Culture Media
 - Affinity Matrices
- Certification

Strategy - process



- Screen End of Production Cells for Endogenous and Adventitious Viruses
- Screen Purification Starting Materials for Endogenous and Adventitious Viruses
- Perform Validation of Virus Clearance/Inactivation

Validation of Virus Safety



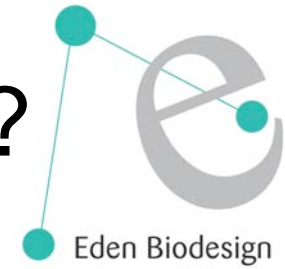
- Technical and Regulatory Implications
- Viral Safety Strategy
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Method Selection - Process Considerations



- Effective clearance methods should be introduced early in development.
 - At least 1 and preferably 2 effective clearance steps
 - Maintain product integrity
 - Reliable and Robust
 - Ease of Validation (viral clearance and process)
 - Ease of scale up/ scale down
 - Ease of engineering

How much clearance is required?

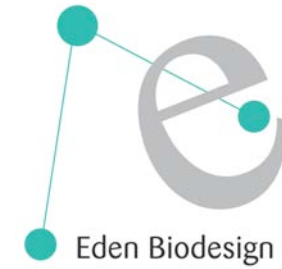


Aim for 1 viral particle per million doses (1×10^{-6})

Example

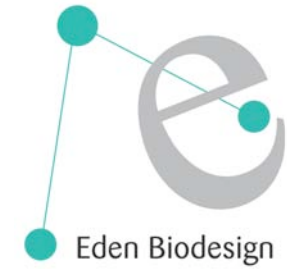
- Bulk Harvest 2,000 L per batch
- 20,000 doses per batch
- 100 mL bulk harvest per dose
- 1×10^6 Viral particles/mL (by EM or similar)
- 1×10^8 particles per dose
- Clearance required = 10^{14}

Method Selection -Inactivation



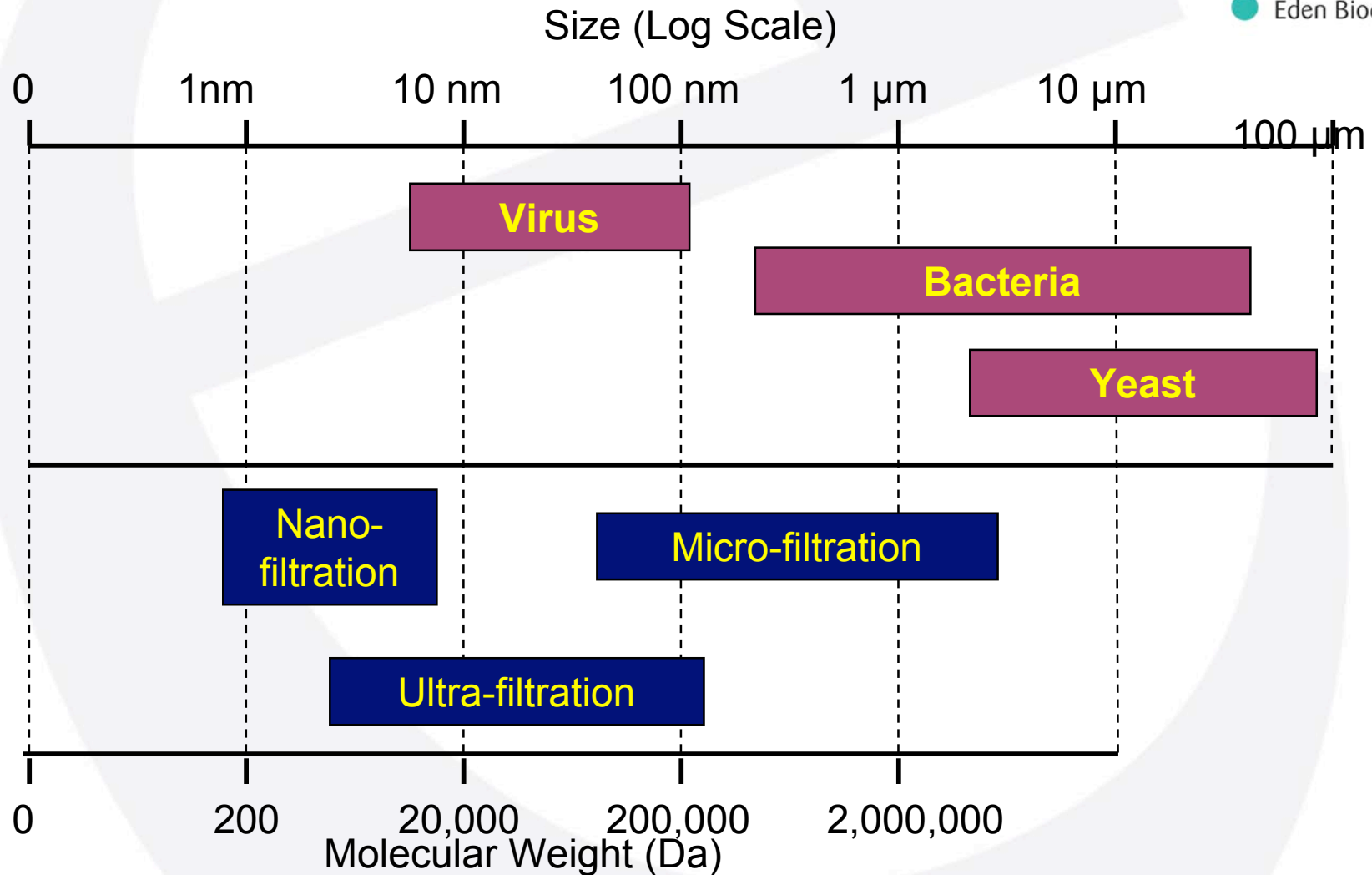
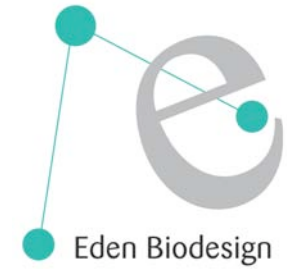
- Preferred option
 - Inactivation is generally irreversible
 - More robust
 - Easier to validate
- Heating
 - Pasteurisation
 - Dry Heat
- Chemical
 - Solvent/detergent
 - Ethanol/Methanol
 - Low pH

Clearance Methods



- Can have issues with cleaning and sanitisation of equipment and robustness
- Partitioning
 - Precipitation
 - Chromatography
 - Ultracentrifugation
- Filtration
 - Ultrafiltration
 - Microfiltration
 - Nanofiltration

Filtration

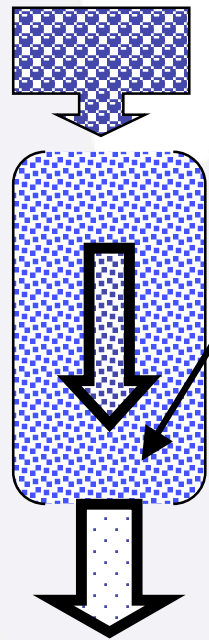


Filtration - Mechanism

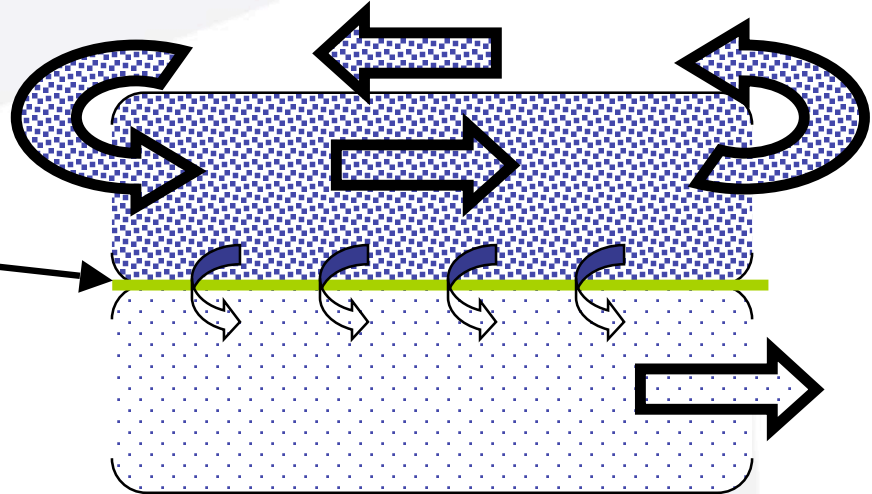


- Can be used for viral clearance in many protein solutions, not suitable for particulate products.
- Combination of size exclusion and adsorptive retention.
- Size exclusion is method of choice
 - Less dependant on product or process conditions
 - More robust
- Type of filtration dependant on membrane

Filtration - Modes

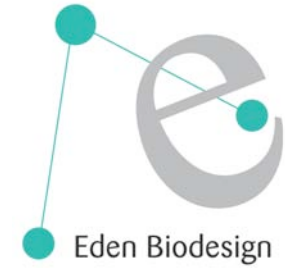


Direct Flow
Filtration (DFF)



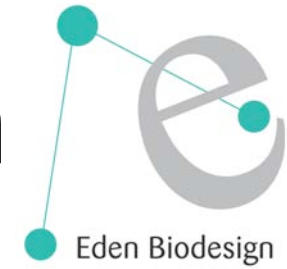
Tangential (Cross)
flow filtration (TFF)

Direct Flow filtration



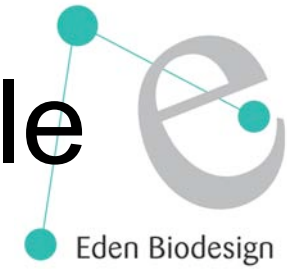
- Advantages
 - Ease and speed of use
 - Low shear /High product recovery
 - Ease of scale down
 - Single use – no cleaning or cleaning validation
 - No retentate
- Disadvantages
 - Requires relatively 'clean' process materials

Tangential flow filtration



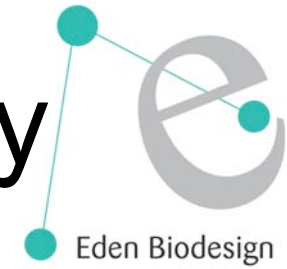
- Advantages
 - Can handle a higher particulate load than DFF
 - Relatively easy to scale-down
 - Theoretically re-useable
- Disadvantages
 - Cleaning and sanitisation is difficult
 - Equipment requires CIP and validation
 - Complex validation issues associated with membrane reuse (viral removal)

Other techniques available



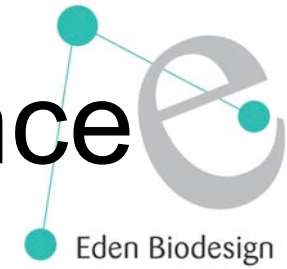
- Microwave technology – High temperature short time.
- UVC Irradiation – has been shown to inactivate parvovirus.
- BPL and Imines – widely used in veterinary products.

Validation of Virus Safety



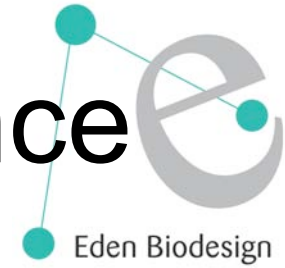
- Technical and Regulatory Implications
- Viral Safety Strategy
- Selection of Methods for Viral Removal/Inactivation
- Validation of Viral Removal/Inactivation

Validation of Viral Clearance



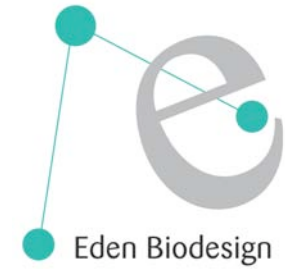
- Determine the capability of the production process to reliably clear potential contaminants using a range of model viruses.

Validation of Viral Clearance



- Viral Burden of purification starting materials
- Scale down Model
- Choice of Viruses
- Study design
 - Cytotoxicity testing
 - Viral Spike
 - Test Samples
- Analytical techniques
 - Infectivity assays
 - QPCR

Viral Burden



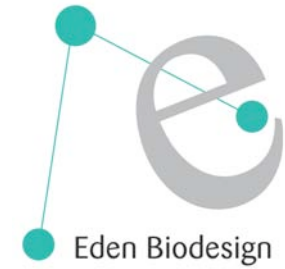
- Performed on post fermentation or biological source material
- Quantitative determination of known or potential viruses.
 - Infectivity assays
 - Electron microscopy (retroviral particles)
 - QPCR

Scale – down model



- Accurate scale down of process materials and equipment.
- Qualify against full scale batches
- Ensure product and impurity profile is comparable to full scale
- Set specifications for scale-down model
- Use ‘worst case scenario’ in viral clearance evaluation e.g. Lowest temperature, High contaminant profile etc

Choice of Viruses



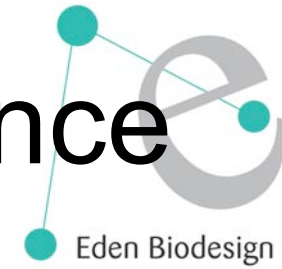
- Panel of viruses with different physioco-chemical properties.
 - Size
 - Ss/ds RNA/DNA
 - Enveloped/non-enveloped
 - Chemical resistance
- Relevant to the cell line and raw materials
- Number of viruses dependant on clinical trial phase

Choice of Viruses (ICH)



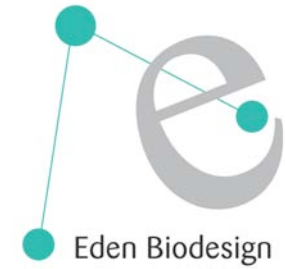
Virus	Family	Genus	Host	Genome	Env	Size (nm)	Shape	Resist
Vesicular stomatis virus	Rhabdo	Vesiculovirus	Equine/ Bovine	RNA	Yes	70 x 175	Bullet	Low
Parainfluenza virus	Paramyxo	Paramyxovirus	Various	RNA	Yes	100-200	Pleo/Spher	Low
Human immuno deficiencyvirus	Retro	Lentivirus	Man	RNA	Yes	80-100	Spherical	Low
Murine Leukaemia virus (MuLV)	Retro	Type C oncovirus	Mouse	RNA	Yes	80-110	Spherical	Low
Sinbis virus	Toga	Alphavirus	Man?	RNA	Yes	60-70	Spherical	Low
Bovine diarrhoeal virus (BVDV)	Toga	Pestivirus	Bovine	RNA	Yes	50-70	Pleo/Spher	Low
Pseudorabies virus	Herpes	Varicellovirinae	Swine	DNA	Yes	120-200	Spherical	Med
Poliovirus Type 1	Picornia	Enterovirus	Man	RNA	No	25-30	Icosahedral	Med
Encephalomyocarditis virus (EMC)	Picornia	Cardiovirus	Mouse	RNA	No	25-30	Icosahedral	Med
Reovirus 3	Reo	Orthoreovirus	Various	RNA	No	60-80	Spherical	Med
Hepatitis A	Picornia	Hepatovirus	Man	RNA	No	25-30	Icosahedral	High
SV40	Papova	Polyomavirus	Monkey	DNA	No	40-50	Icosahedral	V. High
Parvoviruses (Canine/Bovine)	Parvo	Parvovirus	Canine Bovine	DNA	No	18-24	Icosahedral	V. High
Minute virus of Mouse (MVM)	Parvo	Parvovirus	Mouse	DNA	No	18-24	Icosahedral	V.High

Cyto-toxicity/Viral interference



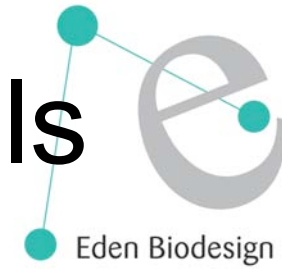
- Test starting materials, buffers and inactivating agents for toxic effects on the virus and indicator cell lines.
- The more toxic the material the higher the dilution required to demonstrate the viral titre pre and post clearance, resulting in a low calculated viral reduction
- Treatment of the sample pre titration

Viral Spike



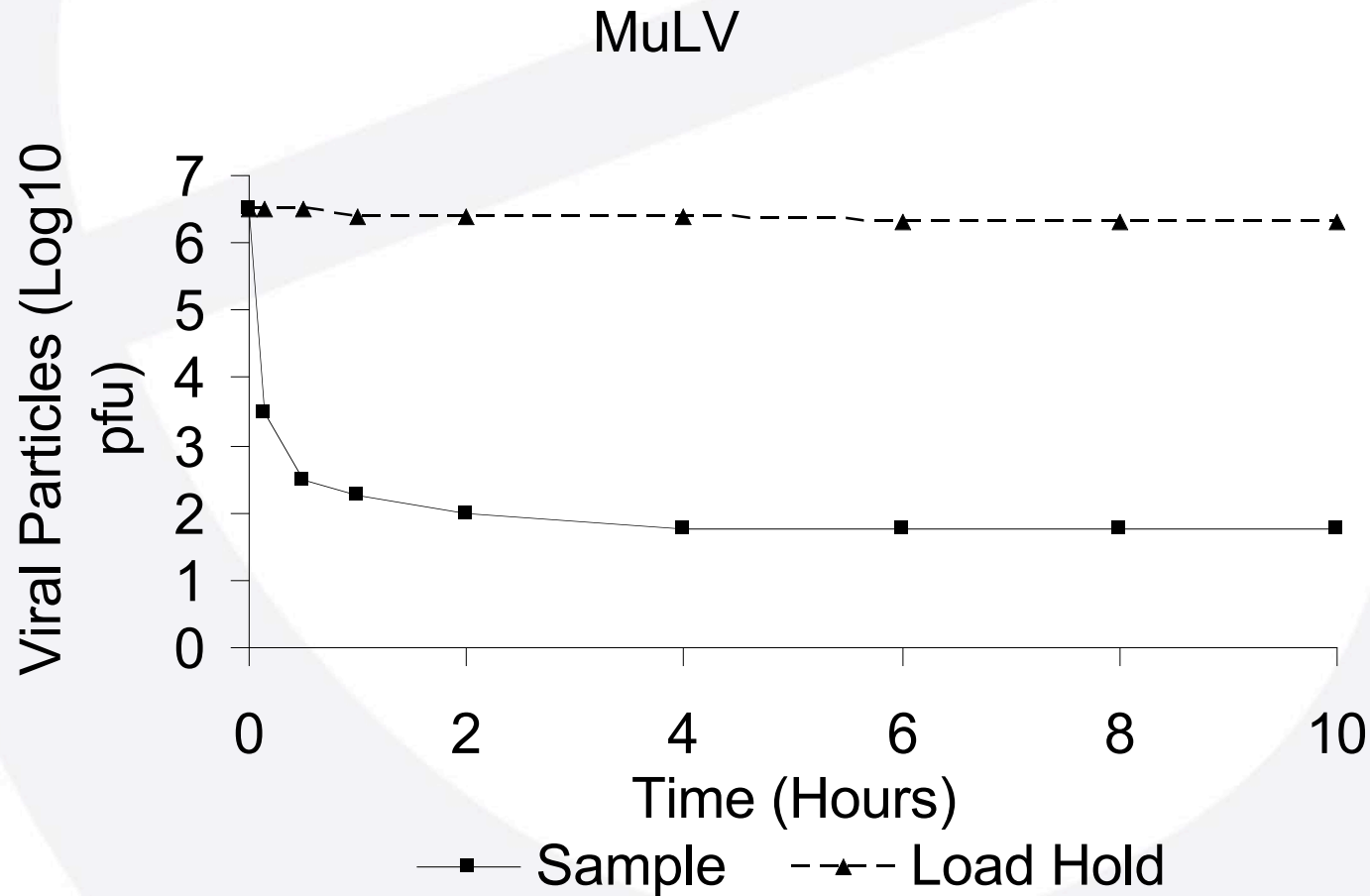
- High Viral Titre
- Ensure no aggregation
- Volume generally 5-10% of the starting material

Test Samples and controls

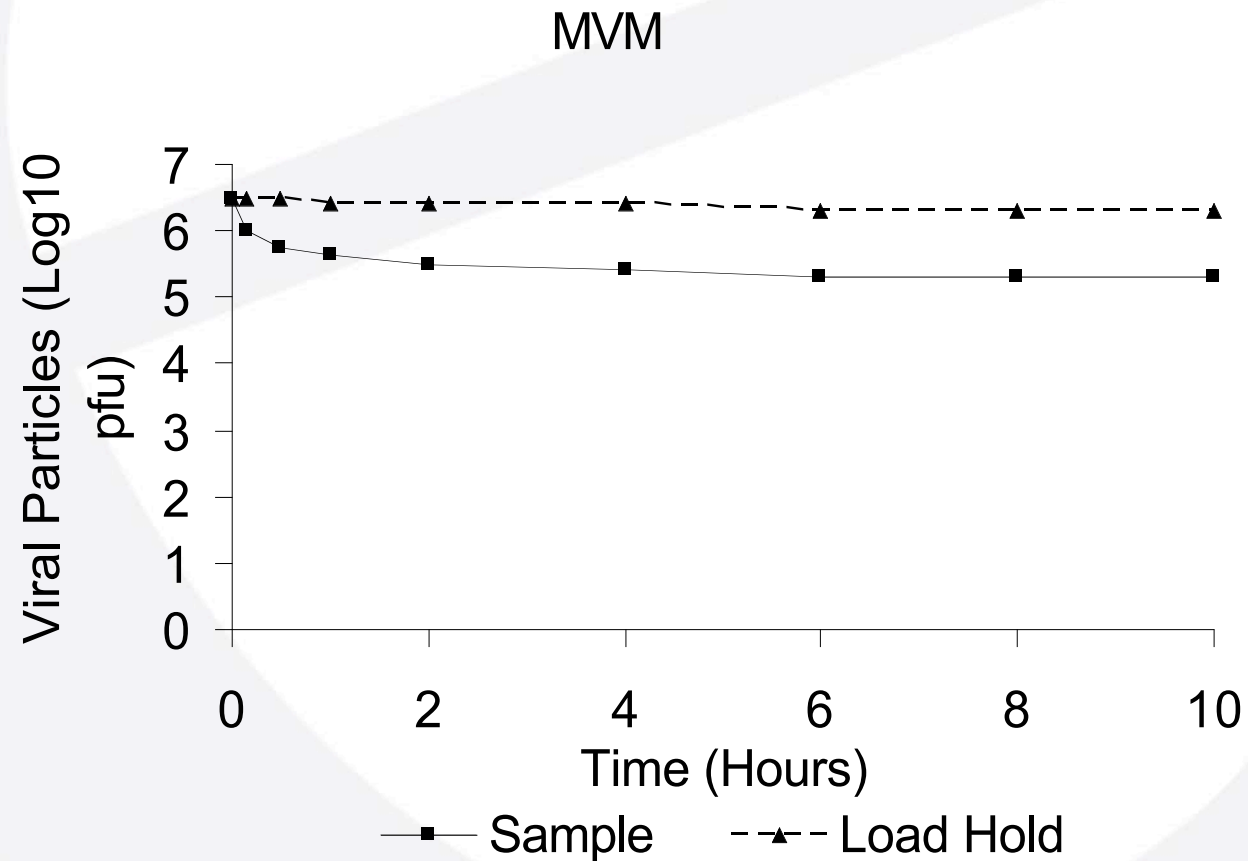
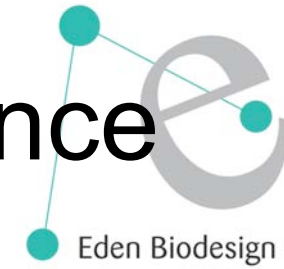


- Inactivation
 - Start (T=0)
 - Timepoints (to determine inactivation kinetics)
 - Endpoint
 - Virus media control (to determine virus stability)
 - Load hold control (to determine virus stability in presence of test materials)

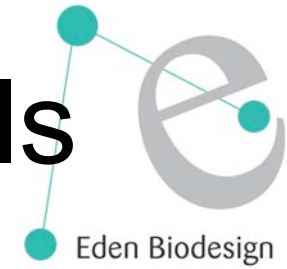
Pasteurisation – Low resistance



Pasteurisation – High resistance

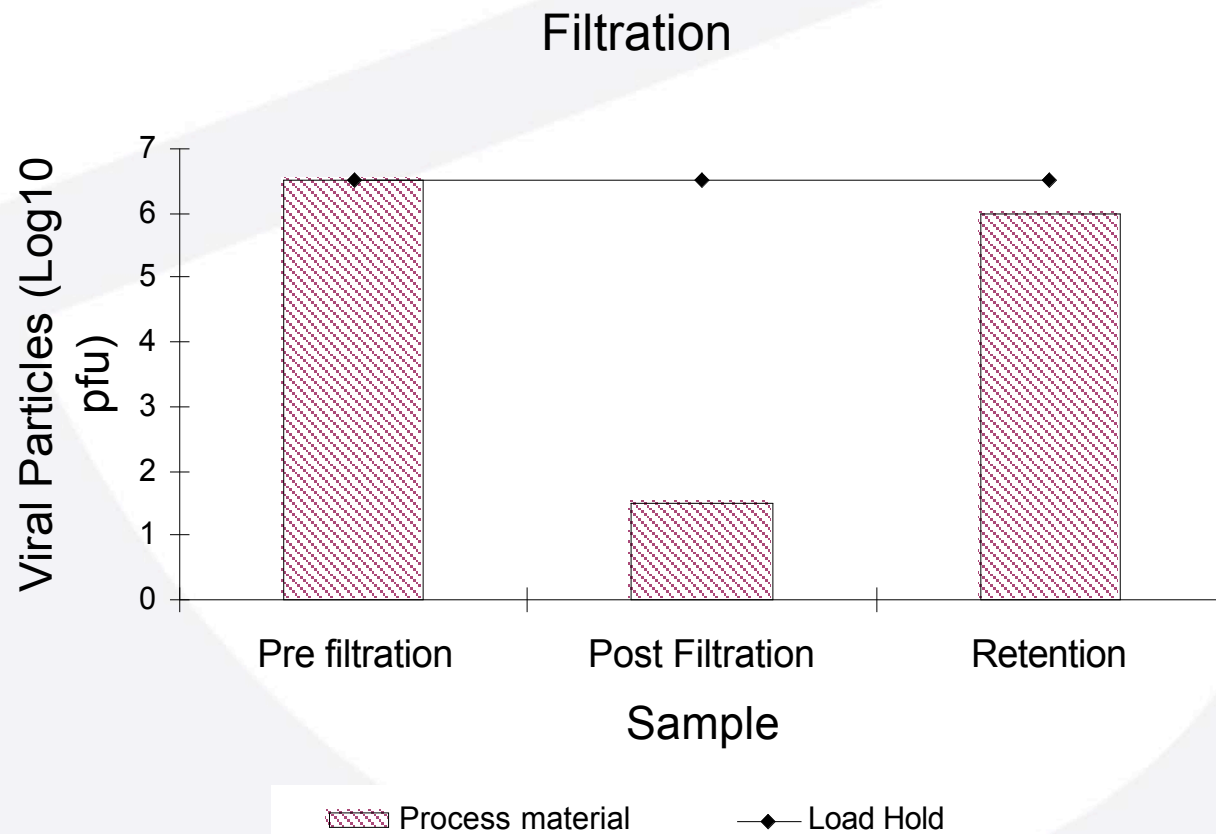
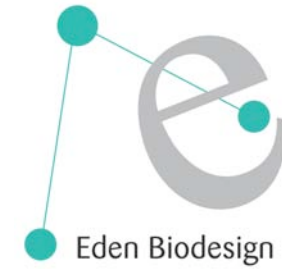


Test samples and controls

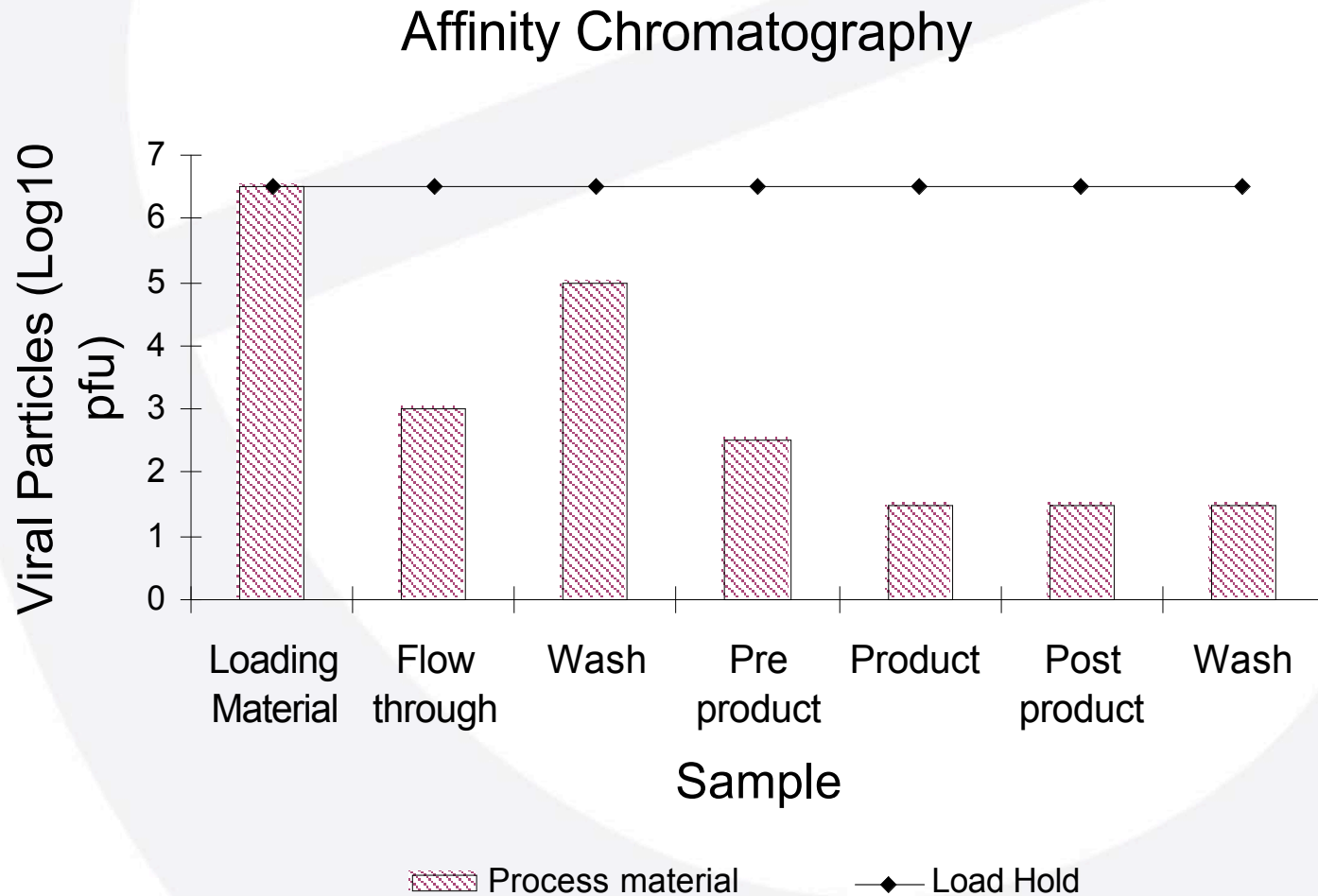
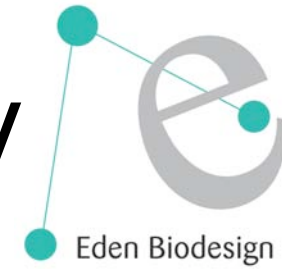


- Clearance
 - Pre Clearance step
 - Fractions or eluates (to determine to determine effective partitioning)
 - Post Clearance step
 - Virus media control (to determine virus stability)
 - Load hold control (to determine virus stability in presence of test materials)

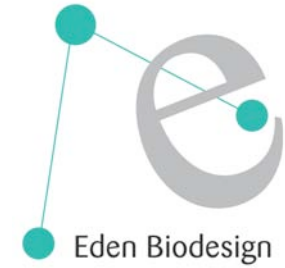
Filtration



Affinity Chromatography

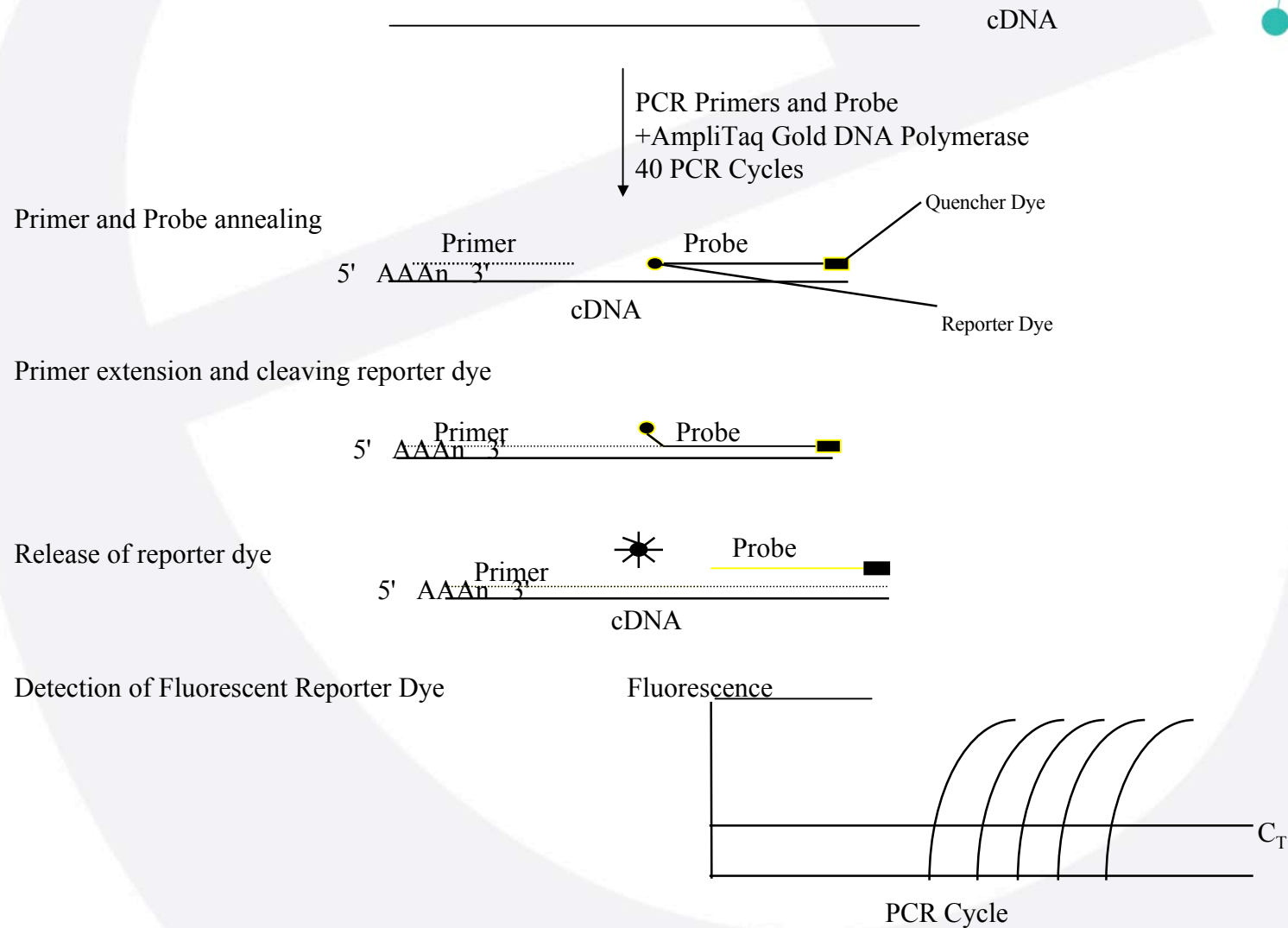


Analytical Techniques

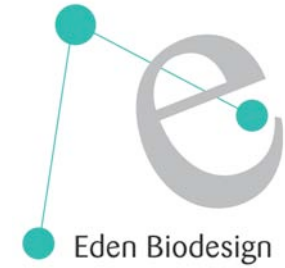


- VALIDATED
- Infectivity Assays
 - Plaque
 - TCID₅₀
 - Can be used for both Inactivation and Clearance steps
 - Cannot be used to determine mass balance
- QPCR
 - Can only be used to estimate virus removal
 - Low limits of detection
 - Shorter time and more robust
 - Can be used to determine mass balance

QPCR



Summary



- Form Viral safety strategy early in development
- Include specific viral removal steps
- Look at all possible removal techniques bearing in mind the commercial process
- Ensure appropriate scale down and analysis of validation samples
- Ensure mechanism for viral removal is known