A Risk Based Approach to the Management of Virus Risk in a cGMP Manufacturing Facility

Anne Stokes (PhD) GSK Senior Fellow TSE and Virus Control GlaxoSmithKline

Objectives	Outline the control framework for adventitious or endogenous viruses, novel microbial agents and transmissible spongiform encephalopathies (TSE, or prion diseases) in the manufacturing process for Biopharmaceuticals and the tools and strategies for their detection, characterization and control.

Introduction

Control Framework for Virus, TSE and Novel Microbial Agents

- Why Virus, TSE and novel microbial agent control is Important during the manufacture of Biopharmaceuticals
- Control Framework in the Clinical Manufacture of Biopharmaceuticals in a Multi-Product Facility
  - Vendor Management and control
  - Transport, distribution and storage of raw materials and consumables
  - Removal and substitution of Animal Derived Raw Materials from manufacturing processes
  - Governance, Quality, Compliance and Certification, segregation and disinfectant procedures
- Virus Safety Testing of Cell Banks and Production Samples using standard methods
- Novel Molecular Methods for Virus Detection
- Overview of Manufacturing Process and Single Use Bioreactors versus stainless steel
- Virus clearance validation requirements in Biopharmaceutical manufacturing processes
- Conclusions

Why Virus Control is Important during the Manufacture of Biopharmaceuticals

Implications of virus/bacteria Contamination to the Pharmaceutical Industry

- [1]Genentech Contamination with Mice Minute Virus (MVM)
  - Facility clean down and decontamination of plant (cost and time)
  - Testing for MVM
- [2]Genzyme Contamination with Vesivirus
  - Facility clean down (significant down time) running to $M losses and supply chain interruption
- [3]Genentech contamination with Leptospira
  - Agency Notification
  - Root cause analysis
  - Industry response

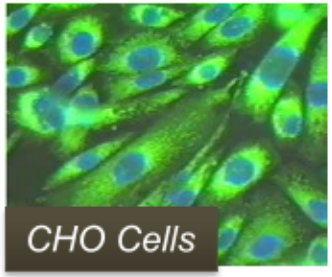
1 Garnick R.L. Raw Materials as a Source of Contamination Dev. Bio. Stand 1998 ; 93: 21-29

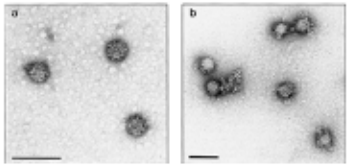
2 Genzyme Press Release: Genzyme Reports Progress Related to Allston Plant June 2009

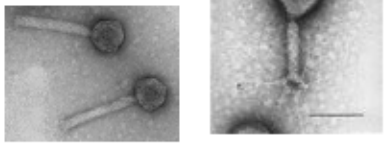
3 Vinther Anders (2012) Novel Contamination Investigation in Upstream Drug Substance Manufacturing Facility. PDA Annual Meeting in Phoenix Arizona 16th to 20th April 2012.

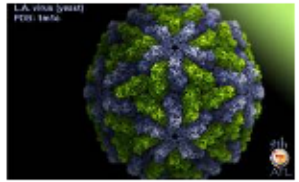
Biopharmaceuticals are Produced By Microbial or Mammalian Cells which are Host to Virus or other Microbial Agents

- CHO cells are host to mammalian viruses and microbial agents
- E.coli are host to bacteriophage and can be contaminated with other agents
- Yeast are host to retroviral like elements and others including L-A virus









Control Framework in the Clinical Manufacture of Biopharmaceuticals

Raw Materials and Components transport to Manufacturing Facility

- Vendor audit and approval procedures
- Chemically defined media
- Gamma irradiation of components
- Control of transportation
- Certification

Receipt of Materials at Manufacturing Facility

- Warehouse Receipt Procedures
- Warehouse monitoring
- Pest Control program
- Cleaning Program

Movement of Materials and Equipment into Manufacturing Facility

- Spray/clean down Procedures
- Materials, personnel and equipment flow
- Pest Control/cleaning program
- Disinfectant and operator health policy
- Segregation and iso classification

Buffer, media, cell bank and manufacture and Filling

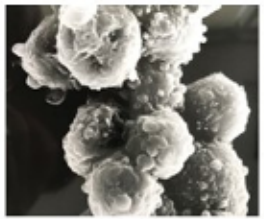
- Media and Buffer filtration (0.1/0.2um)
- Cell Bank and EOP testing
- Virus Clearance validation/segregation
- Drug Substance purification
- Resin usage and storage
- Facility de-contamination procedure

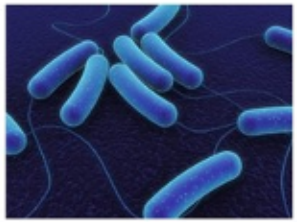
cGMP Manufacturing of Biopharmaceuticals

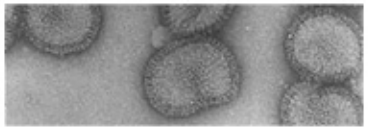
- ICH, FDA and JP Guidelines
- GMP Philosophy and Facility reviews
- Quality Risk Management and Compliance
- QA Oversight

- **Biopharmaceuticals**
  - Recombinant proteins
  - Monoclonal antibodies
  - DNA vaccines

- Host Production System is susceptible to infection with Virus/bacteriophage



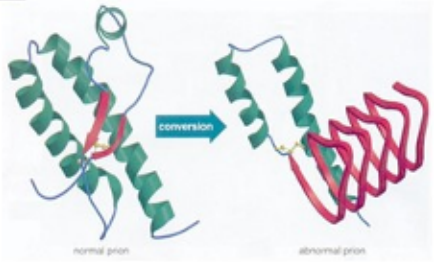




EM Influenza virus

EM Bacteriophage

- **Virus risk is addressed according to ICH Q5A**
  - Selecting and testing cell lines and other raw materials, including media components for the absence of undesirable viruses which may be infectious and/or pathogenic for humans ✓
  - Assessing the capacity of the production processes to clear infectious viruses ✓
  - Testing the product at appropriate steps of production for absence of contaminating infectious viruses ✓
- **Facility and raw material risk assessment**
  - Vesivirus-Genzyme and MVM Genentech
  - Upstream and downstream processing



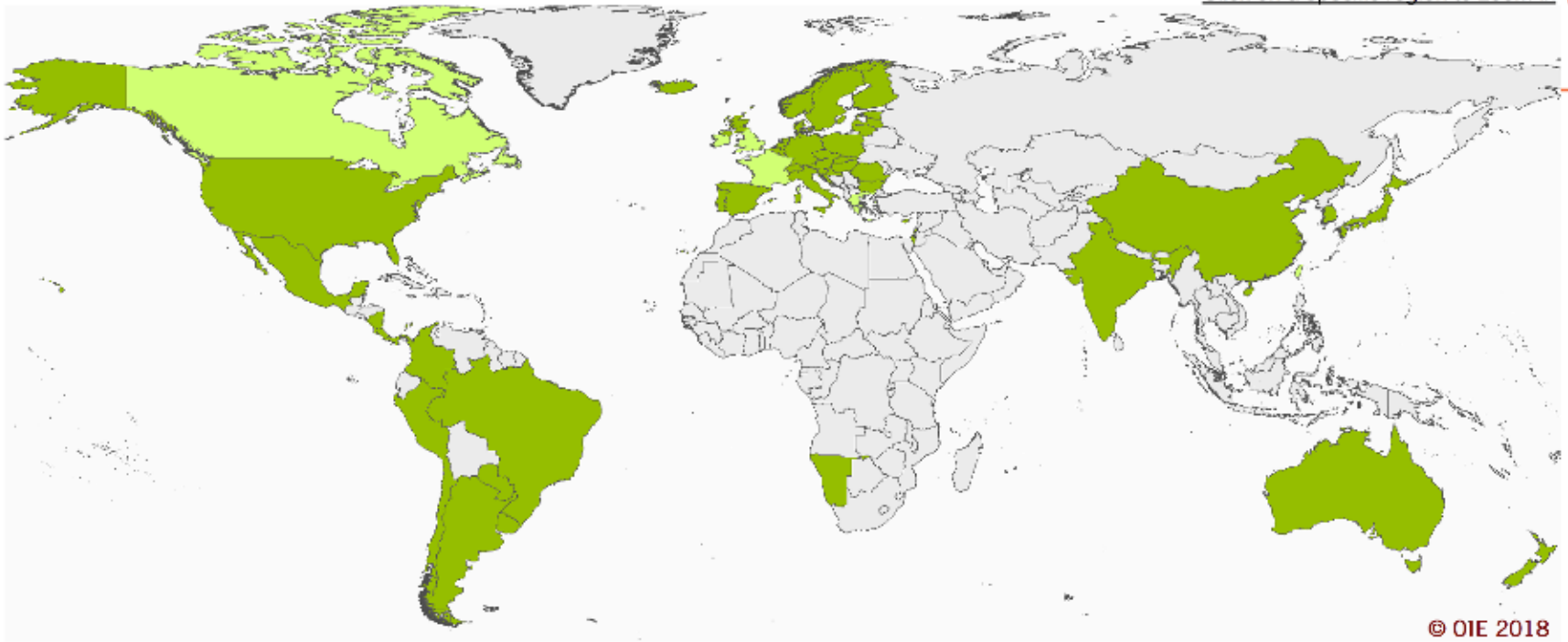
Transmissible Spongiform Encephalopathy (Note for Guidance on Minimising the risk of TSE).

- Chronic degenerative nervous diseases due to an abnormal isoform of a cellular glycoprotein (known as PrP or prion protein) (PrPSc) that is infectious in the absence of nucleic acid.
- Poses a risk to the Pharmaceutical Industry via the potential use of contaminated raw materials
- Cholesterol in media-Sheep wool lanolin (risk from ruminant materials)

OIE Members' official BSE risk status map

Last update May 2018

Click on a specific region to zoom in



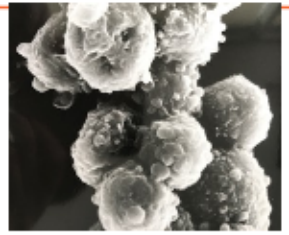
Members and zones recognised as having a negligible BSE risk status

Members and zones recognised as having a controlled BSE risk status

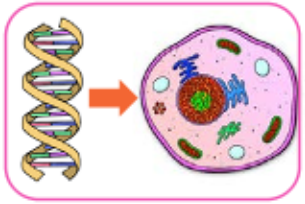
Countries and zone without an OIE official BSE risk status

Production of Cell Banks

CHO Platform

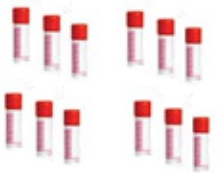


CHO Host Cell Line transfected with DNA construct



Clone Selection

Expansion and scale up



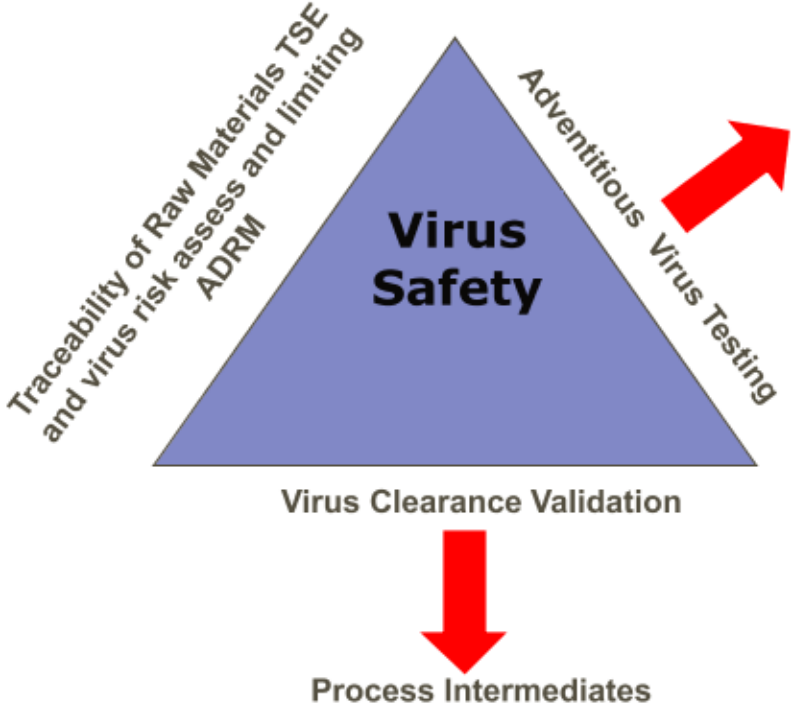
Pre-Master Cell Bank

Expansion

GMP Master and Working Cell Bank Production

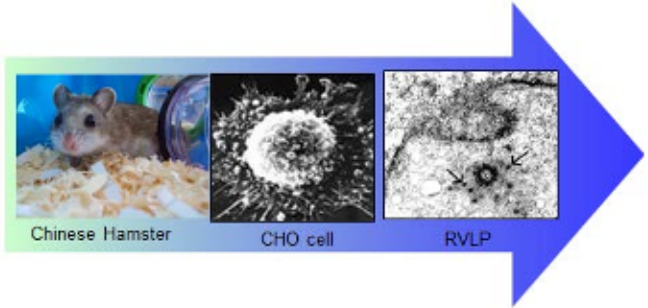
Split Storage in Liquid Nitrogen for Life of Product-ICH Testing

ICH Q5A The 3-pronged Approach to virus safety of Biopharmaceuticals (a holistic approach)



- Cell Banks
- Bulk Harvest
- IVCA testing

CHO Endogenous Viruses



Monoclonal Antibody Production

Virus Safety of Cell Banks (starting material)

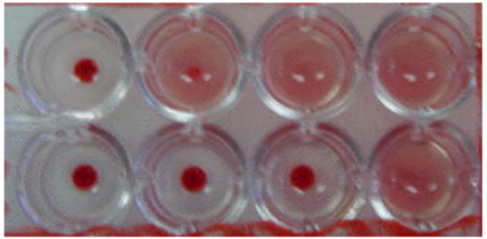
Test	Method
Adventitious viruses	28 day 4-cell line in vitro assay (incorporates 324K cells for detection of Minute Mouse Virus)-Species specific (CHO), Human Diploid (MRC-5), Simian cell line (Vero)
	In vivo assays (adult and suckling mice, guinea pigs and embryonated eggs)
Retroviruses	Extended S+L- focus assay on mink lung cells ((infectious xenotropic) non rodent cells Extended XC plaque assay (infectious ecotropic) rodent cells Mus Dunni (all types of infectious retrovirus)
	Reverse Transcriptase, QFPERT (Retrovirus broad screen)
	TEM (A & C Type particles)
	Co-cultivation with a human cell line or other species
Bovine Viruses	In vitro assay for bovine viruses
	QPCR and/or in vitro assay for bovine polyomavirus
Porcine Viruses	In vitro assay for porcine viruses and porcine circovirus
Species Specific Viruses	Hamster Antibody Production (HAP) test
	Mouse Antibody Production (MAP) test
	Lymphocytic choriomeningitis Virus (LCMV) challenge test

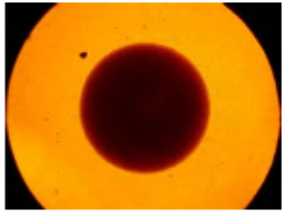
In Vivo Virus Assay

Test for viruses with endpoint

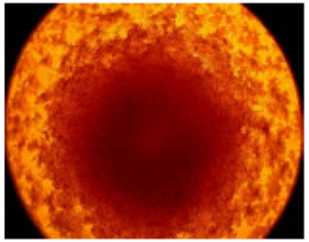
- Uses live animals and eggs
  - Adult Mice - direct inoculation
  - Suckling Mice - inoculation followed by second passage of homogenised animals in to second litter
  - Embryonated Eggs - SPF eggs Inoculated by 2 routes
    - Allantoic
    - Yolk sac with inoculation into a second batch of eggs.
  - Guinea Pigs - direct inoculation
  - A positive result is extremely rare
  - Hemagglutination assay is prone to false positives

- Hemagglutination of red blood cells





- Negative



- Positive

Adventitious Virus: Culture Reagents

Move to Chemically Defined media

Bovine viral diarrhoea virus (BVDV)
Bovine adenovirus (BAV)
Bovine parvovirus (BPV)
Bovine herpesvirus (BHV-1)
Bovine respiratory syncytial virus (BRSV)
Bovine polyomavirus
Bluetongue virus (BTV)
Rabies virus
Reovirus (Reo-3)

Porcine parvovirus (PPV)
Porcine enterovirus
Porcine reoviruses
Porcine adenoviruses
Pseudorabies

- Testing according to 9CFR-adequate?
- MP Seq has uncovered additional viruses in serum
- Test MCB for porcine and bovine viruses using risk based approach

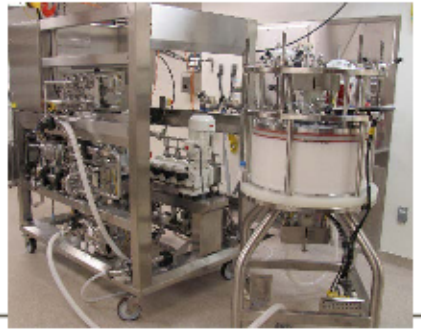
Examples of Animal Derived Raw materials

Material	Manufacturing Process	Risk Assessment/Compliance
Sucrose used as an excipient in Drug Product	Decolorized using bovine charcoal	Carbonization of animal tissues (bone) using temperatures >800°C
Cholesterol used in cell culture media as an additive	Extracted from sheep wool (lanolin)	Treatment at pH ≥ 13 (NaOH concentration of at least 0.1M) at 60°C for 1 hour
Foetal Bovine Serum	Extracted from bovine blood	Sourced from category A countries and supported by an EDQM Certificate of Suitability
Tallow used in plastics during manufacturing	Hydrolysis at not less than 200°C for not less than 20 minutes	In Compliance with Note for Guidance

Biopharm Process Equipment

Primary Equipment Used for Bulk Drug Mfg

- SS Bioreactors & Harvest Vessels
- Single-Use Bioreactors (SUB)
- Chromatography Systems
- Ultra Filtration/Diafiltration Systems
- CIP systems
- Parts Washers & Autoclaves
- Environmental and Stability Chambers
- Water Purification Equipment



Single Use Bioreactors Versus Stainless Steel Fermenter

Facility is at lower virus risk (impact) with the introduction of SUB's

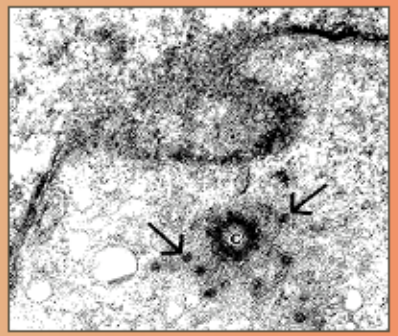
Single Use Bioreactor vs Stainless Steel Fermenter

- Speed to mitigation
  - SUB allows for fully closed processing and can be easily disposed of for decontamination purposes
    - Compartmentalization of contaminant
  - Disposables minimize the potential for propagation of the contaminant
  - Stainless steel bioreactors, equipment and piping require break down and cleaning to mitigate a current or further contamination

CHO Endogenous Retroviruses

Chinese Hamster

CHO cell

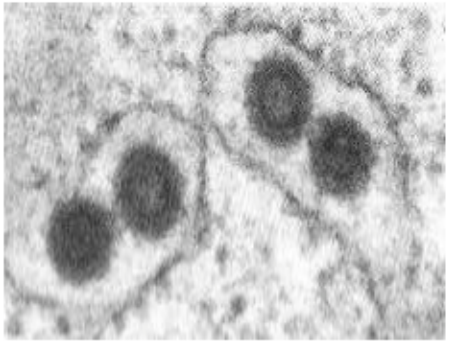


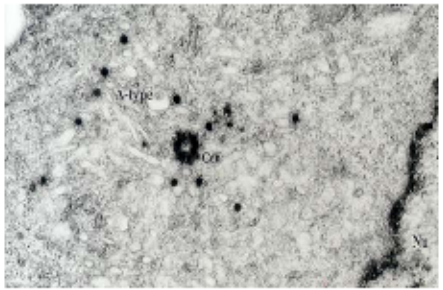
RVLP

Monoclonal Antibody Production

CHO Endogenous Retroviruses

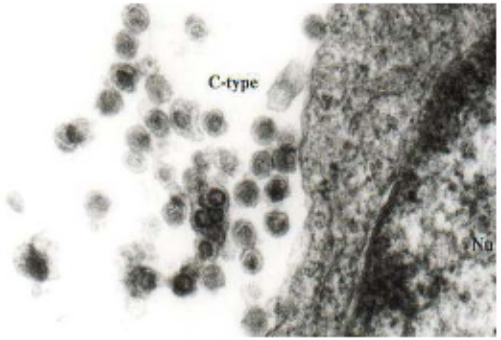
A-Type and C-Type Particles

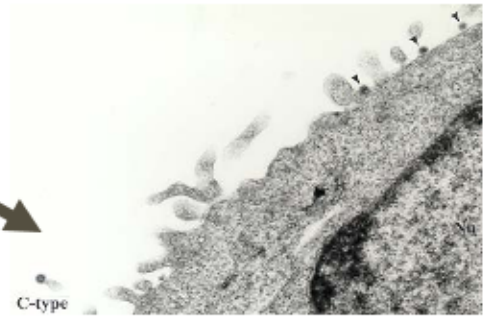




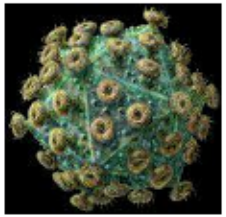
A-type: Immature, cytoplasmic particles with electron dense perimeter - "doughnut" appearance

C-type: Round, enveloped, mature particles with electron-dense core - characterised by 'budding'



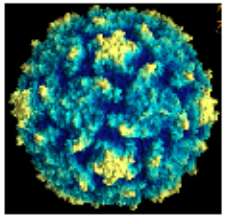


Model Virus Selection for virus clearance validation (Phase I/II/III studies)



XMuLV (Xenotropic Murine Leukaemia Virus)

- Model of the retrovirus-like particles in CHO cells
- Enveloped with surface glycoproteins, acid-labile
- Genome is ssRNA
- Size = 80-110nm
- Spherical shape



PPV (Porcine Parvovirus)

- Model of murine parvovirus Minute Virus of Mice (MVM)
- Non-enveloped, resistant to solvents, pH & temperature
- Genome is ssDNA
- Size = 18-24nm
- Icosahedral shape

Schematic of retrovirus and parvovirus

- For Phase I/II/III submissions 2 viruses are required and validation of virus reduction should be performed prior to the onset of the clinical trial (First Time in Man)
- For BLA/MAA 4 model viruses expected with a range of physicochemical properties
- BLA/MAA include spike clearance on end of column lifetime to show the claim hasn't changed

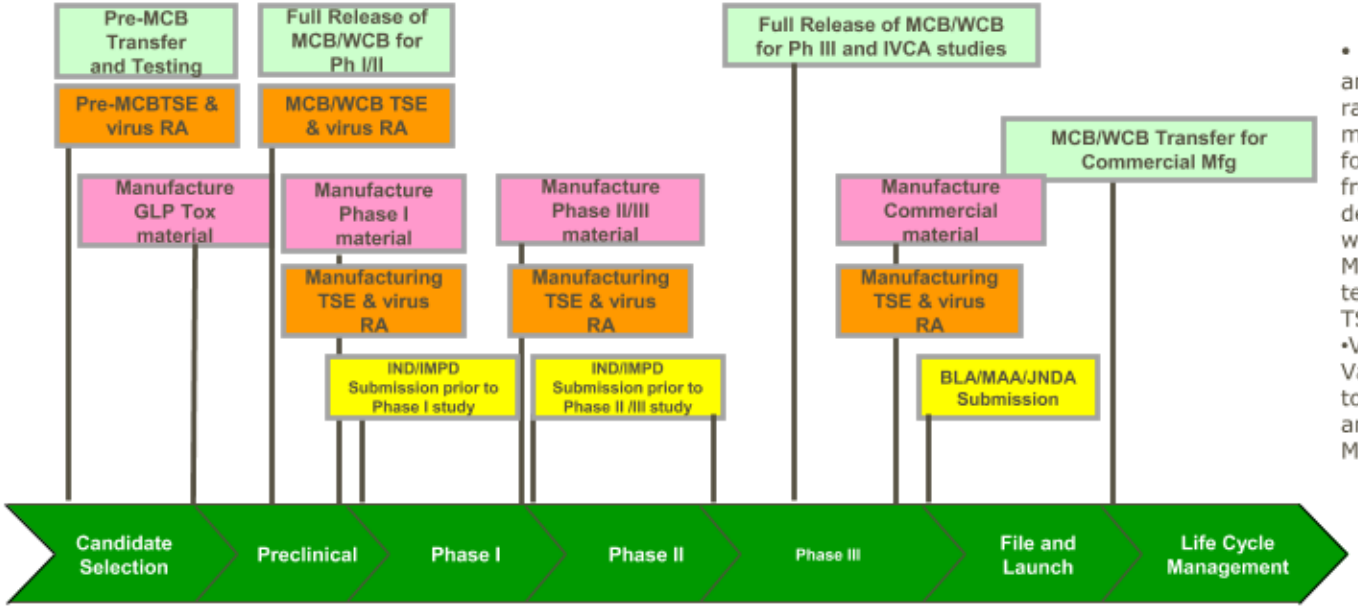
Phase I/II/III requirements

Documented Virus (and TSE) Risk Assessments

Host Cell line, Pre-MCB, MCB/WCB, Manufacturing Facility and Manufacture of Drug

- Prepare documented and approved TSE and virus risk assessments for the following materials and manufacturing stages before drug manufacturing can commence in a GMP Facility
- Host Cell Line
  - CHO, E.coli or yeast cell lines have originated in a research facility and challenging to fully trace exposure to materials of animal origin such as foetal bovine serum and media which may contain e.g. human transferrin, however need to trace to the best extent possible and document.
- Pre-Master Cell Bank
  - Cell line is exposed to cloning reagents and equipment and transfected with DNA which has been exposed to restriction enzymes (recombinant) but BSA can be used in digestion
- GMP Master or Working Cell Bank
  - Chemically defined media (may contain insulin that could expose to secondary or tertiary Animal Derived Materials) and testing program should be adequately defined due to historical exposure according to ICHQ5A
- Manufacturing Facility and Manufacture of Biopharmaceutical Drug (GMP)
  - Raw and product contact materials have the relevant certificates and have been appropriately manufactured i.e. tallow derived components EMA/410/01-Rev. 3

Virus (and TSE) life cycle of product and Regulatory Requirements



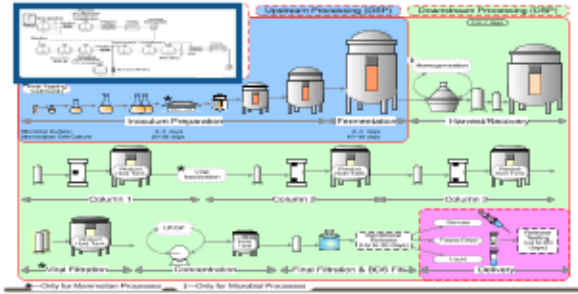
- Pre-MCB, MCB/WCB and Manufacturing raw and starting materials assessed for TSE and virus risk from use of animal derived materials which informs MCB/IVCA virus testing strategy and TSE risk assessment.
- Virus Clearance Validation carried out to support Phase I/II and Phase III and MAA/BLA submissions

TSE and Virus Safety in a Manufacturing Facility

Single use bioreactor and chromatography

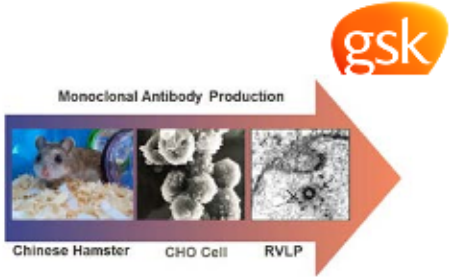
Primary Equipment Used for Bulk Drug

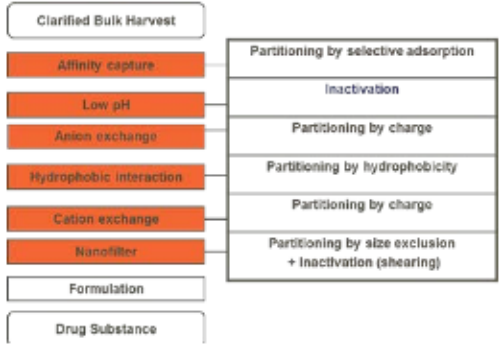
- Single-Use Bioreactors (SUB)
- Buffer & Media Prep Systems (SU)
- Chromatography Systems
- CIP systems
- Parts Washers & Autoclaves
- Disinfectant and cleaning
- Raw and product contact material certs



Closed Processing

Virus and bacteriophage testing (novel molecular methods-MPS and 3R's)





Virus Clearance Validation

TSE and Virus Control Framework

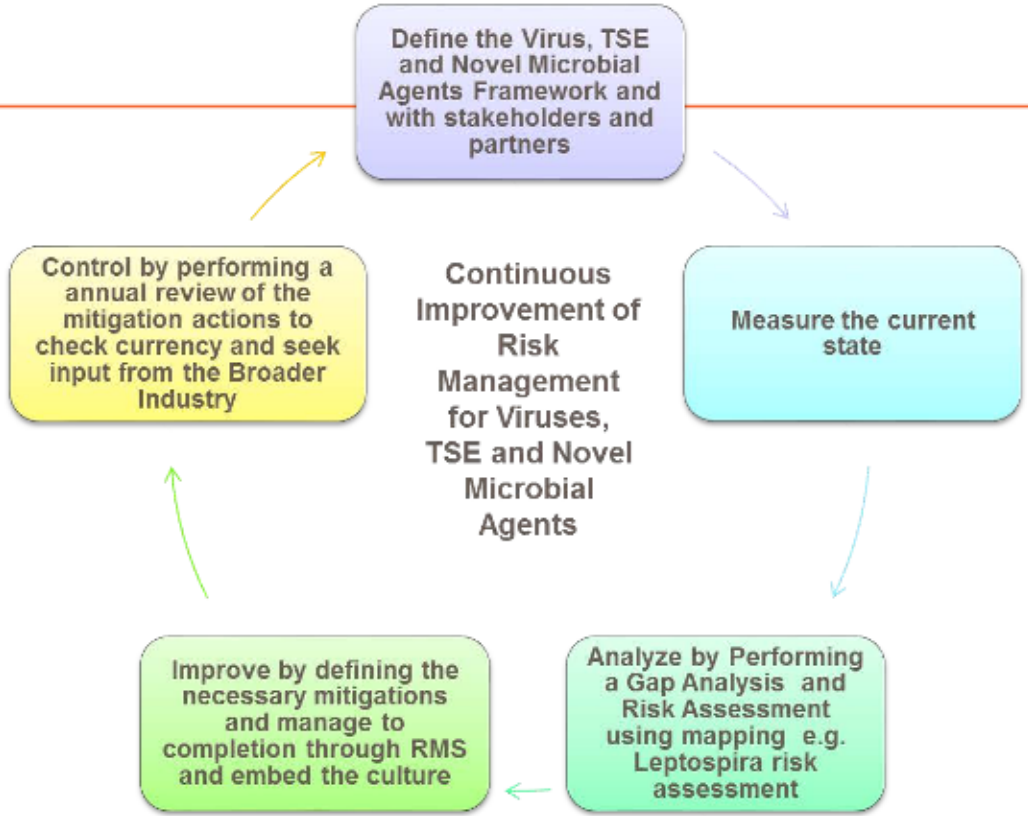
Facility Risk Assessment

Safe Product for Humans

Conclusion

- GSK uses a holistic approach to TSE and virus safety for Biopharms
- Processes are in place that comply with global regulatory requirements
- Virus de-contamination procedure is in place.
- The TSE and virus safety processes are in place to meet our our business requirement which is delivering safe products to patients.

Building a Continuous Improvement Culture



Conclusions

The Control Framework

- Biopharmaceuticals are produced from cells which have the potential to become infected with viruses, microbial agents (novel) and TSE and the risk to the patient needs to be mitigated
- Virus (and TSE) risk assessments need to be in place and risk managed holistically to ensure patient safety, facility, personnel safety and Regulatory acceptance by a combination of:
  - Raw materials sourcing and use of well characterized cell lines
  - Testing of pre-MCB, MCB, WCB and intermediates according to ICH Q5A and Q5D and novel detection methods
  - Virus clearance validation (<1 virus like particle per 10^6 doses)
  - Personnel Gowning procedures and facility cleaning, closed systems and airflows-use of disinfectants provides anti-viral assurance
- Virus (and TSE) risk can be carried for the life time of the product (from Research cell lines) and evidence of clear mitigation of risk must be demonstrated to the Regulatory Agencies within the relevant Dossiers