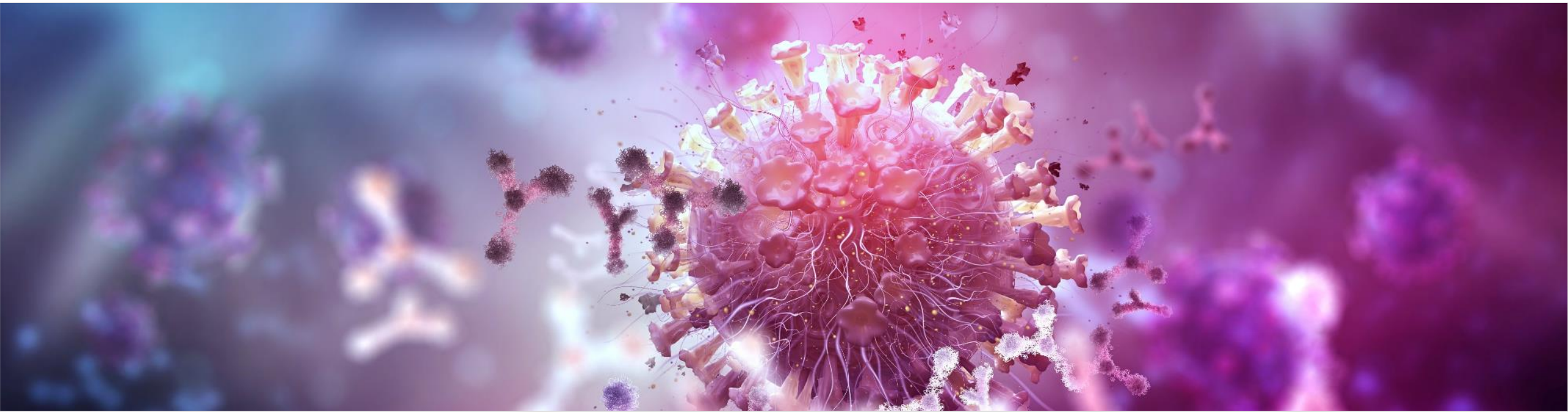

Introduction to Virus Safety in the ICH Q5A (R2) Era

Dr Andy Bailey (Andy_Bailey@virusure.com)

April 8th 2024

ViruSure 3rd Virus Safety Workshop

The Pioneering Difference

AsahiKASEI BIOPROCESS

Unrivaled Product Quality

Deep Technical Support

Scientific Knowledge Sharing

Trustworthy Quality Management System

A Tradition of Innovation

Dependable Global Business Network

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Asahi Kasei Bioprocess Portfolio

Asahi Kasei Bioprocess

PLANOVA™

Virus Removal Filters

Assurance Beyond Expectation

BioOptimal™ MF-SL

Microfilters

FLUID MANAGEMENT

Oligonucleotide Synthesis

MOTIV™ Inline Buffer Formulation

Chromatography

Virus Filtration

Built For You™

BIOSAFETY TESTING SERVICES

VIRUSURE

Virus/Prion Clearance Studies

bionique

Mycoplasma Testing Services

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BIOLOGICS DEVELOPMENT & MANTUFACTURING

Bionova Scientific®

End-to-end Process Development

GMP Manufacturing

Where concept becomes cure

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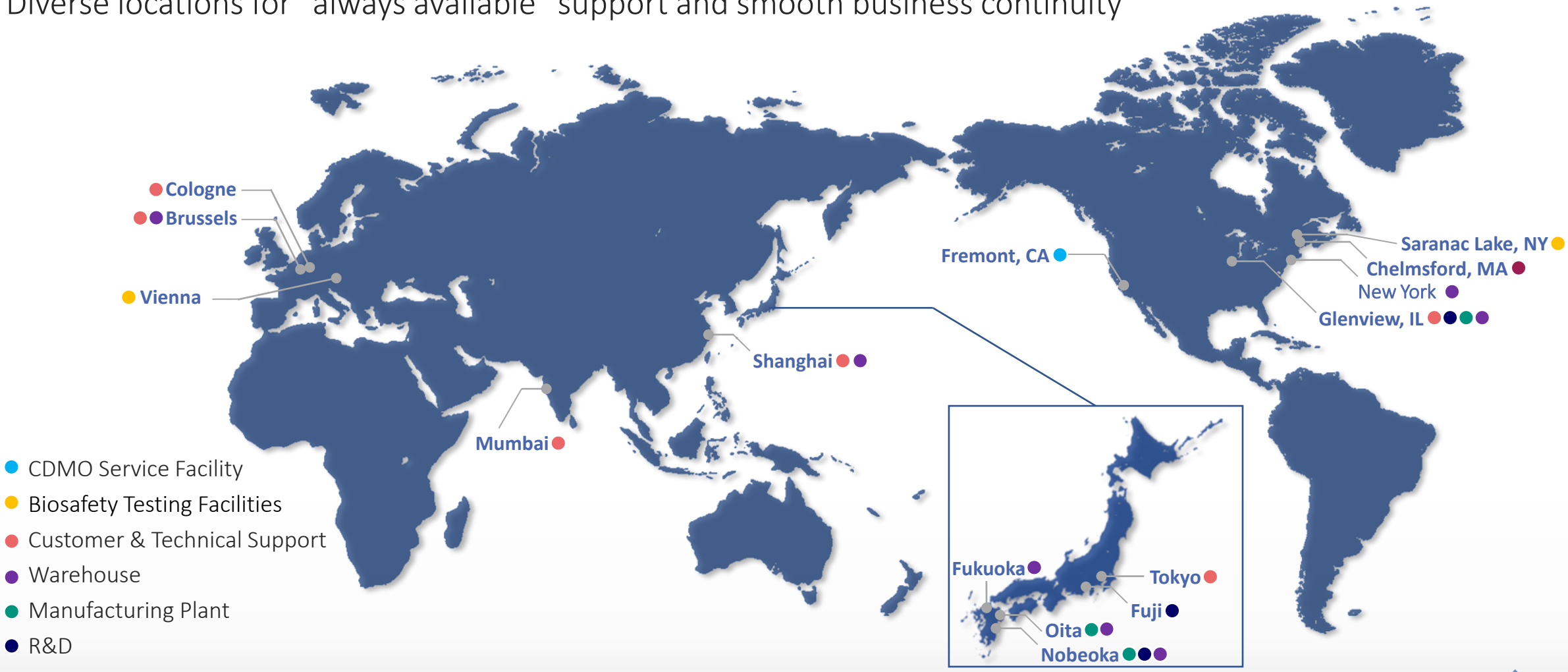
Global Network Locations

Diverse locations for “always available” support and smooth business continuity



Biosafety Testing Services

- The Biosafety Testing Services (BTS) division of Asahi Kasei Bioprocess is a global provider of contract testing services to GMP/GLP standards, ensuring the purity, safety and efficacy of raw materials and biological derived products
- The BTS division provides state-of-the-art specialized testing and consultancy services focusing on mycoplasma (Bionique) or virus (ViruSure) safety release tests as well as virus clearance studies and a range of other biosafety testing services
- A strong commitment to quality, customer service and rapid turn-around times are the hallmarks of the high standards to which we hold our quality systems and the studies we perform.

Quality is no coincidence.

Presentation Overview

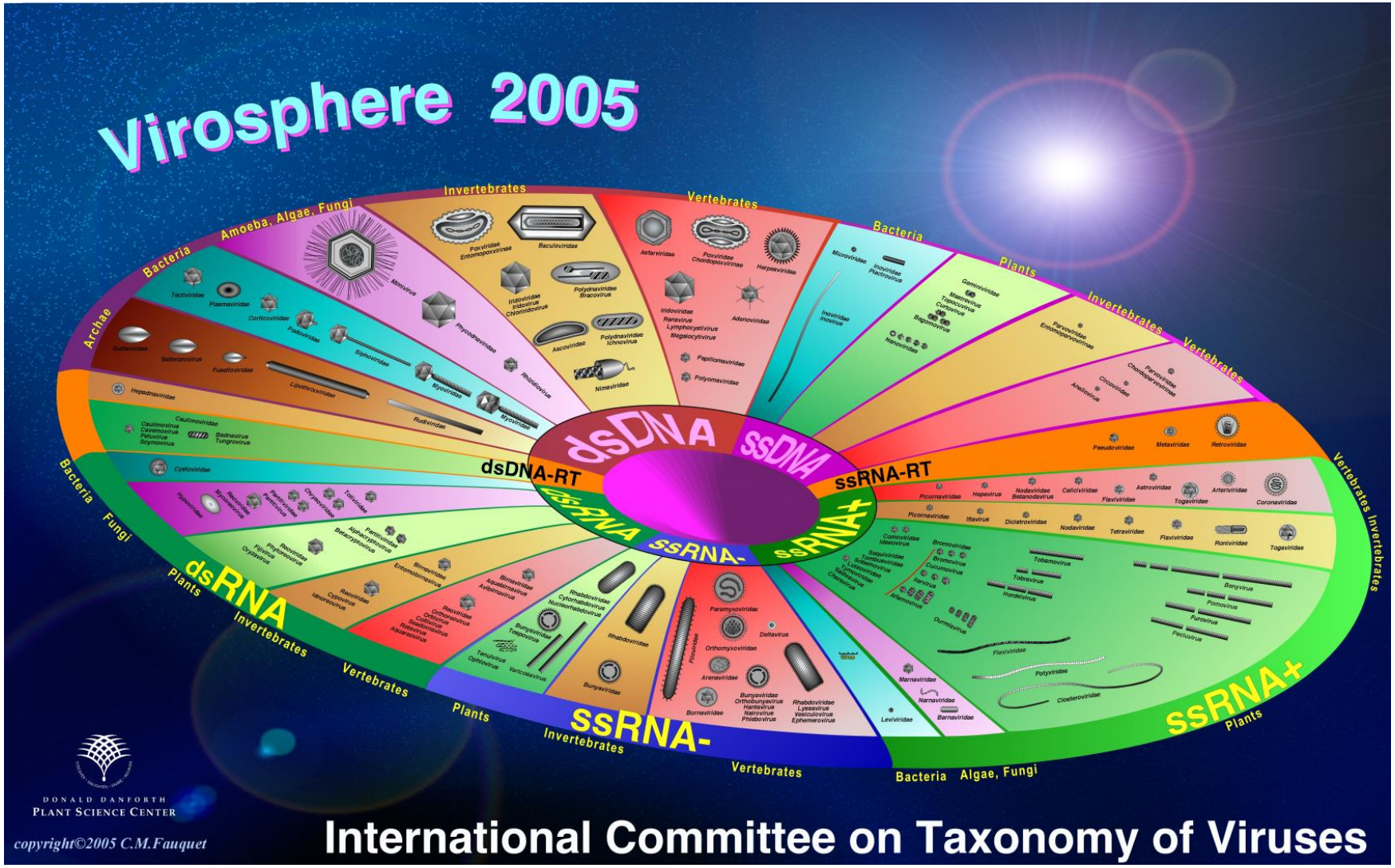
- **Why virus safety?- the importance of risk management**
  - The importance of virus safety and the basic strategies for controlling virus risk:
    - Sourcing
    - Testing
    - Virus clearance
  - Understanding that risk assessment needs to be product specific

- **Historical incidences of virus contamination and the source of contamination**
  - How did they occur?
  - How best can they be prevented?
  - Considering all sources of virus risk in a risk management program

The Patient- the Heart of Virus Safety Guidelines

- Historically (80-ies)
  - 1,000's of patients (primarily haemophiliacs) infected with HIV, HCV, HBV, HAV & other viruses
- Recent past
  - Variant Creutzfeldt Jakobs (vCJD), B19 & other viruses continue to challenge the safety of plasma products
  - New and emerging viruses continue to challenge plasma, recombinant or cell-based therapy products
- Most of the regulations in place today stem out of lessons learned from prior contamination events

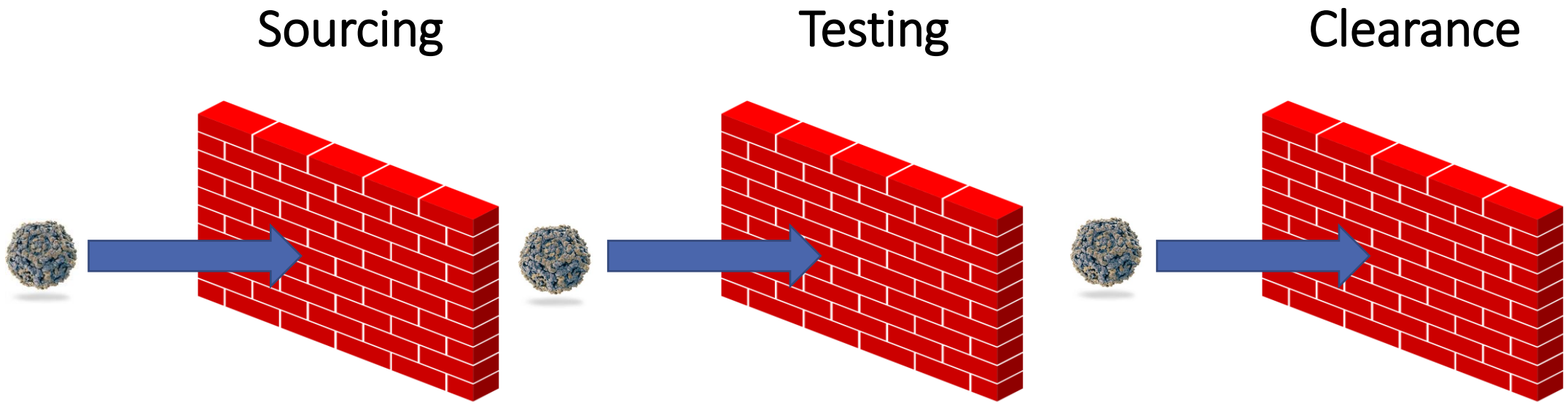
The Real of Viruses



- Viruses are found in all spheres of life in all shapes and sizes
- Testing for every possible contaminant is a virtual impossibility- you need a holistic approach

Biologics Manufacturing Safety

Complementary Measures Assures Appropriate Levels of Risk Reduction



- Reliance on only 1 or 2 of these measures increase the chance that virus contamination might slip through
- The strategy of how each of these measures can be effectively implemented is the main theme of the ICH Q5A guideline

Examples of Contamination: The Use of Animal/Human Tissue Sources

Identification and characterization of avian retroviruses in chicken embryo-derived yellow fever vaccines: investigation of transmission to vaccine recipients

Althaf I Hussain [1], Jeffrey A Johnson, Marcos Da Silva Freire, Walid Heneine

Affiliations + expand

PMID: 12502826 PMCID: PMC140796 DOI: 10.1128/jvi.77.2.1105-1111.2003

Iatrogenic transmission of spongiform encephalopathies has been reported. In sheep, scrapie has been accidentally transmitted by the use of Louping Ill vaccine prepared from pooled, formaldehyde treated ovine brain and spleen in which material from scrapie-infected sheep had been inadvertently incorporated. Also, transmission of scrapie to sheep and goats occurred following use of a formol-inactivated vaccine against contagious agalactia, prepared with brain and mammary gland homogenates of sheep infected with *Mycoplasma agalactiae*. In man, cases of transmission of CJD have been reported which have been attributed to the parenteral administration of growth hormone and gonadotropin derived from human cadaveric pituitary glands. Cases of CJD have also been attributed to the use of contaminated instruments in brain surgery and with the transplantation of human dura mater and cornea.

EMA/410/01 rev.3

Contamination of Poliovirus Vaccines With Simian Virus 40 (1955-1963) and Subsequent Cancer Rates

Howard D. Strickler, MD, MPH; Philip S. Rosenberg, PhD; Susan S. Devesa, PhD; et al

» Author Affiliations

JAMA. 1998;279(4):292-295. doi:10.1001/jama.279.4.292

CORRESPONDENCE

Creutzfeldt-Jakob Disease Infectivity of Growth Hormone Derived from Human Pituitary Glands

Published February 4, 1993 | N Engl J Med 1993;328:358-359 | DOI: 10.1056/NEJM199302043280520

VOL. 328 NO. 5

- These transmission events highlight the issues when working with animal or human derived source materials with minimal virus clearance downstream- e.g. also cell-based therapies

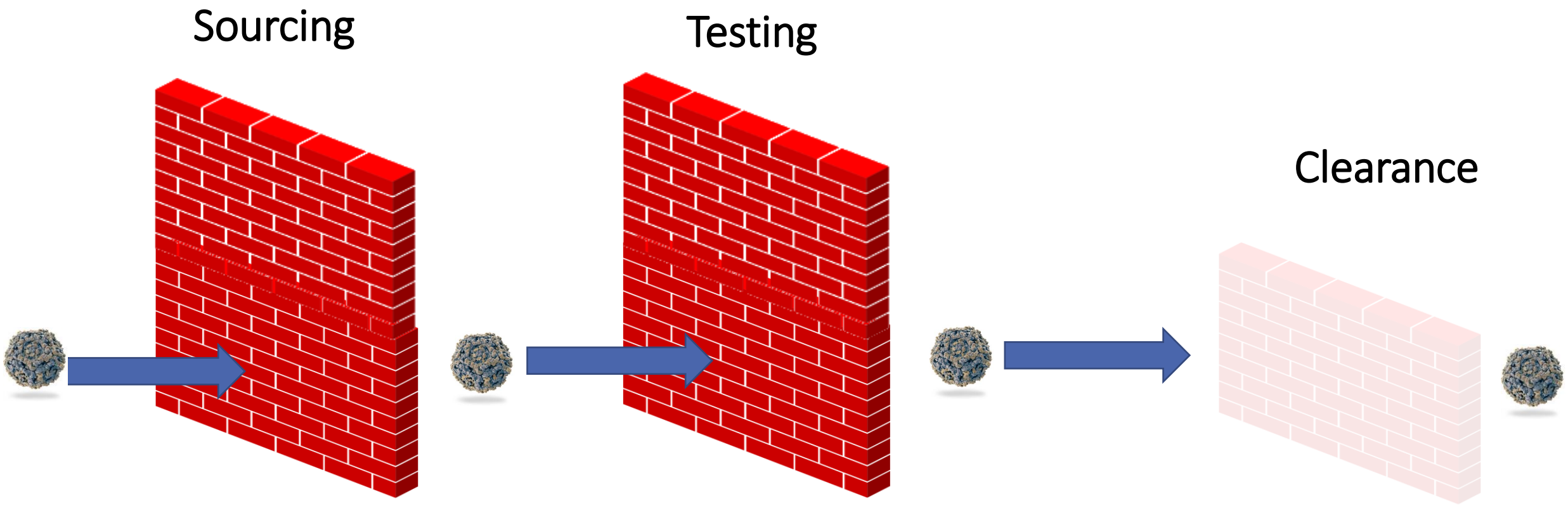
ATMPS- Available Risk Reduction Measures for Assuring Virus Safety?

The only place to control virus risk

	Gene therapy vectors (enveloped)	Gene therapy vectors (unenveloped)	Somatic cells
Upstream:			
QC testing of start materials?	☑	☑	☑
QC testing of cell culture medium & components?	☑	☑	☑
Barrier technologies for cell culture medium?	☑	☑	☑
Downstream:			
Virus filtration	X	☑	X
Detergent treatment	X	☑	X
Chromatography	☑	☑	X
UV inactivation	X	X	X
Gamma-irradiation	X	X	X
HTST	X	☑???	X

Within Scope of ICH Q5A

Virus Safety for Cell & Gene Therapy Products



Cell Therapy - not possible to include downstream steps to remove or inactivate virus as it would destroy the product

Gene Therapies – depends on the vector

AAV (non-enveloped viral vector)- inactivation by detergent or low pH

Lentivirus (enveloped viral vector) - downstream inactivation or removal steps are not compatible with the product

The Importance of Risk Management in Virus Safety

- **Risk management plays a key role in designing and implementing an effective virus safety strategy**
  - No two products are identical
  - The risks for each product need to be clearly defined and evaluated
  - Measures for reducing risk at the level of sourcing implemented
  - The correct testing strategy implemented based on the defined risks
  - Proactively design the manufacturing process to reduce risk wherever possible

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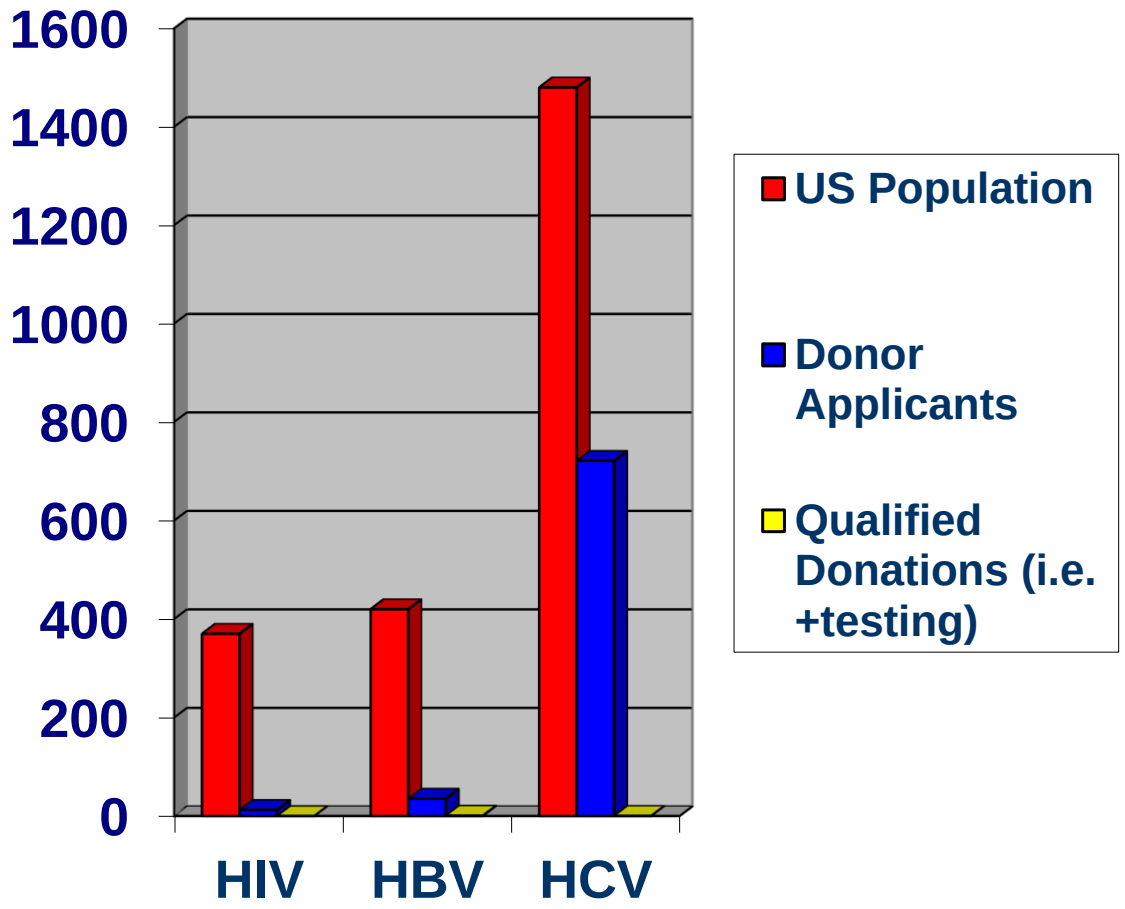
Virus Contamination Events- What can we Learn from the Past?

AsahiKASEI
BIOPROCESS

The Contribution of Sourcing and Testing to Risk Reduction

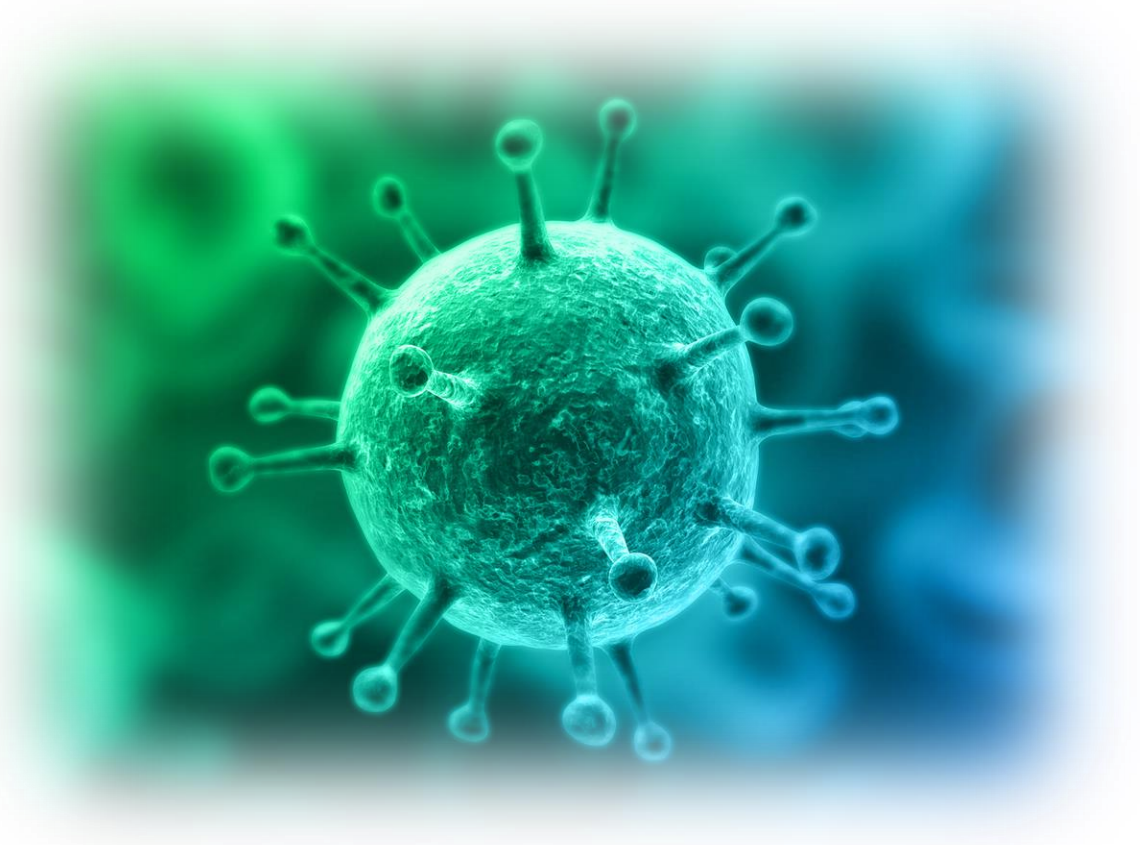
- Donor sourcing and testing can provide a significant reduction in viral marker rates and therefore risk
- However, even with improved donor sourcing, transmissions have occurred without additional measures to control risk:
  - i.e. virus inactivation or removal
- Reduction of risk:
- Donor selection: ~1-2 $\log_{10}$
- Donor testing: ~1-2 $\log_{10}$



Data from Waytes *et. al.* Dev Biol Stand 2000;102:37-51

Cell Culture Derived Products: Examples of Contamination

- **Cell culture derived products**
  - Cell line derived:
    - Endogenous Retroviruses
    - Latent Herpesviruses (e.g. EBV)
- **From animal derived components:**
  - Porcine parvovirus (PPV)- e.g. trypsin
  - Porcine circovirus (PCV) - e.g. trypsin
  - Bovine viral diarrhoea virus (BVDV)
  - Bovine polyomavirus (BPyV)
  - Equine haemorrhagic disease virus (EHDV)
  - Cache valley virus (CVV)
- **Of as yet non clearly defined aetiology**
  - Mice minute virus (MMV)
  - Vesivirus 2117 (probably animal derived)



The Impact of Contamination Events?

- **The consequences of a contamination event can be severe, including:**
  - Incapacity to manufacture the product and maintain clinical supply
  - Significant resources needed to investigate the root cause
  - Clean-up operations last for months and may restrict or even halt the ability to manufacture multiple products in a multi-product facility
  - Company image and stock price can be affected

The History of Virus Contamination Events in Mammalian Cell Culture Derived Biopharmaceuticals

Year of Contamination	Contaminations (virus / host cell)	Total
1985-1989	Bluetongue / CHO EHDV / CHO	2
1990-1994	Herpesvirus / Primary Monkey Herpesvirus / Vero MMV / CHO (x2) Parainfluenza virus / MRC-5 Reo3 / MRC-5 Simian adenovirus / Primary monkey	7
1995-1999	Cache valley virus / CHO Reovirus / Human primary kidney Vesivirus 2117 / CHO	3
2000-2004	CVV / Unknown (x2) Human adenovirus / HEK293	3
2005-2010	CVV / CHO MMV / CHO (x2) Vesivirus 2117 / CHO (x3)	6
2010-Present	MMV / CHO MMV / BHK-21 PCV-1 / Vero	3
Unknown	MMV / BHK-21 Reovirus / Unknown	2
	Total:	26

Data from Barone et. al.; Nature Biotechnology (2020); Vol 38; pp 563-572

ViruSure has in recent years identified 2 additional contamination events not reported in this table

What were the Sources of Contamination in these Historical Events?

Contaminated Cell Line	Contaminating Virus	Pathogenic to Humans?	Suspected and Confirmed Sources of Contamination					
			Serum	Recombinant Medium Component	Undetermined Medium Component	Operator	Host Cell Line	Not Found
Viruses found to contaminate CHO cell culture								
CHO	Bluetongue virus	No	1					
CHO	Cache valley virus	Yes	2					
CHO	Minute virus of mice	No		1	3			1
CHO	Vesivirus 2117	No	4					
Viruses found to contaminate human or primate cell lines								
Primary monkey, Vero	Herpesvirus	Yes				1	1	
HEK293	Human adenovirus type 1	Yes				1		
MRC5	Parainfluenza virus type 3	Yes				1		
MRC5	Reovirus type 3	Yes				1		
Primary monkey	Simian adenovirus	No					1	

Comments:

- Data from Barone *et. al.*; Nature Biotechnology (2020); Vol 38; pp 563-572

PCV Contamination of Rotarix Vaccine: An example of contamination that was missed

- **PCV1 was identified as a contaminant of the Rotarix vaccine through massively parallel sequencing**
  - Investigation by GSK identified contamination to have originated during preparation of the MCB back in 1983. All subsequent materials produced from this MCB (e.g. WCB, Master Seed) were also positive
  - Replication of PCV on Vero cells has been reported and the presence of infectious virus was confirmed for the suspected cell banks
- **It has not been reported if the Trypsin used in the preparation of the 1983 MCB was tested for the presence of porcine viruses (unlikely as such testing was not implemented until later)**
  - Even if tested, it is unlikely that the PCV contamination would have resulted in a positive result as the cells would show no cpe

Preventing PCV-Like Contamination Events?

- **PCV is known as a contaminant in pigs with a high sero-prevalence and high titre viraemia**
  - A risk analysis of potential viruses of concern focusing on those viruses at high sero-prevalence and high titre would identify PCV as a potential virus of concern
  - Importance of coupling epidemiology with testing (as for plasma products)
  - Other testing methodologies (e.g. PCR) could then be employed to evaluate potential contamination
  - Possible controls at the herd level?
- **Implementation of robust virus removal steps for viruses like PCV into the manufacturing process for Trypsin**
  - EMA: Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products (2014)

MMV Contamination: The Facts

- **Contamination events started to be noted by Genentech in the 1990's in CHO fermenters**
  - Multiple contamination events (not just a single isolated event)
  - A number of other manufacturers have also had incidences of contamination (maybe as many as 50% of the large manufacturers!)
- **Source of contamination was never clearly identified, but it was assumed to be caused by facility rodents:**
  - Facility rodents present in the GMP manufacturing facility?
  - Rodents present in the suppliers for excipients or media components (i.e. fomite transmission)?

MMV as an Endemic Virus in Mice?

- **Sero-prevalance in mice is up to 70% (Besselson et al. 2006)**
  - Virus present in high titres in multiple tissues (titres as high as 107/ml)
  - Virus is excreted in the urine of infected animals
- **Potential source of contamination where exposure to mice is not controlled- measures need to be in place:**
  - How far back in the supply chain though?
  - Parvoviruses are among the most resistance viruses used in virus validation studies and will also survive for extended periods in the environment

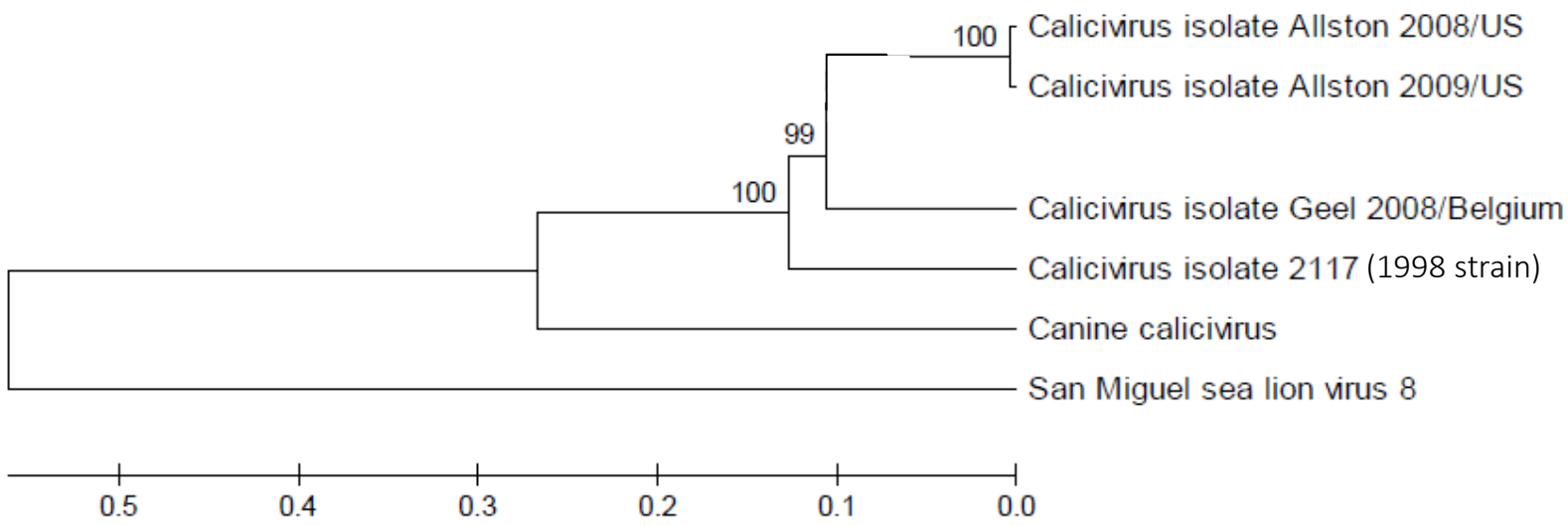
Vesivirus 2117

- We have had a total of 4 reported contamination events with Vesivirus 2117
- Originally observed as 40nm virus particles CHO bioreactor cultures demonstrating cytopathic changes
- Timing of contamination events:
  1. ~1998- Boehringer Ingelheim (Oehmig et. al.; 2003)
  2. 2008- Genzyme (US)
  3. 2008- Genzyme (Belgium)
  4. 2009- Genzyme reported another contamination with the same strain as found in the 2008 US event
  - The contamination events in the US & Belgium were with different 2117 strains (see next slides)
  - No identifiable shared components in use at both facilities
- Direct data to support a bovine origin for the contamination is limited
  - Phylogenetic analysis shows closest relationship with Canine Caliciviruses

Sequence Similarity* Among Vesivirus 2117 Isolates

A:

Status 2010



* Prepared using the UPGMA method using the virus capsid protein alignment. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

Sequence Similarity Among Vesivirus 2117 Isolates

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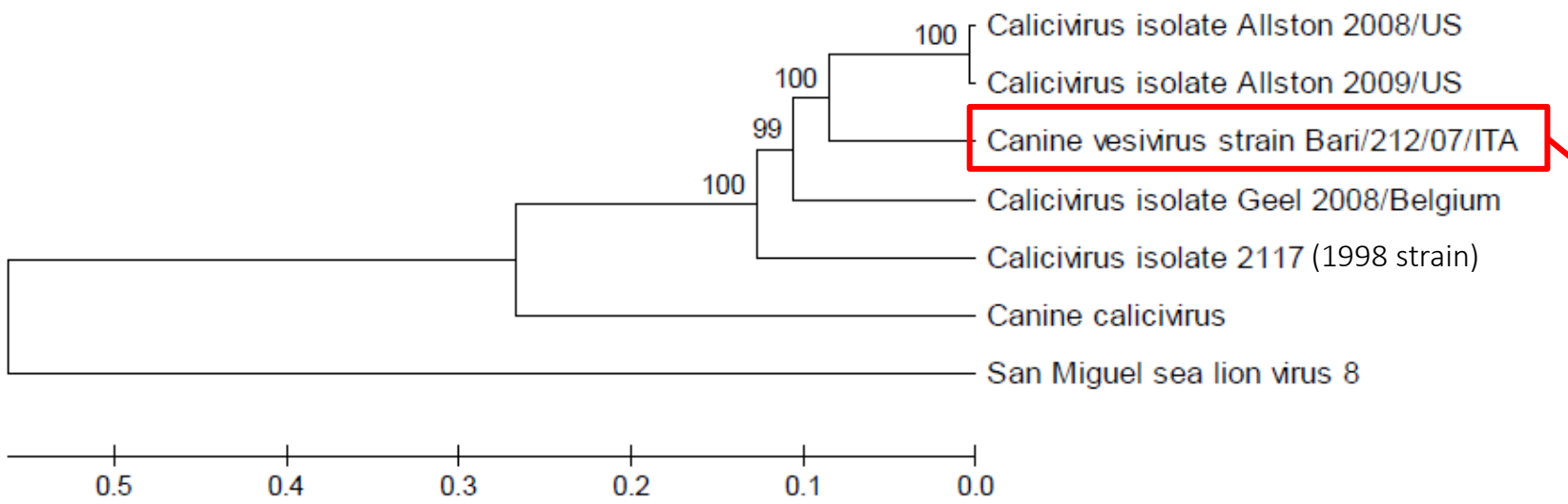
Status 2011



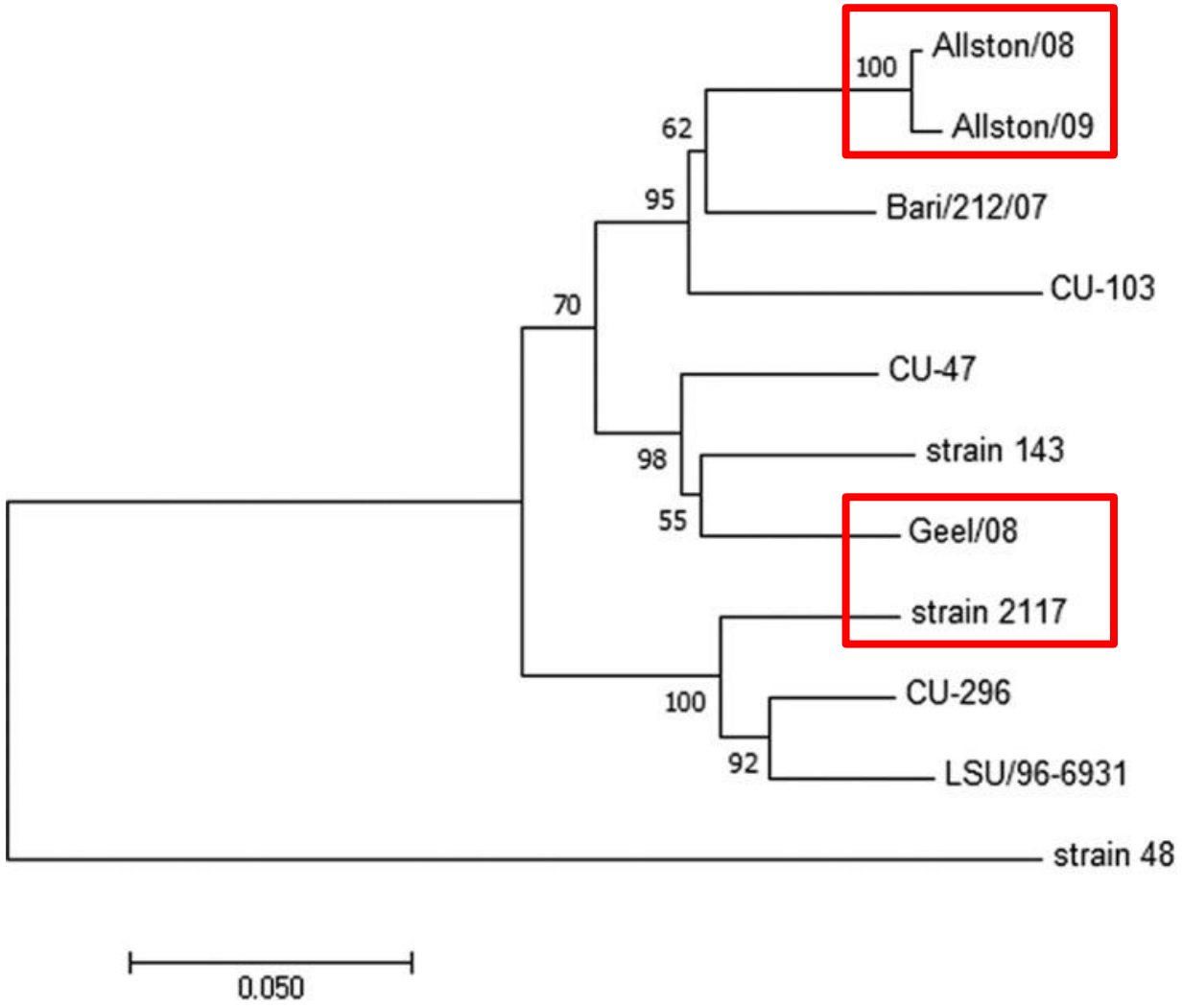
Sequence not deposited until 2011. Virus isolated from a dog in Italy in 2007 with gastroenteritis

i.e. Virus safety is not static- it is always changing

What is the Origin of Vesivirus 2117 Contamination?

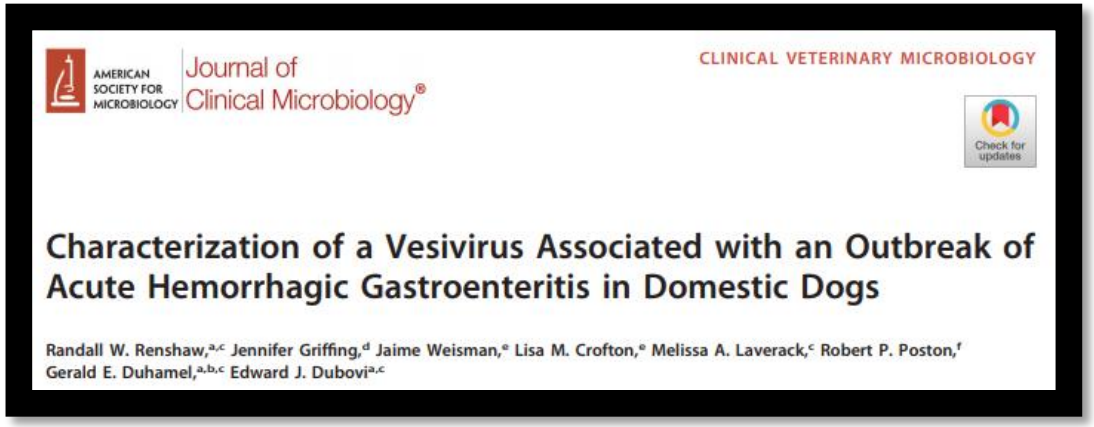
- **The observation of two different strains of Vesivirus 2117 occurring simultaneously at two different locations with no shared components raises a number of aspects:**
  - Is the Vesivirus 2117 more prevalent in the environment than has been reported?
  - The absence of any homologies with bovine viruses does not support the conclusion that this is coming from bovine derived components
- **The MMV precedent has shown us that we should be prepared to expect unusual sources of potential contamination**
  - Environmental contamination? Maybe dogs!

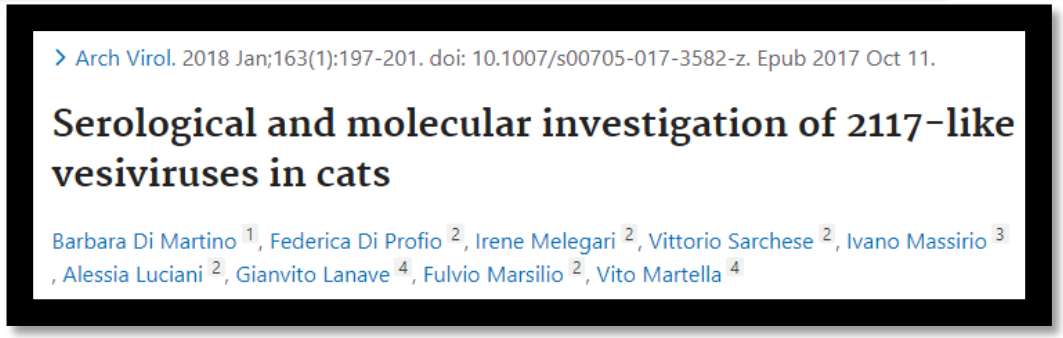
Vesivirus 2117: Prevalence in Dogs



- Figure from: Renshaw et. al. (2018): "Characterization of a Vesivirus Associated with an Outbreak of Acute Hemorrhagic Gastroenteritis in Domestic Dogs"
- Original 2117 and Genzyme isolates highlighted in Red
- All these viruses are >85-90% homologous at the DNA level

Exposure to Vesivirus 2117 appears in Multiple Species





- ~67 % seropositivity for 2117-like viruses in dogs (Martella et. al. 2015: Detection and Full-Length Genome Characterization of Novel Canine Vesiviruses)
- 37.3% of cats carry antibodies to 2117-like viruses
- 7.8% of humans carry antibodies to 2117-like viruses
- The potential sources for environmental contamination with Vesivirus 2117 are growing!

What Sources of Contamination need to be Considered?

- For cell culture medium, contamination could arise from 2 sources:
  - Animal or human derived medium components (e.g. FBS, Albumin, other growth factors)
  - Environmental sources (→ MMV and Vesivirus 2117 are classical examples of this)
- Arboviral insect vectors
  - A large number of viruses are Arboviruses (transmitted by insects). Dead insects present in e.g. powdered media could therefore be a source of contamination
  - Virus though needs to survive for long periods in the environment (non-enveloped viruses like Bluetongue; EHDV could be potential candidates here)
- Infected workers at the GMP plant or supplier
  - Many viruses are asymptomatic and so would not be apparent, but viruses could also be carried by fomites from infected pets or other animals
- Controlling the supply chain for media additives
  - How far back in the supply chain to go? Some viruses have high resistances to inactivation and so could survive for extended periods in the environment
- Conclusion: Controlling such risks in medium is almost impossible!

Summary

- **Understanding the historical causes of virus contamination in biological products is key to establishing a risk-based approach to virus safety:**
  - Much of what is now found in regulatory guidance stems from what we have learnt over the last 30-40 years
  - Virus risks exist outside of the sphere of animal-derived components, often considered to be the main source of virus risk, e.g.:
    - MMV
    - Vesivirus 2117
  - Unusual sources of contamination need to be considered
  - Contaminating viruses can be introduced either through contaminated start materials/components or through operators

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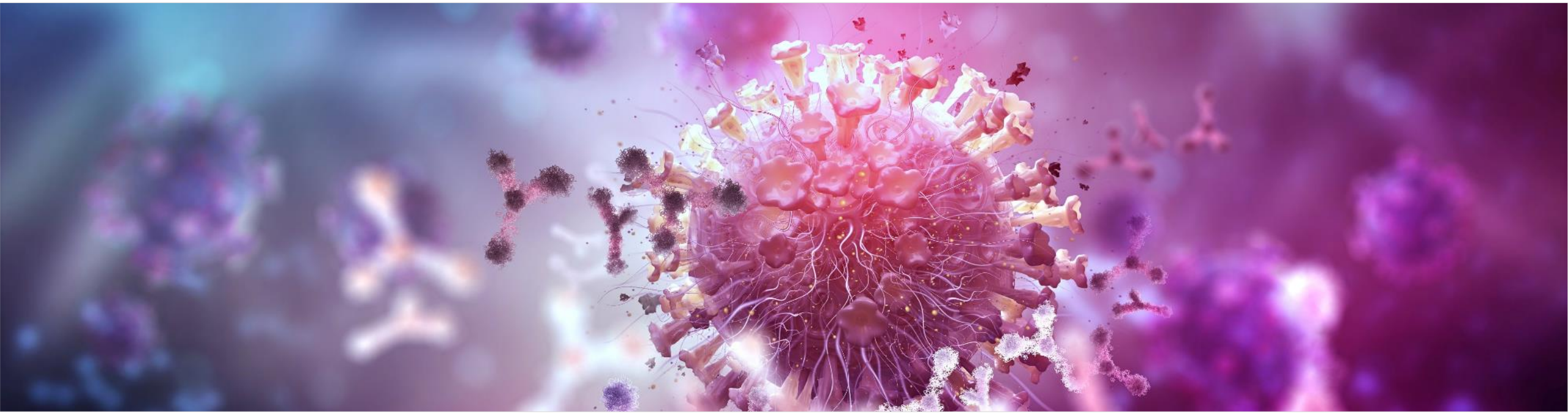
감사합니다 Gracias Danke

Благодаря 谢谢 Tack धन्यवाद Dziękuję

Спасибо Thank You Obrigado

Děkuju Grazie Ευχαριστώ Merci Köszönöm

ありがとうございました Teşekkür ederim



Part 2: ICHQ5A R2: Regulatory Perspectives

Andy Bailey

Presentation Overview

1. Introduction to the new ICH Q5A R2 document
2. Upstream risks
3. Platform approaches
4. Continuous manufacturing
5. Testing: Controlling the Contamination Load in a Manufacturing Process and the Importance of Robust Virus Clearance in the ICH Q5A (R2) guideline

ICH Q5A

ICH Guideline Q5A(R2) on viral safety evaluation of biotechnology products derived from cell lines of human and animal origin

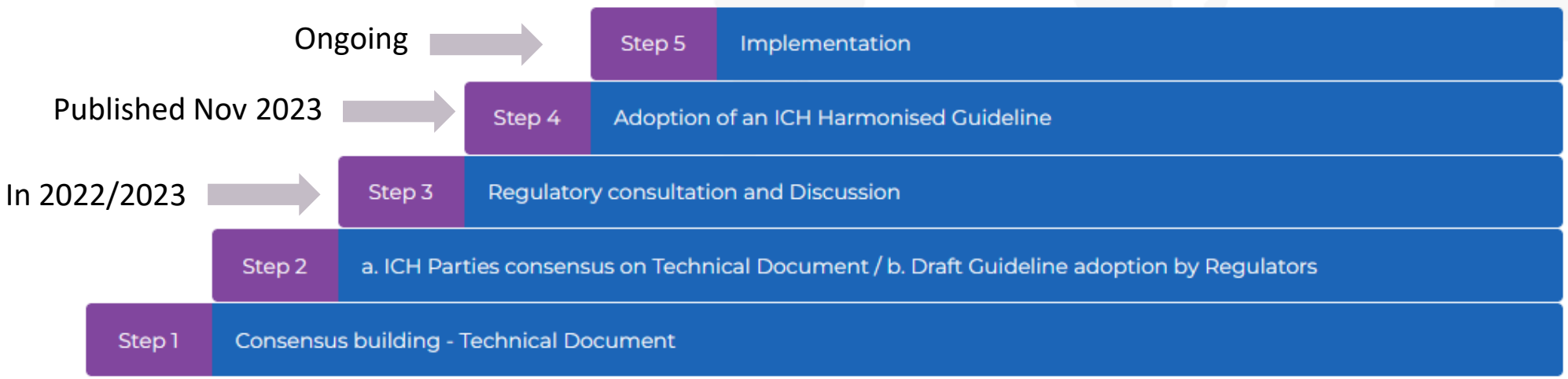
1997 – Implementation

23 years

1999 – Revision 1

2022 – Revision 2 *(release for public consultation)*

Nov 2023 – Final version released



https://ich.org/page/formal-ich-procedure

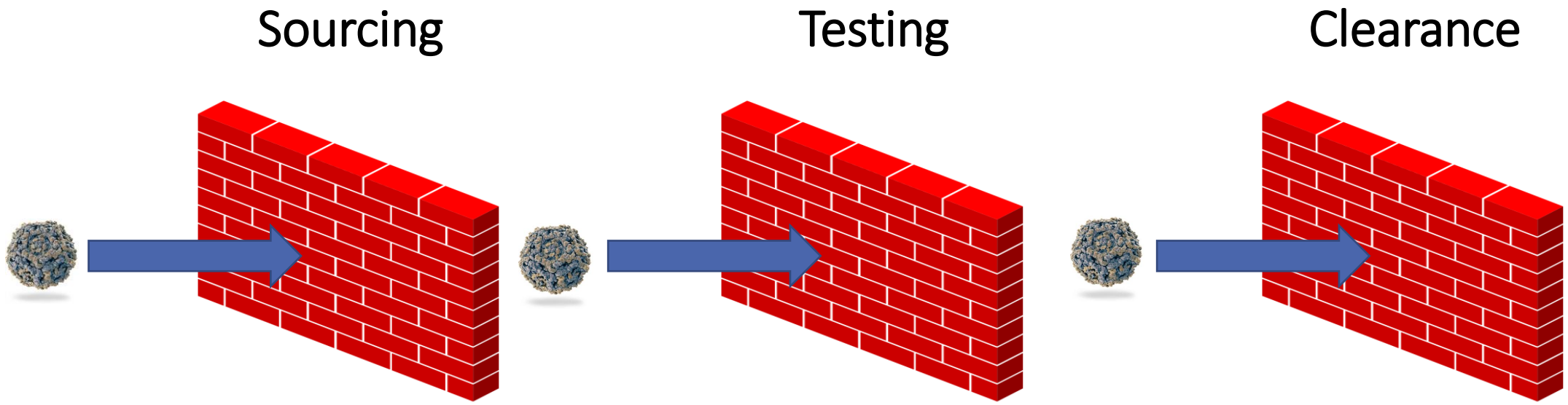
ICH Q5A (R2)- Key Messages

Impact of the new ICH Q5A R2 guideline?

- The new ICH Q5A R2 guideline has brought biosafety testing up the current state of the art
- It is a document which will be been signed off at all major regulatory agencies, including FDA, EMA, PMDA, MFDS and many other around the world
- The broadened scope of the guideline means that it can now be applied to products beyond the classical recombinant derived products (e.g. gene therapy vectors that can be subject to purification)
- Advanced detection technologies such as NGS and PCR play an important role in the new ICH Q5A document and can be used as replacements for in vivo and in vitro adventitious agent testing ***without any direct head-to-head*** comparison
  - How technologies like NGS impact on the overall virus safety profile is important to understand
- For virus clearance studies, there are new important additions that will impact on how platform virus clearance studies should be performed as well as expanding applicability to continuous biomanufacturing

Virus Safety in ICH Q5A

Complementary Measures Assures Appropriate Levels of Risk Reduction



- Reliance on only 1 or 2 of these measures increase the chance that virus contamination might slip through
- The strategy of how each of these measures can be effectively implemented is the main theme of the ICH Q5A guideline

2

Sourcing / Upstream Risks

Upstream Virus Contamination Risks

Recombinant Products:

- Steps are implemented downstream of cell culture to reduce virus risk and medium is often chemically defined:
  - Chemically defined though does not necessarily mean there is no virus risk:
    - MMV
    - Vesivirus 2117
    - The concentration of these contaminants was probably very low (e.g. ~3 IU/Litre according to some estimates)

- The impact of a contamination even upstream can be significant:
  - Supply of product is impacted
  - Significant investigation / clean-up costs
  - Impact on company image

Upstream Virus Contamination Risks

Cell- or Virus-Based Therapies :

- Steps downstream for controlling virus risk may not be feasible

- Virus might be inherently carried in the cells (latent or inapparent infections)
- Medium is often complex including human or animal derived components where the virus risk is higher
  - FBS or purified bovine / porcine proteins
  - Platelet derived growth factor

- → there is a greater need to control the virus risk in such components

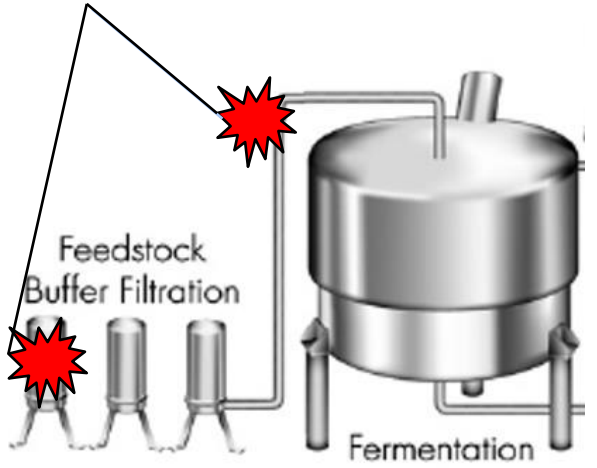
ICH Q5A (R2)

Sourcing and Upstream Controls:

- *Manufacturers should avoid using human- and animal-derived raw materials (e.g., human serum, bovine serum, porcine trypsin) in their manufacturing processes when possible. When this is not possible, the use of animal-derived raw materials should be supported by the relevant documentation or qualification of the material, commensurate with risk. Information such as the country of origin, tissue of origin, virus inactivation or removal steps applied during the manufacturing process of the material, and the types of virus testing that have been performed on the raw material should be provided.*

- *When possible, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, or ultraviolet C irradiation can be used as additional virus risk mitigation measures.*

Barrier Technology

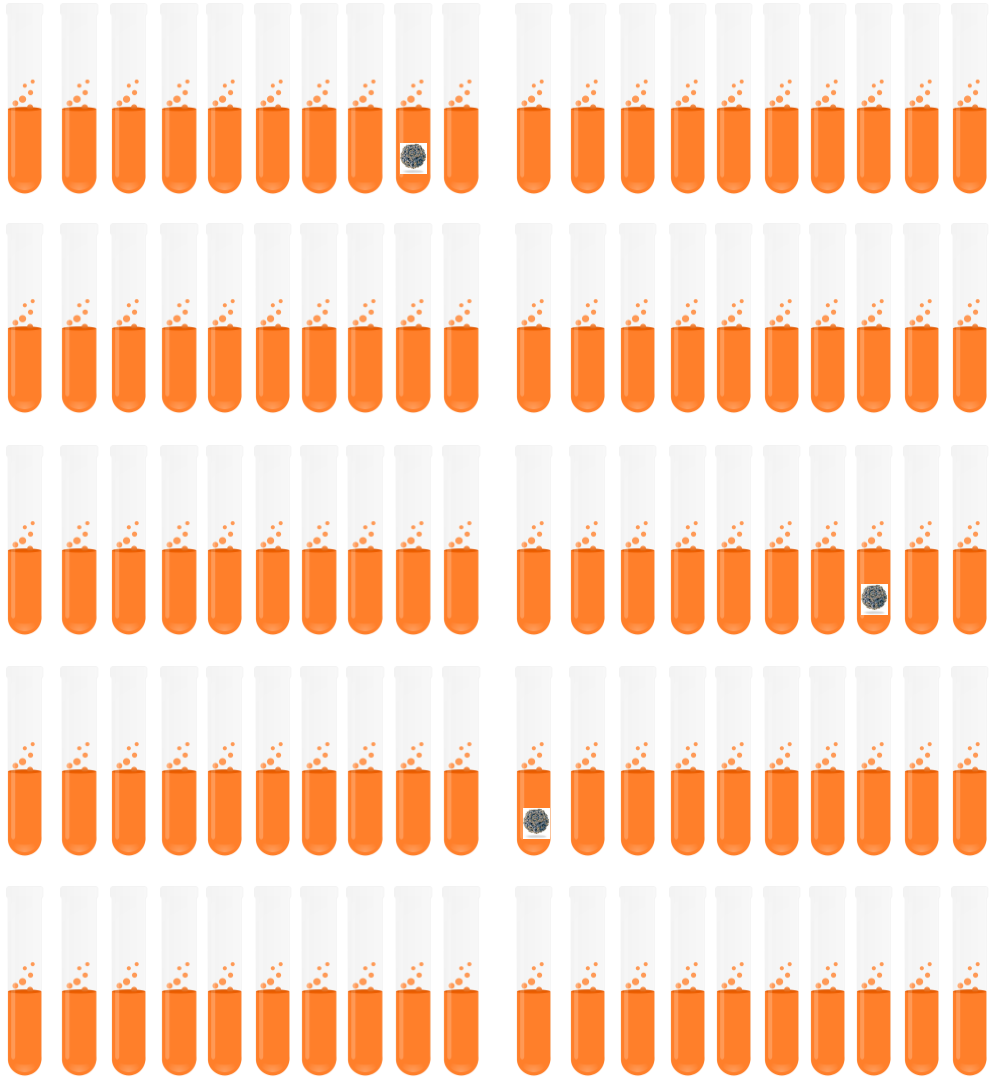


The Challenge of Detecting Low Virus Concentrations

Total volume of medium (1,000ml)

Volume for testing: 10ml

Concentration of virus: 3IU/Litre



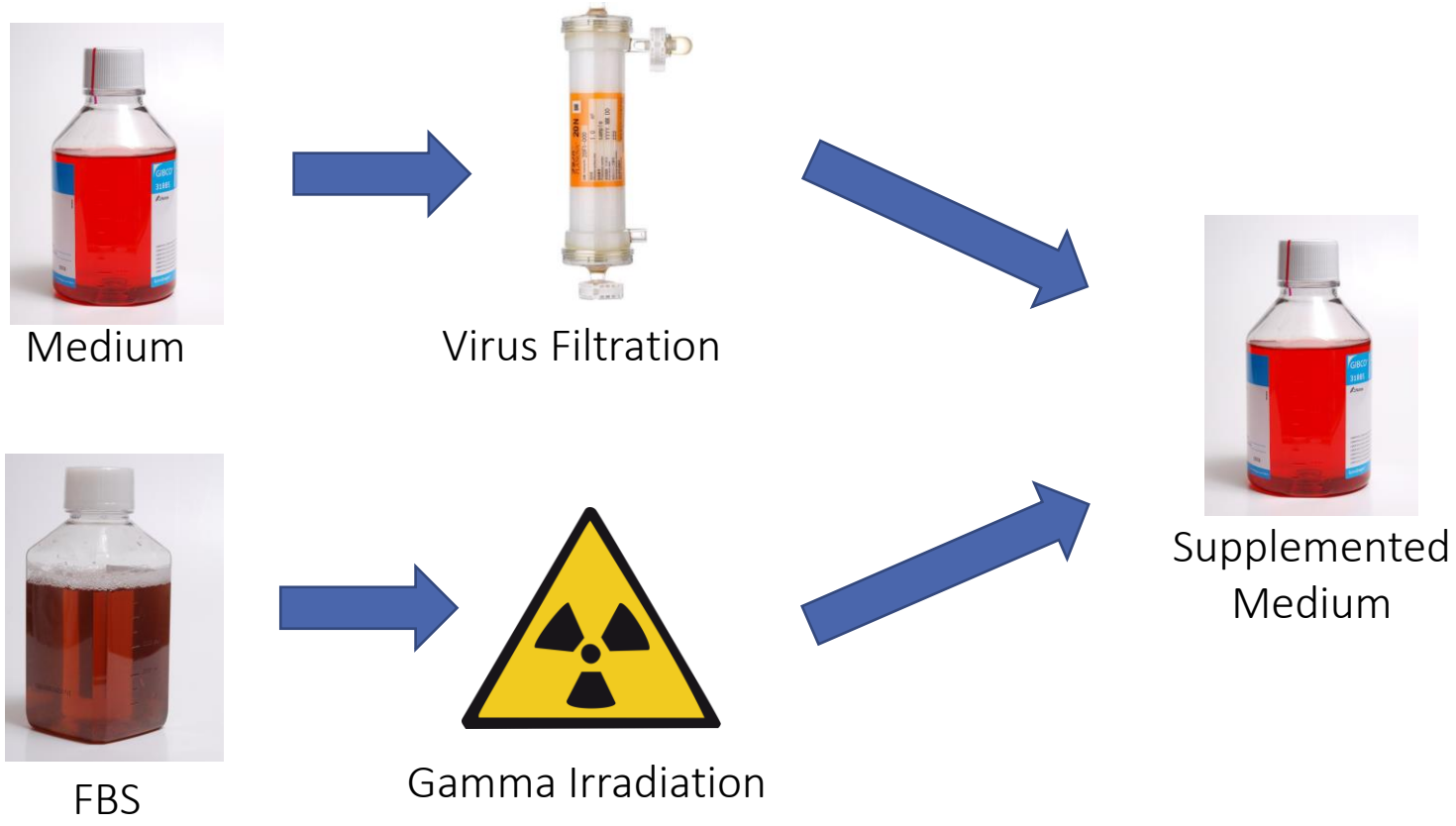
The volume you would need to test to have a 95% probability of detecting the contaminant would be impossible for most tests

The virus contaminant would go undetected but is high enough to initiate an infection in a bioreactor

Barrier technologies become critical for controlling such low risks

Combined Strategies for Multi-Component Medium

- For multi-component medium, strategies where components are treated separately can also be considered



3

Platform Approaches (Prior Knowledge)

ICH Q5A (R2)

Platform Approaches:

- The ICH Q5A revision contains more details around how platform approaches can be considered in reducing the amount of spiking studies required
  - *Process steps dedicated to virus clearance (e.g., inactivation by detergent, low pH and removal by viral filtration) are suitable for a platform validation approach.*
  - *Factors that impact efficient retrovirus removal by small-virus filters are well understood with respect to variation of process parameters such as membrane type, flow- or pressure-controlled filtration mode, and pressure interruptions. Based on predictability and robustness of virus removal this process step is considered suitable for a platform validation approach.*
  - *For virus removal using small virus filters, one option is to apply parvovirus log reduction values for larger spherical/icosahedral viruses and enveloped viruses. However, sometimes this could result in underestimating virus removal capacity (e.g., retrovirus removal capacity) as a result of parvovirus passage. Given the size-based mechanism of action, and industry's experience of robust complete retrovirus removal with small virus filters, companies could use their in-house data from parvovirus and retrovirus removal to build a platform retrovirus clearance claim for commonly used small virus filters.*

ICH Q5A (R2)

Platform Approaches (cont.):

- *A thorough understanding of the impact of pressure interruptions, as well as volume throughput and filter flush volume reflecting good manufacturing practice conditions should be conserved*

- *If using prior knowledge and in-house experience from other products to claim parvovirus removal, at least one confirmatory product-specific validation run using a parvovirus should be performed.*
  - i.e. these approaches help to reduce the burden of virus clearance studies for manufacturers

- *The type of virus filter is important for virus reduction and its robustness with respect to impact of process parameters and should be considered when designing platform data.*

4

Continuous Biomanufacturing

ICH Q5A (R2)

Continuous Biomanufacturing:

- The ICH Q5A revision contains a significantly expanded section on continuous manufacturing and how to implement virus clearance studies for such processes
- *Chromatography:*
  - *For the process of repeating sub-batches (e.g., multi-column), a batch process could serve as a scale-down model with well-justified target process conditions (e.g., flow rate, resin load vs column overload, resin cleanability);*
  - *Simultaneous validation of two or more connected unit operations could be an option according to the equipment design and system integration (e.g., bind and elute mode of Cation Exchange Chromatography (CIEX) and flow through mode of Anion Exchange Chromatography (AEX)), but only when all unit operations are to be validated for viral clearance. For connected unit operations, if the loading of the challenge material does not differ from batch operation, it is possible to evaluate with a conventional scale-down model*
- *Virus filtration:*
  - *Validation as a batch process could be appropriate if settings of parameters which have impact on virus clearance do not vary beyond ranges tested in the virus clearance study (e.g., worst case setpoint)*

5

Testing:
Controlling the Contamination Load in a Manufacturing Process and the Importance of Robust Filtration/Virus Clearance

ICH Q5A (R2)

Use of Next Generation Sequencing

- *Next Generation Sequencing (NGS) and Nucleic Acid Amplification Techniques (NATs) such as Polymerase Chain Reaction (PCR) may be appropriate for broad and specific virus detection, respectively. The introduction of these tests may be done without a systematic head-to-head comparison with the currently recommended in vitro and in vivo assays. In particular, a head-to-head comparison is not recommended for in vivo assays to meet the intent of the global objective to replace, remove, and refine the use of animals. Because of the assay sensitivity and breadth of virus detection, NGS may also be used to replace cell-based infectivity assays, to overcome potential assay limitations, or to detect viruses without visible phenotypes in the assay system. Positive results should be investigated to determine whether detected nucleic acids are associated with an infectious virus.*
  - The reasons why a head-to-head comparison is not beneficial is explored in the following slides

- NGS provides broad ranging detection of all virus contaminants in a system, and can be used to establish the baseline risk in process samples (see following slides)
  - A validated NGS method detects a broader spectrum of potential contaminants than e.g. the traditionally uses in vitro adventitious agent test

Can the in vitro Adventitious Agent Test (AAT) Detect all Viruses?

How broad spectrum is the in vitro AAT test?

- The assumptions with the in vitro AAT test are that the utilisation of a number of different detector cell lines (normally 3 but sometimes 4) provides a high assurance that most viruses would be detected:
  - The assay has been successful in identifying a number contamination events, but not all

- **The reality though is that:**
  - We validate the test using cell culture adapted strains of virus that have been adapted over many generations to grow in the detector cell lines- titration on a completely different cell line yields a different titre!
  - In contrast, it is known that many wild type strains of virus will be detected at a much lower sensitivity and adaptation to cell culture normally requires several rounds of replication
  - The standard in vitro assay relies on 3 end-points:
    - Cytopathic effect
    - Haemadsorption
    - Haemagglutination

    There are though many non-cytopathic variants or viruses that do not cause any of these effects
  - Even where a specific antibody for virus detection is included, it is assumed that the antibody will cross-react with all variants, which is often not the case

Detecting MMV by Standard in vitro Assays

MMV detection in standard in vitro assays

Received: 21 August 2023 | Revised: 20 September 2023 | Accepted: 2 October 2023
DOI: 10.1002/bit.28573

ARTICLE

BIOTECHNOLOGY BIOENGINEERING WILEY

Detection of *Minute virus of mice* strains in different cell lines: Implications for adventitious agent testing

Maria R. Farcet[1] | Jens Modrof[1] | Gerhard Antoine[1] | Cecilie Klausen[1] | Astrid Kerschbaum[1] | Martina Kopp[2] | Houman Dehghani[3] | Thomas R. Kreil[1]

- Not all MMV strains are the same and replicate to the same extent using in vitro detection systems
- PCR was shown to be better suited for broad ranging detection of MMV at high sensitivity
  - NGS could also be considered here as a broad range detection method

"Silent" Infections

CONFERENCE PROCEEDING

Proceedings of the PDA/FDA Adventitious Viruses in Biologics: Detection and Mitigation Strategies Workshop in Bethesda, MD, USA; December 1–3, 2010
Guest Editors: Arifa Khan (Bethesda, MD), Patricia Hughes (Bethesda, MD) and Michael Wiebe (San Francisco, CA)

Mouse Minute Virus (MMV) Contamination—A Case Study: Detection, Root Cause Determination, and Corrective Actions

MARK MOODY*, WASHINGTON ALVES, JOSE VARGHESE, and FAZAL KHAN

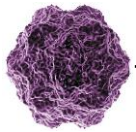
Merrimack Pharmaceuticals, Inc.

ABSTRACT: The production of biologic drugs using mammalian cell production systems offers the benefits of high yield, proper protein folding, and faithful post-translational modifications. However, mammalian cell culture is vulnerable to contamination with adventitious agents, including mouse minute virus (MMV). The case study presented here demonstrates that MMV is a ubiquitous threat to CHO (Chinese hamster ovary) cell-based production of biologic drugs and that animal-free media components can be a contamination source. Compounding the risk posed by MMV, the contamination may be "silent," with no impact on cell viability and product titers. Furthermore, contamination may not be detected using in vitro virus assays, and assays based on PCR (polymerase chain reaction) are required for reliable detection. The development of effective corrective and preventative action (CAPA) was greatly aided by the identification of the source of the contamination as an animal-free recombinant media additive. The execution of a CAPA that included disposal of contaminated materials, decontamination of the facility, and replacement of the contaminated raw material allowed the resumption of MMV-free production.

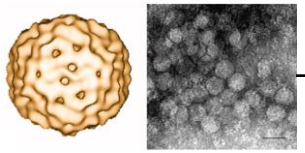
- Silent MMV infections can only be detected by PCR

Non-Cytopathic Viruses (Silent Infections)

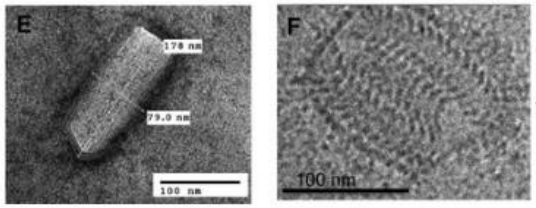
Virus Contaminant



Mouse minute virus (MMV)



PCV



Sf9 Rhabdovirus

Vesivirus 2117

How Identified?

- Examples of silent, non-cytopathic contamination events with MMV have been reported

PDA Journal of Pharmaceutical Science and Technology

Mouse Minute Virus (MMV) Contamination—A Case Study: Detection, Root Cause Determination, and Corrective Actions

Mark Moody, Washington Alves, Jose Varghese, et al.

PDA J Pharm Sci and Tech **2011**, *65* 580-588
Access the most recent version at doi:10.5731/pdajpst.2011.00824

- PCV was only identified as a non-cytopathic contaminant growing in Vero cells using NGS
- The Sf9 Rhabdovirus grows without cytopathic effect and was only found by NGS
- Vesivirus 2117 has a very weak cytopathic effect and carries the possibility of being missed

- All of these examples demonstrate that the standard *in vitro* adventitious agent assays carry the risk that not all viruses will be detected

Limiting the Virus Load: Parvovirus B19

A real-life example from the plasma industry

THE JOURNAL OF AABB
transfusion.org
TRANSFUSION

High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation

Thomas Weimer, Sigrid Streichert, Charles Watson, Albrecht Gröner

First published: 24 November 2002 | https://doi.org/10.1046/j.1537-2995.2001.41121500.x |
Citations: 48

- Parvovirus B19 is a common infection in plasma donors, with plasma titres sometimes as high as 10^{13} per ml of plasma
- Prior to the implementation of high titre screening by PCR, manufacturing pool levels of B19 could be as high as 10^{6}-10^{8} per ml
- As much as 10,000 ml of plasma is required to manufacture a single dose of product, resulting in a virus loads of 10^{10}-10^{12} per dose
  - Such titres overloaded the manufacturing process resulting in transmissions
- With the introduction of PCR and limiting the manufacturing pool load to no higher than 10^{4} IU/ml, the safety of plasma products with respect to B19 has been significantly improved

Implementing the ICH Q5A in Practice?

The Safety Tripod

- The safety tripod involves complementary mechanisms to provide assurances with regards to virus risk
  - Sourcing: All risks from any inputs into the manufacturing process need to be evaluated and measures implemented to minimise risks
  - Testing: Complements the sourcing and confirms that indeed the input risks have been appropriately controlled- testing should establish the baseline residual risk
  - Clearance: Sourcing and testing can never eliminate risk 100%- at best they define a baseline to ensure that any downstream steps for virus clearance are not overloaded
    - Understanding the robustness of the virus clearance steps provides the assurance that this residual risk is indeed effectively controlled

- Ensuring that any adventitious agent would be effectively detected by the testing methods is one of the key elements of the new ICH Q5A (R2) guideline:
  - NGS can play an important role here in ensuring that all adventitious agents are detected

Comparison of NGS with current testing portfolio

Parameter	In vivo AAT	MAP/HAP	In vitro AAT	9 CFR	Retroviruses (PG-4)	TEM	RT activity	qPCR	NGS
Detection of unknown virus	✓	-	✓	-	✓	✓	✓	-	✓
Detection of replicating virus	✓	✓	✓	✓	✓	-	-	-	✓/ -
Identification of virus	-	✓	-	✓	-	-	-	✓	✓
Specificity of Detection	unspecific	specific	unspecific	specific	unspecific	unspecific	unspecific	specific	Unspecific
Sensitivity	+	+	+++/???	+	+	-	+++	+++	+++

- Traditional testing portfolio is a good set-up to minimize the risk of adventitious viral contaminations
  - Very time consuming process to get to the final results
- NGS combines the advantages of most assays:
  - Non-targeted approach → detection and identification of all contaminants (including silent infections)
  - High sensitivity
  - Depending on the set-up it can already give an indication on whether virus is actively replicating or not
  - It establishes a baseline for “all” possible virus contaminants which can then be directly related to virus clearance data, not just for retroviruses but for all virus types

Overall Risk- Combining Testing with Clearance

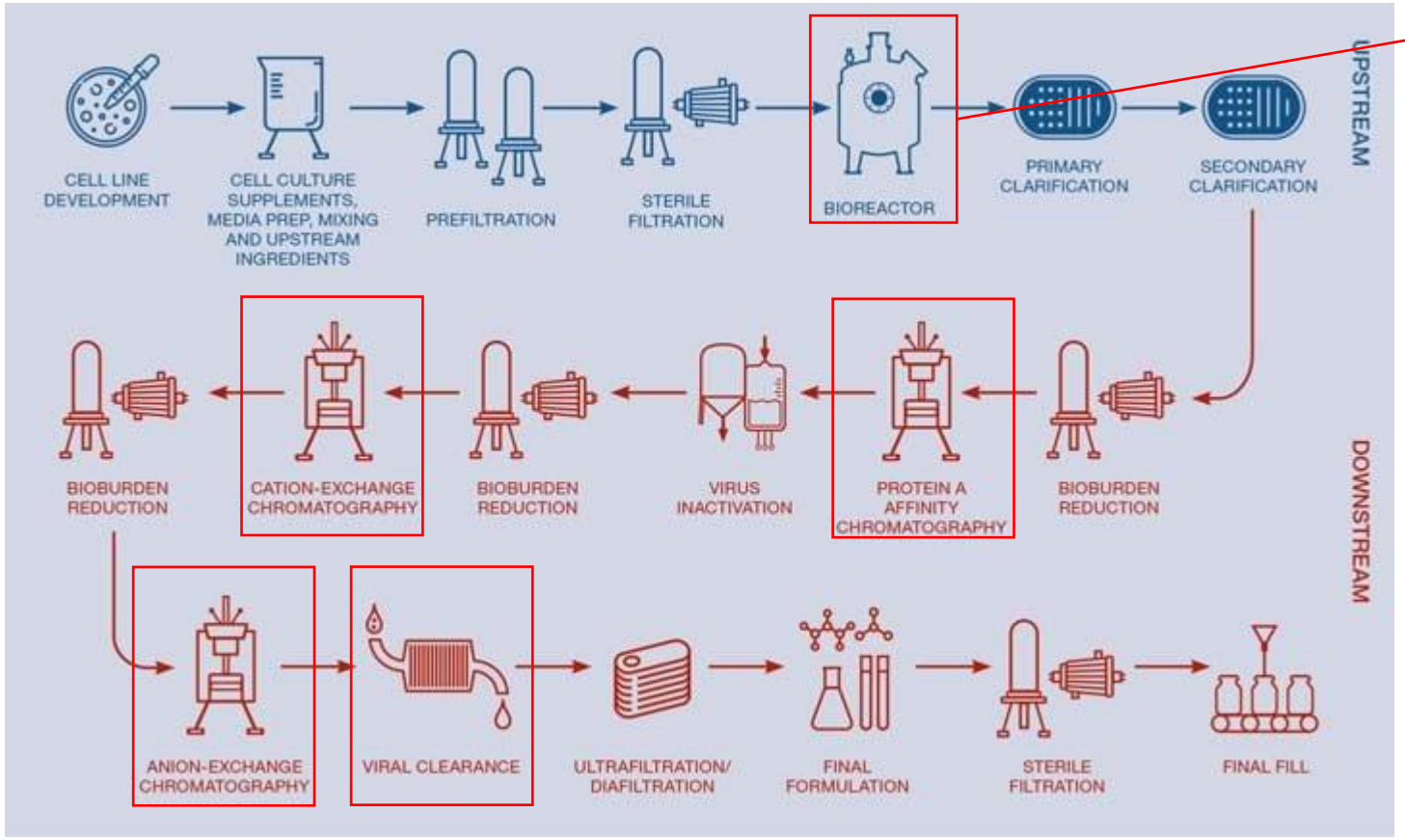


Image courtesy of: https://www.thechemicalengineer.com/features/an-introduction-to-the-biopharmaceutical-industry/

- The goal of testing at the bioreactor stage is to ensure that the capacity of the manufacturing process to clear virus is not overloaded
  - NGS is ideally suited for this
- Typical RFs for small resistant viruses (e.g. MMV):
  - Protein A: ~2 logs
  - CEX chromatography: 0-4 logs
  - AEX Chromatography: 0-4 logs
  - Virus filtration: 5 logs
- Worst case overall RF: ~7 logs
- Once the virus load exceeds 7 logs, the capacity of the manufacturing process is exceeded without any margin of safety
- The robustness of the individual steps is a key component of the safety profile

ICH Q5A (R2) and Filtration- Key Messages

Impact of the new ICH Q5A R2 guideline

- The new ICH Q5A R2 guideline has bought biosafety testing up the current state of the art and provides a balanced approach to virus safety that enables manufacturers to reduce the burden of virus safety testing:
  - Expanded scope beyond only recombinant biopharmaceutical products
  - Using risk management to reduce the burden of testing for adventitious agents
  - Replacement of outdated, insensitive assays like the in vivo tests with new technologies like NGS
  - Defining how platform data can be effectively used to reduce the burden of virus clearance studies for new products
  - Expanded sections for continuous manufacturing processes

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Благодаря 谢谢 Tack धन्यवाद Dziękuję

Спасибо Thank You Obrigado

Děkuju Grazie Ευχαριστώ Merci Köszönöm

ありがとうございました Teşekkür ederim