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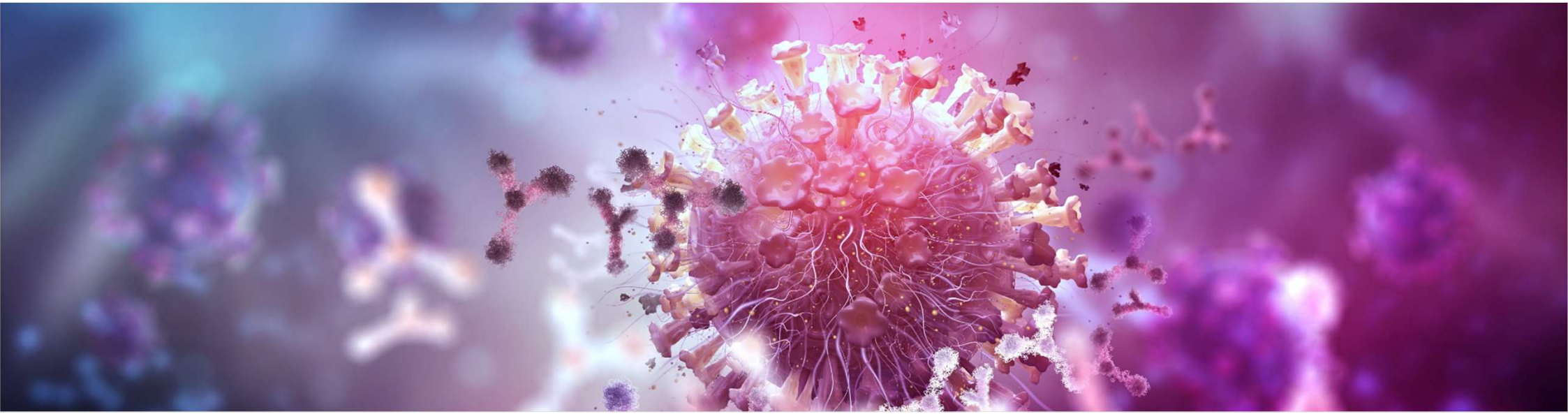

ICH Q5A R2 - viral safety evaluation of biotechnology products derived from cell lines of human or animal origin – ein Überblick

AQPA Vereinstreffen, Wien, 15. Mai 2025
Regine Tomasits, ViruSure GmbH

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PLANOVA™

Virus Removal Filters

Assurance Beyond Expectation

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Microfilters

FLUID MANAGEMENT

Oligonucleotide Synthesis

MOTIV™ Inline Buffer Formulation

Chromatography

Virus Filtration

Built For You™

BIOSAFETY TESTING SERVICES

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Virus/Prion Clearance Studies

bioniique

Mycoplasma Testing Services

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BIOLOGICS DEVELOPMENT & MANUFACTURING

Bionova Scientific

End-to-end Process Development

GMP Manufacturing

Where concept becomes cure

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ViruSure Facilities

In vitro Testing Facility

Tech Gate Technology Park

- ~ 2000 m^2 state of the art laboratory (BSL 2 + BSL-3) and lab office space
- Strict segregation of air handling for different labs (HEPA filtered)

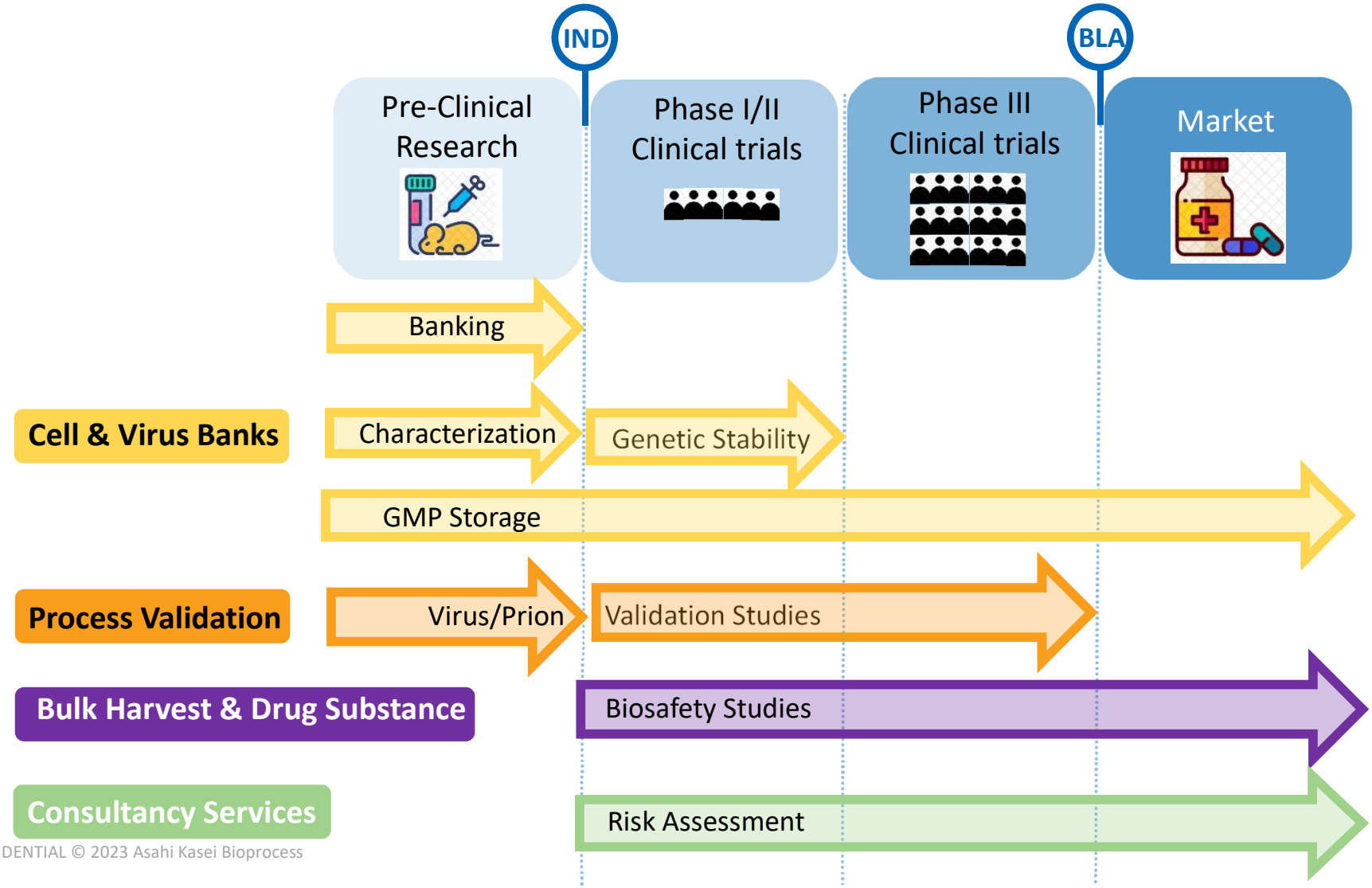
Animal Facility

Boku Biotech Site

- In 2017 constructed space dedicated to ViruSure
- ~190 m^2 animal rooms for *in vivo* experiments
- Individual air handling for each cage (IVC's)

- GMP/GLP certification of both facilities
- Inspections are performed every 3 years

viral safety – Testung und Evaluierung bei ViruSure



viral safety – Testung und Evaluierung bei ViruSure

Cells & Virus Banks

- Banking
- Storage
- Characterization (e.g., mycoplasma, sterility, TEM)

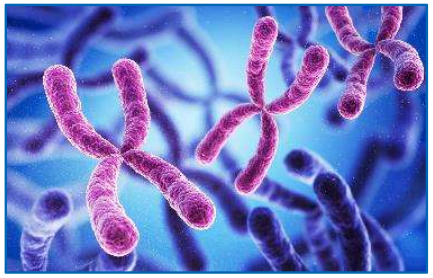
***In vitro* Safety Studies**

- Adventitious Agent testing
- Retrovirus Infectivity Testing
- 9CFR Bovine/Porcine in *vitro* Assay

***In vivo* Safety Studies**

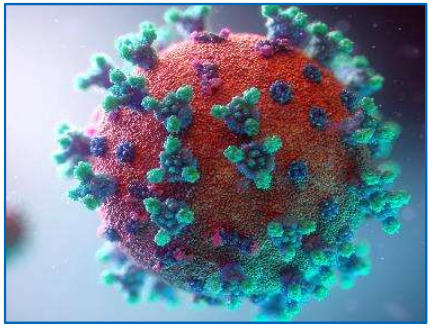
- Adventitious Agent testing
- MAP/HAP
- Tumorigenicity & Oncogenicity Studies
- Biodistribution Studies for ATMPs
- *In vivo* prion studies

viral safety – Testung und Evaluierung bei ViruSure



Molecular Tests

- qPCR for Virus/Pathogen Detection (> 130 qPCR)
- Identity Testing
- FPERT
- Genetic Stability/Sequencing
- NGS



Virus/Prion Clearance Studies

- Virus Clearance studies (phase I/II & phase III clinical trials)
- Prion Western Blot Testing Services
- *In vivo* Prion Bioassays

Consultancy Services

- Biosafety Risk Assessments
- Expert Reports

Agenda

1. Kontrolle & Risikomitigation als zentrales Prinzip
2. ICH Q5A(R2) – Was ist neu?
   a. Neue Produktklassen
   b. Neue Testmethoden
   c. Neuerungen in der Virus-Clearance-Validierung
   d. Weiterentwicklungen bei “Herstellungsverfahren”
   e. (Neue Definitionen)
3. Die ICH Q5A(R2) Prinzipien und die QP
4. Herausforderungen für die Umsetzung der ICH Q5A (R2)?
5. Zusammenfassung

Quellenangaben

- **ICH Q5A (R2) final version was adopted by ICH on 1 November 2023 & Step 4 Presentation**
  https://www.ich.org/page/quality-guidelines

- Q5A(R2)

  Training Materials

  Q5(R2) Training Materials Module 1-3, developed in April 2025.

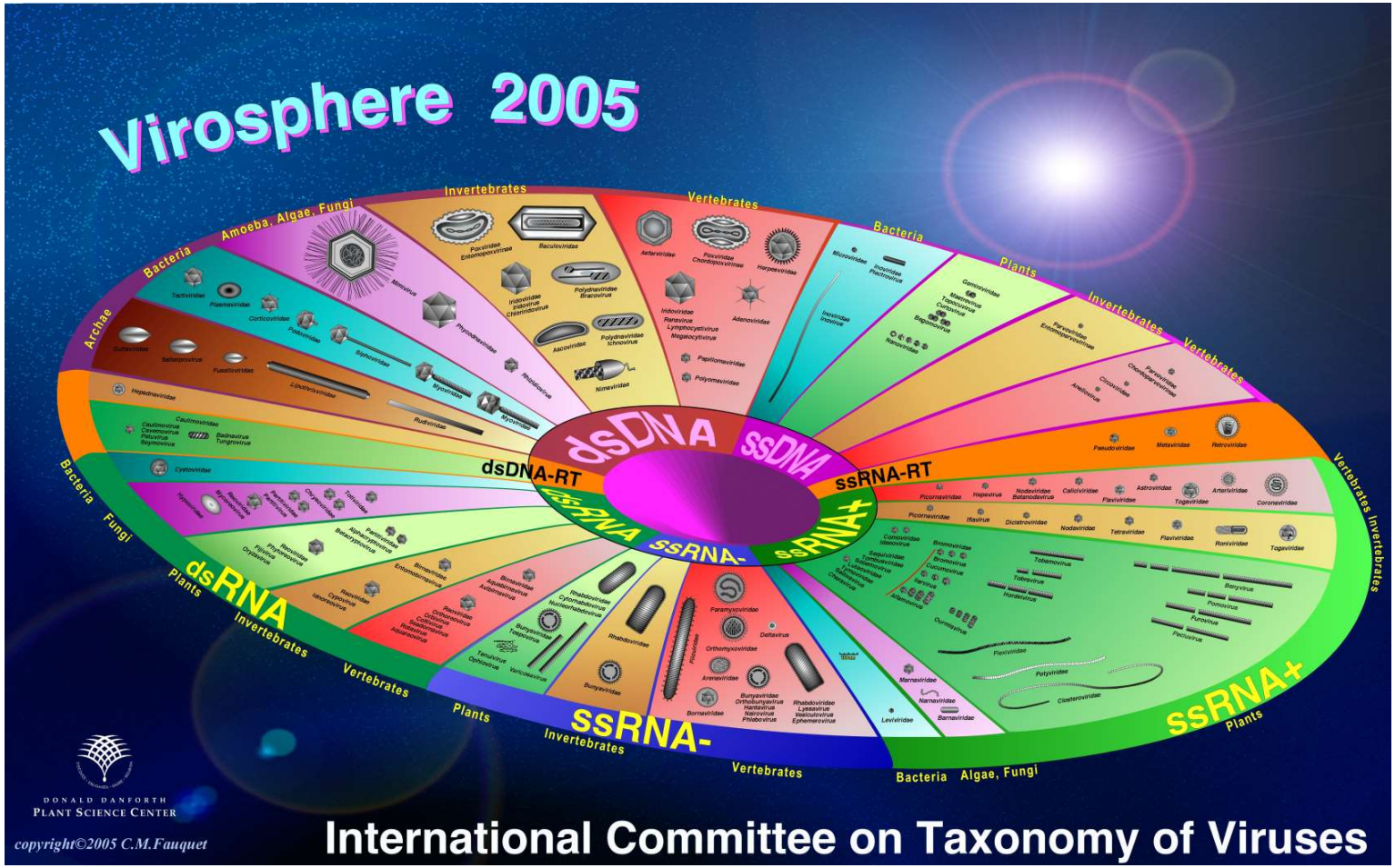
  https://www.ich.org/page/training-library

- 4th ViruSure Workshop, Vienna, April 2025

  Andy Bailey, CEO ViruSure GmbH

  - Practical considerations in application of the new guideline to ensure virus safety
    (slide 9, 15, 16, 17, 18, 21, 26, 27)

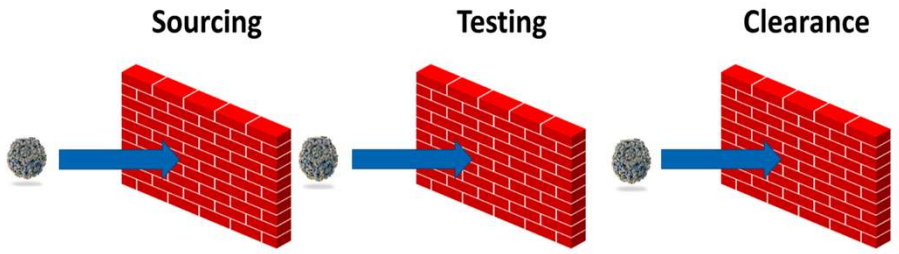
The Realm of Viruses



- **Viren verschiedenster Formen und Größen sind in allen Bereichen des Lebens zu finden.**
- **Eine Testung jeder möglichen Kontamination ist daher unmöglich.**
- **Darum erfordern Maßnahmen zur Kontrolle der Virussicherheit einen ganzheitlichen Ansatz!**

1. Kontrolle & Risikomitigation als zentrales Prinzip

- *three principal, complementary approaches to control potential viral contamination*
  - *Selecting and testing* ***cell lines and other raw materials****, including* ***media components*** *for ensuring the absence of undesirable infectious viruses*
  - *Assessing the* ***capacity of the production processes*** *to clear adventitious and endogenous viruses*
  - *Testing the* ***product*** *at appropriate steps of production for the absence of contaminating infectious viruses* *ICH Q5A*



- virengeprüften Zelllinien
- tierfreie Medien

- Bekannte und unbekannte Viren frühzeitig zu identifizieren

- Inaktivierung (pH, Temp)
- Entfernung (Filtration, Chromatographie, Herstellverfahren)

durchgängiges, mehrschichtiges Kontrollsystem
case-by-case & step-by-step

Viral Safety Assessment
Es umfasst präventive, kontrollierende und korrektive Maßnahmen über die gesamte Entwicklung & Herstellung eines biotechnologischen Produkts – QP-Relevanz

2. ICH Q5A(R2) – Was ist neu?

Key updates (Step 4 presentation)

Section	Title	Comment
Section 1	Introduction	Major Changes
Section 2	Potential Sources of Viral Contamination	Minor Changes
Section 3	Cell Line Qualification: Testing for Viruses	Major Changes
Section 4	Testing for Viruses for Unprocessed Bulk	Major Changes
Section 5	Rationale and Action Plan for Viral Clearance Studies and Virus Tests on Purified Bulk	Major Changes
Section 6	Evaluation and Characterisation of Viral Clearance Procedures	Major Changes
Section 7	Points to Consider for Continuous Manufacturing	New
Section 8	Summary	Minor Changes
Section 9	Glossary	Major Changes
Section 10	References	New

Annex	Title	Comment
Annex 1	The Choice Of Viruses For Viral Clearance Studies	Minor Changes
Annex 2	Statistical Considerations For Assessing Virus And Virus Reduction Factors	Minor Changes
Annex 3	Calculation Of Reduction Factors In Studies To Determine Viral Clearance	Minor Changes
Annex 4	Calculation Of Estimated Particles Per Dose	Minor Changes
Annex 5	Examples Of Prior Knowledge Including In-house Experience To Reduce Product-specific Validation Effort	New
Annex 6	Genetically-engineered Viral Vectors And Viral Vector-Derived Products	New

- Section 1 / Annex 6 – Produkte
  - Impfstoffe, monoklonalen Antikörper
  - Zell- und Gentherapeutika
- Section 3-5 - Test Methoden
  - Molecular Methods
  - e.g. Next Generation Sequencing (NGS); *Ph.Eur. Chapter 2.6.41. High-Throughput Sequencing*
- Section 6 / Annex 5 – Alternative Virus Clearance Validierungs Strategien
  - prior knowledge and Platform Technologie
- Section 7 - Entwicklungen bei Herstellungsverfahren
  - e.g. continuous manufacturing

QP-Relevanz

2a. ICH Q5A (R2)- Produktklassen

Product Categories/Types	Examples
Included: • Products derived from in vitro cell culture using recombinant DNA technologies: • **Genetically-engineered viral vectors and viral vector derived products provided they are amenable to viral clearance** Excluded: • Inactivated vaccines • All live vaccines containing self-replicating agents • Products derived from hybridoma cells grown in vivo as ascites • **Genetically-engineered viral vectors provided they are not amenable to virus clearance** • Cell therapies	Included: • mAbs • Recombinant proteins • Recombinant subunit vaccines • Certain vaccines • Cytokines • **Helper-dependent recombinant AAV and recombinant AAV produced by transient or stable transfection** • **Baculovirus produced Virus Like Particle (VLP) vaccines and gene therapies e.g., baculovirus expressed recombinant AAV vector (genetically engineered viral vector)** • **Protein subunits expressed in baculovirus (genetically engineered viral vector derived product)** Excluded: • Inactivated viral vaccines • Live attenuated vaccines: Measles, Mumps, Rubella • Cell therapies • **Viral vectors not amenable to viral clearance such as Retroviral vectors e.g., Lentivirus**

[a] Bold text indicates new product types that are included or excluded in the Guideline

- **Genetisch veränderte virale Vektoren**
- **Produkte viraler Vektoren**

Voraussetzung - Viral-Clearance Strategien / Prozesse sind anwendbar

- bei Virus-ähnliche Partikel (VLPs) und Protein-Subeinheiten

diese Viral-Clearance Strategien sind nicht für alle dieser Produkte anwendbar

- zusätzliche Risiken durch Hilfsviren oder replikationskompetente Partikel

→ Evaluierung von weiteren Maßnahmen zur Virus Risiko Reduktion – Annex 6

2b. Neue Test Methoden

Section 3. - CELL LINE QUALIFICATION: TESTING FOR VIRUSES 3.1.1. Master cell Bank
"…Testing for adventitious viruses should ***include both broad and specific virus detection assays*** *as described in Table 1.*

- *In vitro / in vivo Assays* ***or NGS***
- *e.* ***Non-targeted NGS*** *can replace the in vivo assay (Section 3.2.3) and supplement or replace the in vitro assay (Section 3.2.2).*
- *f. …Methods such as cell culture-based infectivity assays, antibody production tests (MAP, HAP, RAP),* ***virus specific NAT or other molecular methods*** *e.g., NGS can be used.*

Section 3.2.5 - Molekulare Methoden

- Polymerase Chain Reaction (PCR)
- **Next Generation Sequencing** (NGS)
  - NGS als bevorzugte Methode zur Virusdetektion empfohlen - übertrifft Sensitivität und Breite traditioneller Tests
  - NGS fördert die Reduzierung von Tierversuchen (3R-Prinzipien *Replace, Reduce, Refine*)
  - NGS kann in vivo Tests und vitro Tests für adventive Viren ersetzen
  - Head-to-head-Vergleich von NGS mit etablierten Methoden nicht gefordert /nicht empfohlen für *in vivo assays*
    - NGS soll Modellviren mit ausreichend hoher Empfindlichkeit erkennen
    - NGS ist als Limit Test (ICH Q2) zu betrachten

2b. Neue Test Methoden

Application of Next Generation Sequencing in these Examples

NGS using non-targeted analysis was used to Replace the In Vivo Assays

The approach used in this example was based on the recommendations of the ICHQ5A R2 guideline: 'Non-targeted NGS can be used to replace the *in vivo* assays and supplement or replace the *in vitro* cell culture assays, without a head-to-head comparison as long as the method is demonstrated to be suitable for its intended purpose. A head-to-head comparison is not recommended due to the different end points of the assay systems and limitations of the breadth of virus detection by the *in vitro* and *in vivo* methods compared to the enhanced capability of NGS for broad virus detection of known and unknown viruses. The results of the *in vitro* and *in vivo* assays rely on virus replication and biological properties for detection in the specific target system which limits the breadth of detection. Replacement of *in vivo* assays by NGS also meets the intent of the global objective to replace, reduce, and refine the use of animals for testing'. Due to the application as a Replacement Assay, the validation package for the NGS assay is provided to demonstrate the assay suitability

- The NGS assay is demonstrated as a scientifically sound method and validated as a limit test, in accordance with, ICHQ2 and as described in the Ph.Eur. 2.6.41 draft general chapter.
- Matrix specific verification was performed to confirm the absence of interference. See Ph.Eur. 2.6.41 draft general chapter (1).
- References used to support not performing the head to head comparison include Charlebois 2020 (2), Gombold (3) and Pei-Ju Chin (2024) in progress (13)

NGS using targeted analysis was used to Replace the Species-Specific Virus Assays

The approach used in this example was based on the recommendations of the ICHQ5A R2 guideline for targeted NGS to replace the cell-based assays and antibody production assays, Mouse Antibody Production (MAP) test, Rat Antibody Production (RAP) test, and Hamster Antibody Production (HAP) for specific virus testing and Nucleic Acid Amplification Technique (NAT) virus testing, without a head to head comparison as long as the method is demonstrated to be suitable for its intended purpose. In this example NGS also complements the *in vitro* cell culture infectivity assays for general adventitious virus detection to enhance the breath of detection and potential assay limitations.

- To demonstrate the suitability of NGS to replace the species-specific virus assay, the example uses the same dataset already established for the non-targeted assay used for the replacement of the in vivo assay by applying bioinformatic analysis to the original non-targeted dataset, by interrogating the dataset for the specific viruses to be detected.
- The use of targeted NGS can help to overcome the limitation of NAT for the detection of virus variants.
- Due to the application as a Replacement Assay for specific virus testing, the validation package for the NGS assay is provided to demonstrate the assay suitability.
- Matrix specific verification was performed to confirm the absence of interference. See Ph.Eur. 2.6.41 draft general chapter (1)

ICH Q5A R2 Training p24

2b. Neue Test Methoden - Vergleich NGS & traditionellen Test-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
Detection of non-cytopathic viruses?	✗	✗	✗	✗/✓	✗	✗	✗	✗	✓
Sensitivity	+	+	+++	+	+	-	+++	+++	++

- Traditionelles Test-Portfolio ist effektiv, aber nicht für alle Kontaminationen
- Zeitintensiv
- Live virus vectors – sehr schwierige Systeme

- NGS kombiniert viele Vorteile:
  - Non-targeted approach → Detektion und Identifikation aller Kontaminationen
  - High Sensitivity

2b. Neue Test Methoden - Detecting Virus at Low Concentrations

Total volume of medium or component (1,000ml)

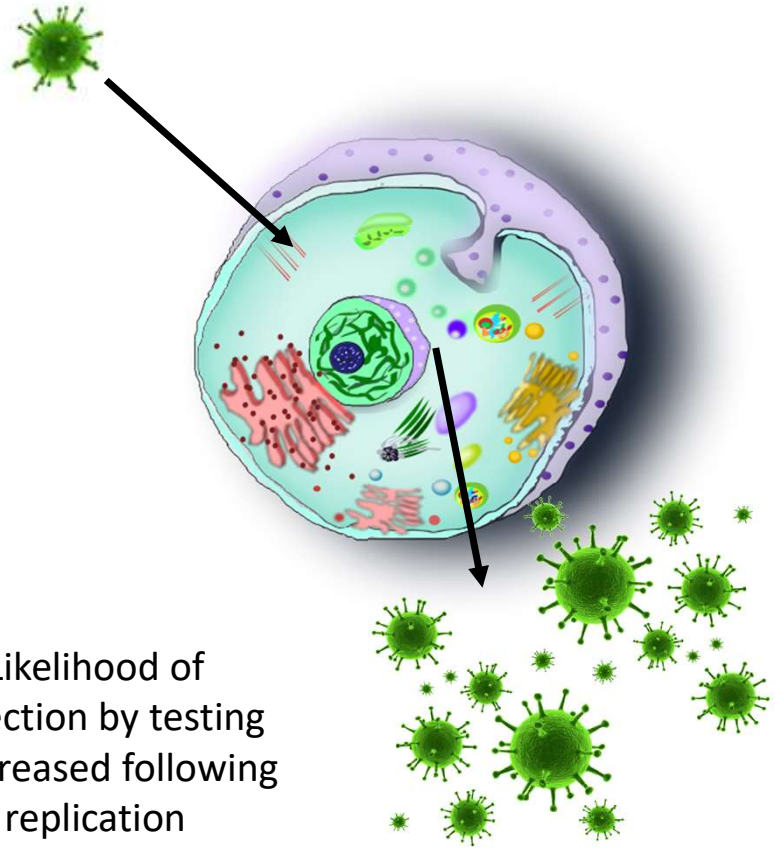
Volume for testing: 10ml

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
- **Understanding both the LOD of a given test as well as the specificity (i.e. what can be detected) are key to establishing the *baseline risk* for virus contamination in a product**

2b. Neue Test Methoden – Infectivity Assays

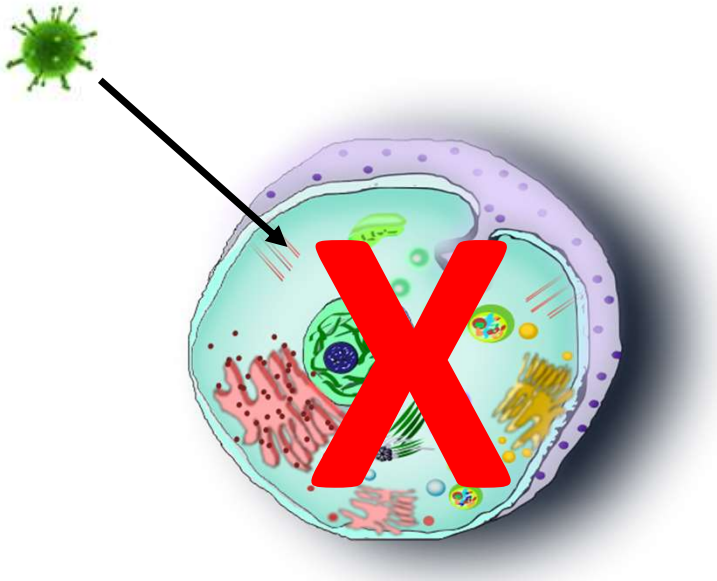
Permissive Cell Line



Likelihood of detection by testing is increased following replication

Either scenario speaks to the implementation of upstream barrier technologies

Non-Permissive Cell Line

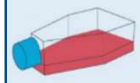


Detectability of a low level virus contamination highly dependent on the LOD and specificty of the assay

2b. Neue Test Methoden - NGS – a case study

OOS Investigation

Identification of contamination



Cell Culture

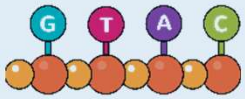
- Limited specificity
- Long

TEM

- Reveals the virus structure
- Low sensitivity

PCR

- High specificity
- Extensive list of potential qPCR



NGS

- High specificity
- Sequence agnostic
- Quick TAT

Virus Safety in ICH Q5A R2

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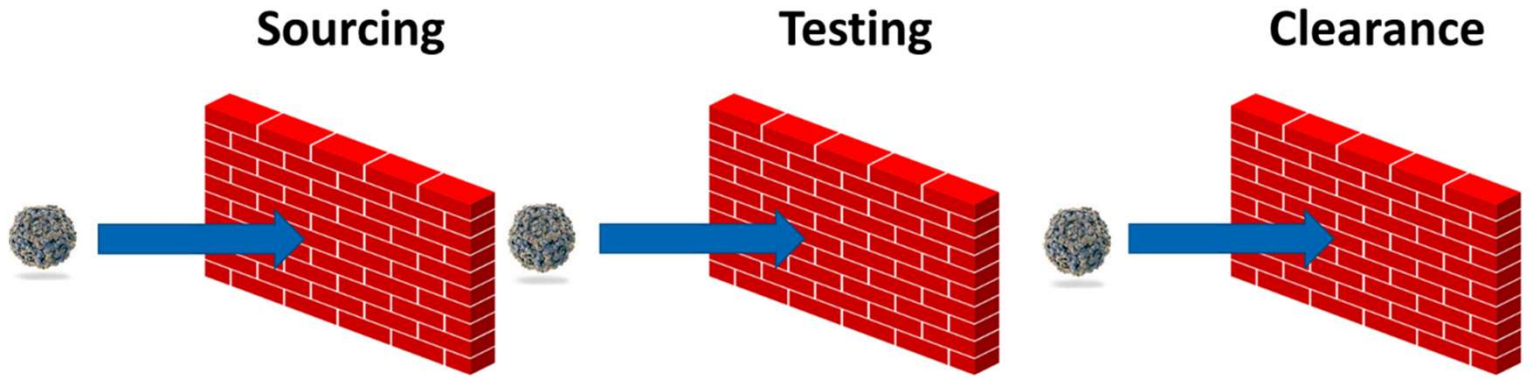
mehrschichtiges Kontrollsystem
zur Sicherstellung einer ausreichenden Risikoreduktion



Prior Knowledge & Platform Technologie

- - angewandtes, modernes Risikomanagement – QP-Relevanz
  - internes Wissen
  - externes Wissen
  - wissenschaftliche Prinzipien

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2c. Prior Knowledge & Platform Technologie

Platform manufacturing (ICH Q11)

- *The approach of developing a production strategy for a new drug starting from manufacturing processes similar to those used by the same applicant to manufacture other drugs of the same type (e.g., as in the production of mAbs using predefined host cell, cell culture, and purification processes for which considerable experience already exists).*

Platform validation

- *Throughout this guideline, this term exclusively refers to validation of the process platform regarding viral clearance. In this context, platform validation is defined as the use of prior knowledge including in-house experience with viral reduction data from other products, to claim a reduction factor for a new similar product, according to current understanding.*

ICH Q5A (R2)

ICH Q5A (R2) and Platform Approaches

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Platform Approaches- Where can this be applied?:

- *There should be an understanding of the mechanism underlying viral clearance.*
- *There should be comprehensive understanding of the process parameters that may affect viral clearance.*
- *It should be clear that interactions between virus and product do not affect viral clearance. If there is a potential risk that the virus-product interaction may affect viral clearance, applying prior knowledge from manufacturing of other products should be justified. If data for more than one product is available for the specific step, the effectiveness of virus reduction should be comparable in each case.*
- *The composition of a specific process intermediate may affect viral clearance. For some process steps, even small differences in buffers, media, reagents, and profile of impurities (for example) may affect viral clearance. Therefore, the representativeness of the composition of the process intermediate(s) from other products should be justified. Processing before the specific step for the new and the established product(s) should follow a similar strategy unless prior knowledge indicates robustness of viral clearance with respect to composition of the process intermediate.*
- *The general limitations of viral clearance studies as outlined in Section 6.4, should be considered when applying prior knowledge to a specific product.*

ICH Q5A (R2) Chapter 6.6

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2c. Prior Knowledge & Platform Technologie

“prior knowledge” – ein angewandtes, modernes Risikomanagement zur Festlegung des den Umfang von Virus-Clearance-Studien

- für vergleichbare Produkte aus etablierten & charakterisierten, repräsentativen Prozessen (platform approach)
- Voraussetzungen zur systematischen Nutzung ist ein belegtes, fundiertes Wissen zu
  - gut charakterisierten Zelllinien
  - etablierten Herstellprozessen (mehrere, nicht ein klinisches Entwicklungsprodukt)
  - validierten Virusinaktivierungsverfahren
  - publizierter Literatur und regulatorisch akzeptierten Daten zu ähnlichen Produkten (unterstützend)
  - Limitationen sind zu evaluieren (Section 6.4)
  - Prozess Change Control & Re-Evaluierung

Aufwandminimierung ohne Produktsicherheitsverlust

Virus Safety in ICH Q5A R2

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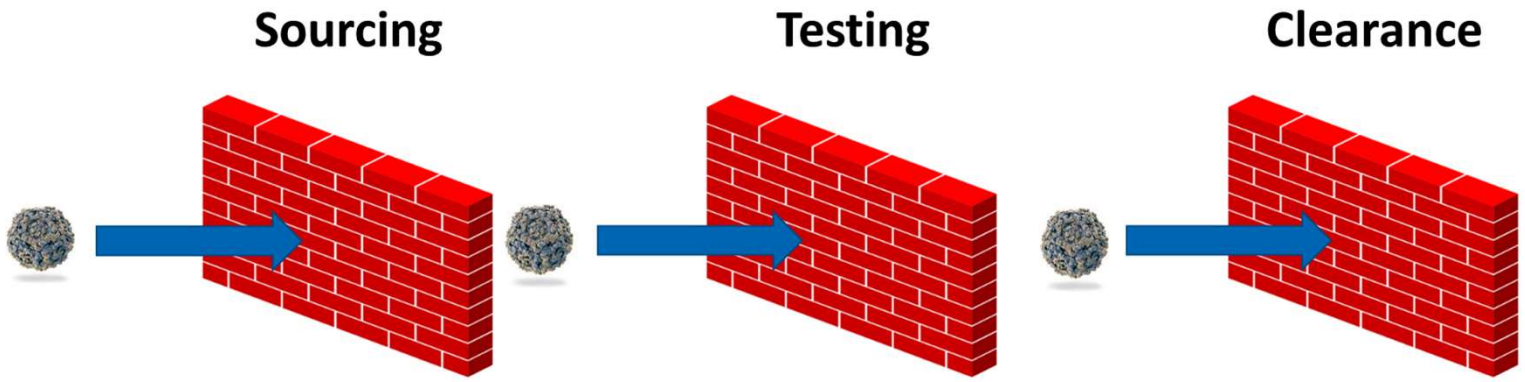
mehrschichtiges Kontrollsystem -
zur Sicherstellung einer ausreichenden Risikoreduktion



7. Points to consider for continuous manufacturing

"CM may involve the continuous feeding of input materials into a manufacturing process comprised of a series of linked unit operations that transform the feed and provide a continuous stream of an output material (i.e., product)." …

"An understanding of the integrated process and its dynamics, in addition to each unit operation, is essential to identify and mitigate the risk to viral safety."

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2d. Herstellungsverfahren - Continuous Manufacturing (CM)

Spezifische “viral safety considerations”

- sind in Verbindung mit ICH Q13 zu lesen
- zu berücksichtigende Aspekte sind u.a.
  - welche Prozessschritte erfolgen kontinuierlich?
  - mögliche Anwendung von Erfahrungswerten & *prior knowledge* aus der Batch Produktion
  - Einfluss der Dauer der Zellkultur
  - Monitoring von Prozessparametern, um Störungen rasch zu erkennen und darauf zu reagieren
  - …

Eine gleichwertige oder überlegene Virussicherheit im Vergleich zur Batch Produktion ist nachzuweisen.

3. Die ICH Q5A(R2) Prinzipien und die QP

Warum sollte die QP mit den ICH Q5A(R2) Prinzipien vertraut sein?

Die Virussicherheit und Virusabreicherung von biotechnologischen Produkten ist ein wesentlicher Aspekt der

- regulatorischen Compliance
  - Anforderungen an die Daten für die Markteinreichung
- Produktqualität von biotechnologischen Produkten
  - Viral Safety Assessment
  - Risikobasierter Ansatz

Die beschriebenen Neuerungen bilden den wissenschaftlichen Fortschritt ab

- Neue Technologien
- Previous knowledge
- Platform approach
- Continuous Manufacturing

Herausforderungen für die Umsetzung der ICH Q5A (R2)?

Die neue ICH Q5A R2-Richtlinie hat die viral safety Testung auf den Stand der Technik gebracht und ermöglicht durch Anwendung der bewährten Prinzipien, Risiko Mitigation und neuer Technologien die Aufwendungen für Virussicherheitsprüfungen zu reduzieren.

ABER …

- Wie ist die Akzeptanz fortschrittlicher Technologien bei Behörden und Industrie?
- Akzeptanz wird mit wachsender Vertrautheit & Wissens über neue Technologien steigen.
  - zB Validierung neuer komplexe Methoden wie NGS:
    Mangel an klaren Richtlinien zur Validierung der Technologie hat die Akzeptanz verlangsamt. Dies ändert sich derzeit mit dem Arzneibuchkapitel 2.6.41 und der Verfügbarkeit zertifizierter WHO-Virusreferenzstandards.

Zusammenfassung

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ICH Q5A(R2) Prinzipien

- Risikomanagement und ein Verständnis für Risiken spielen weiterhin eine entscheidende Rolle in den neuen ICH Q5 R2 Richtlinien.
- Die Umsetzung dieses Risikomanagements erfordert ein grundlegendes Verständnis für folgende Punkte:
  - Woher können Kontamination kommen?
  - Was sind die Einschränkungen der Nachweisgrenze (LOD) und der Spezifität aller durchgeführten Tests?
  - Welche Testmethoden sind die besten zur Kontrolle des Risikos?
    - Welche klaren Empfehlungen gibt es für bestimmte Teststrategien (NGS als Alternative zu in vivo-Tests)
  - Welches Restrisiko verbleibt nach dem Testen
  - Wie wird dieses Restrisiko durch nachgelagerte Prozesse (einschließlich Barriere-technologien und Virus-Clearance im Herstellungsprozess) kontrolliert?

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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

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