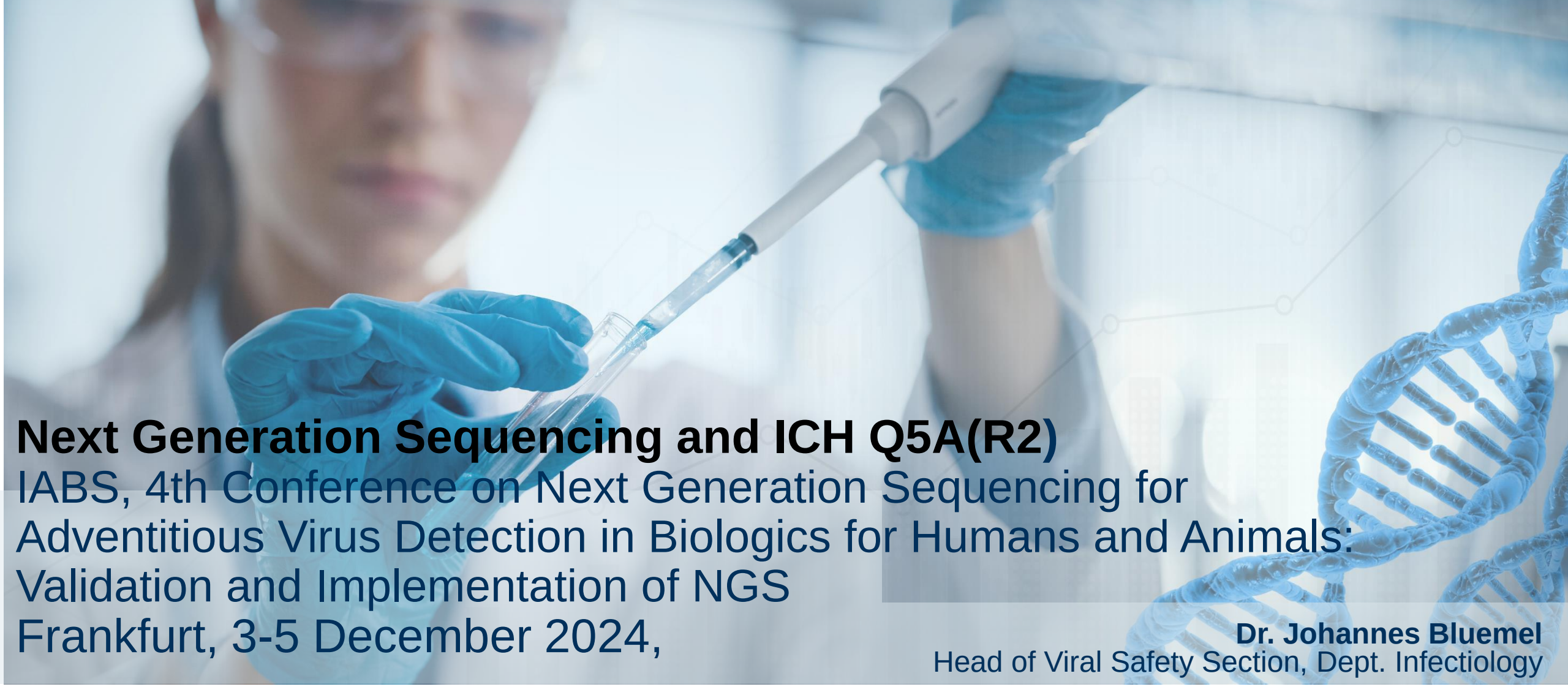

Next Generation Sequencing and ICH Q5A(R2)

IABS, 4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animals: Validation and Implementation of NGS

Frankfurt, 3-5 December 2024,

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Paul-Ehrlich-Institut

Das Paul-Ehrlich-Institut ist ein Bundesinstitut im Geschäftsbereich des Bundesministeriums für Gesundheit.

The Paul-Ehrlich-Institut is an Agency of the German Federal Ministry of Health.

ICH Q5A (R2) final version was adopted by ICH on 1 November 2023

What is new in Revision 2

1. **New product types** that are amenable to viral clearance (**including genetically engineered viral vectors and viral vector derived products**)
2. **New test methods** Potential Analytical technologies (e.g., **Next Generation Sequencing** [**NGS**])
3. Manufacturing (both maturation of the industry and the emergence of **continuous manufacturing**)
4. Alternative virus clearance validation strategies (including **prior knowledge**)

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.*

Introduction of new methodologies such as NGS for detecting a broad range of adventitious viruses is encouraged.

Table 1. Virus Tests Recommended for Characterisation of Cell Substrates

	MCB	*WCB*	*Cells at the LIVCA*
Tests for Retroviruses and Other Endogenous Viruses			
Retrovirus Tests[a]	+	-	+
Other Endogenous Virus Tests[b]	as appropriate[b]	-	as appropriate[b]
Tests for Adventitious Viruses			
In vitro Assays or NGS[e]	+	+[c]	+[c]
In vivo Assays or NGS[e]	+[d]	-	+[d]
Tests for Specific Viruses[f]	as appropriate[f]	-	-

a .Refer to Section 3.2.1 for additional details.

b. As appropriate for cell lines that are known to contain such agents.

c. The *in vitro* virus assay is performed directly on the WCB or on LIVCA cells directly derived from this WCB.

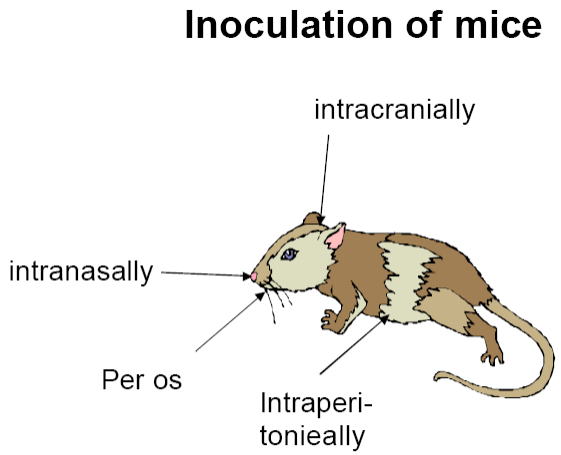
d. The *in vivo* assay may be performed based on risk assessment. If residual risk remains, retention of the test or replacement with non-targeted NGS can be considered to detect viruses that may have been introduced during establishment of the MCB or during culture of the cells at the LIVCA stage. Refer to Section 3.2.3 for details.

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).

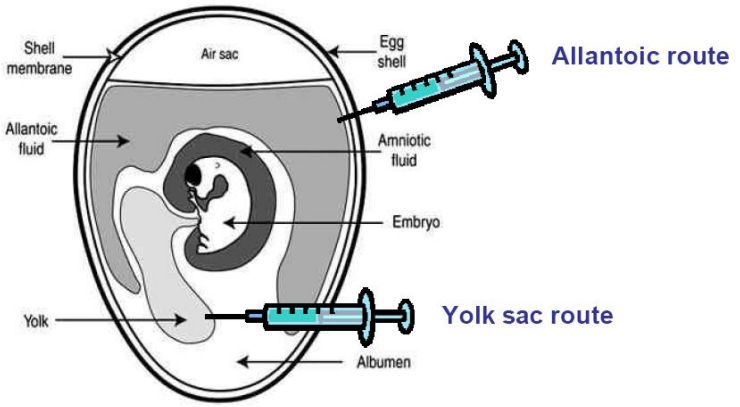
f. Testing is based on risk assessment including the origin and history of the cell substrate, and potential exposure to human or animal derived raw materials. Methods such as cell culture-based infectivity **assays, antibody production tests (MAP, HAP, RAP),** virus specific **NAT or other molecular methods e.g., NGS** can be used. Refer to Section 3.2.4 for details. This may include testing for species-specific viruses, for example, arboviruses in insect cells, and bovine or porcine viruses if serum or trypsin are used, respectively. Refer to Table 4 (Case B, C, and E) for action steps to be taken for virus detection in cell substrates used for production.

In vivo Assays

Inoculation of embryonated eggs, suckling/adult mice, (guinea pigs) via different routes



Presence of virus is indicated by change of health state or death of embryo/animals



In vivo Assays

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Advantage:

• detection of a range of viruses (may include some unexpected/unknown viruses) and non-cell culture adapted viruses

Limitations

• Death by multiple causes: injection trauma, bacterial contamination (eggs)/cannibalization of suckling mice
• Toxicity of media components
• Active Substance (e.g. virus vector/vaccine) can interfere with virus detection (neutralization with animal sera required)
• Limited sensitivity (inoculation volume) except some specific viruses
• Limited specificity
• High number of animals used

ICH Q5A Section 3.2.3 In Vivo Assays

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…Non-targeted NGS is encouraged as a replacement for in vivo assays due to its breadth and sensitivity of virus detection and the limitations of the in vivo assays. Furthermore, this promotes the global initiative to replace, reduce, and refine the use of animal testing.

In Vitro Cell Culture Infectivity Assay

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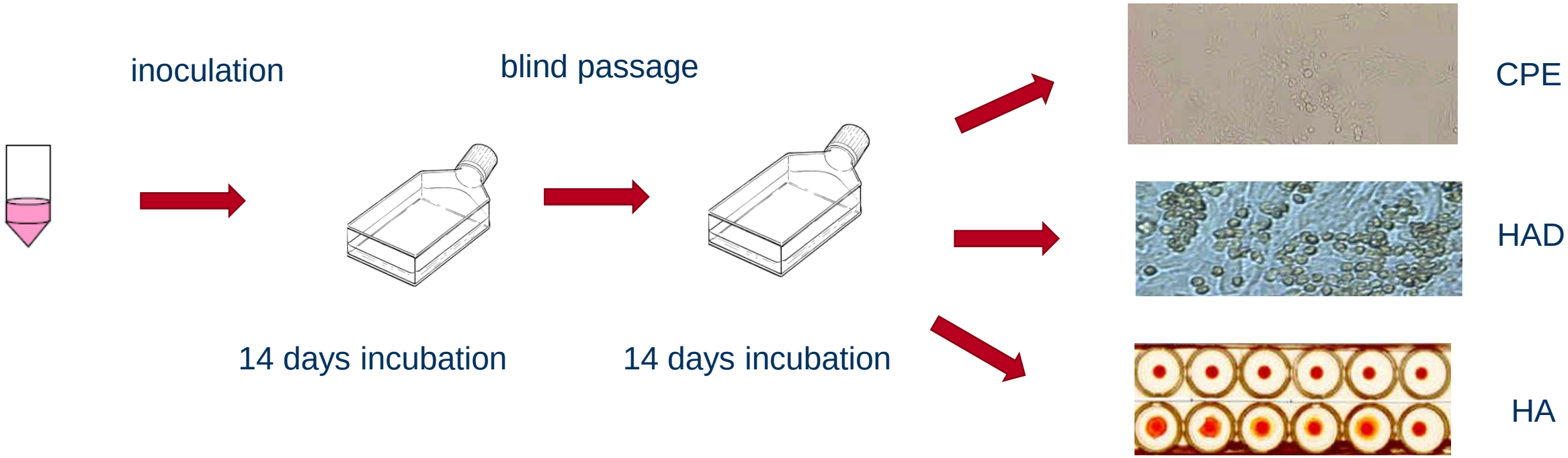

In Vitro Cell Culture Infectivity Assay

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Advantage:

- detection of a broad range of viruses (may include some unexpected/unknown)
- sensitive for cell culture adapted virus strains (consider inoculated volume)

Limitations

- Cell cultures are specific for a range of viruses
- Not all viruses induce cytopathic effects or haemadsorption/haemagglutination
- Time and labor consuming (slow growing viruses)
- Correct transport of test items (cooling)
- Well-trained personal required
- Contamination of indicator cell culture can interfere with virus detection (quality control of indicator cell culture is important!)
- Components of test matrix can interfere with virus detection
- Active Substance (e.g. virus vector/vaccine) can interfere with virus detection
- Problems with neutralization/animal derived serum

3.2.2 In Vitro Cell Culture Infectivity Assays

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*….NGS or other molecular methods may be used to **supplement or replace** the assay. This could address **general limitations of the in vitro cell culture infectivity assay** (e.g., susceptibility of cell lines to infection) and specific limitations of the production system (e.g., test article mediated interference or toxicity).*

How to Validate the NGS (1)

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Suitable reference materials *should be used for method qualification and validation to evaluate performance of the different steps involved in the workflow and to demonstrate sensitivity, specificity, and breadth of virus detection. This can include using currently available reference virus reagents/panels which contain* ***viruses of distinct physical*** *(size, enveloped and non enveloped), chemical (low, medium, and high resistance), and genomic (DNA, RNA, double and single-stranded, linear, circular) characteristics to evaluate the performance of the entire NGS workflow or specific steps.* ***A comprehensive viral database*** *should be used with diverse viral sequences for broad virus detection.* ***Other types of reference materials may be used to evaluate the specific technical and bioinformatic steps.***

Validation/qualification includes testing **of general assay performance** and **matrix-specific verification**.

How to Validate NGS (2)

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*NGS is used as a **limit test** and as such, the performance characteristics (**specificity/breadth of detection** and **sensitivity**) for method validation/qualification should consider the principles of ICH Q2.*

*Method **validation** requires predefined performance criteria while method **qualification** only evaluates the performance characteristics of the method.*

*For any NGS method used, validation/qualification should be provided to support its intended use for the application. This includes method **validation** and matrix-specific verification when used as a **replacement** method. When used as a **supplementary** method, this includes method **qualification** and matrix-specific verification.*

How to replace?

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NGS can provide defined sensitivity and breadth of virus detection and can reduce animal use and testing time. Non-targeted NGS can ***replace*** *the* ***in vivo*** *tests with broad virus detection for unknown or unexpected virus species* ***without a head-to-head comparison.***

Non-targeted NGS ***may also be used without a head-to-head comparison*** *to* ***supplement or replace the in vitro cell culture assays*** *for detection of known and unknown or unexpected viruses. This could address general limitations of the in vitro cell culture infectivity assay (e.g., susceptibility of cell lines to infection) and specific limitations of the production system (e.g., test article-mediated interference or toxicity).*

How to replace?

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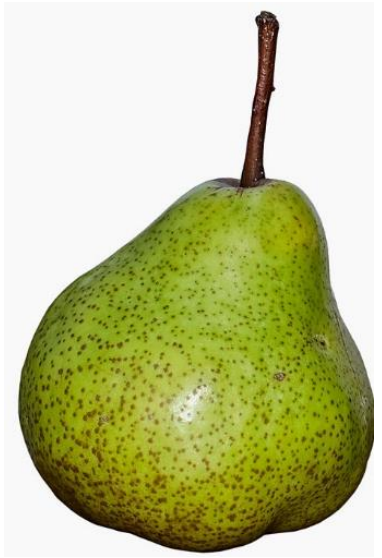
Head to head comparison of Apples with Pears

Detect some (infectious) virus particles
with excellent/high sensitivity



Detect “all” viral nucleic acids
with (equally high?) sensitivity

Summary on ICHQ5A

- ICHQ5A (R2) encourages NGS for testing of cell banks (MCB)
- ICH Q5A(R2) encourages to **replace** the in vivo test with NGS without head to head comparison
- ICH Q5A(R2) allows to **replace** the in vivo antibody production tests (MAP, HAP, RAP) with specific PCRs or NGS without head to head comparison
- ICH Q5A(R2) allows to **supplement or replace the in vitro cell culture assays** without head to head comparison
- Method validation is expected for **replacement** while method qualification is expected for **supplementing** in vitro assays
- Method validation needs predefined criteria.

Replacing in vivo testing

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Both the generic and the matrix-specific validation of the NGS method is performed using a limited set of properly justified model viruses, e.g. the set of model viruses recommended by WHO for NGS method validation.

If properly justone or several of these WHO-recommended model viruses may be replaced by other appropriate model viruses with similar characteristics.

No head-to- head comparison is expected.

It is important that the NGS method is capable to detect the model viruses with sufficiently high sensitivity. In this context, it may be useful to compare the sensitivity of the NGS method with published literature or previously obtained data on the sensitivity of *in vivo* adventitious virus testing. However, “non-inferiority” of sensitivity may not be necessary in each case/for each virus.

no ICH regulation

Replacing *in vitro* testing (1)

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A larger number of model viruses will likely be expected to be included in the spiking studies for the “generic” NGS method validation (not necessarily using the specific test matrix)

The selection and number of model viruses for the spiking studies should be justified based on a risk assessment considering the nature of the product and the manufacturing process (e.g. source of starting materials, use of animal-derived materials, presence of virus removal/inactivation steps during purification.

Matrix specific validation using the WHO virus panel (or equivalent panel as justified) is expected.

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Replacing *in vitro* testing (2)

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In vitro cell culture testing may have an excellent sensitivity for certain cell-culture adapted virus strains. In general larger volumes can be tested with culture methods than with molecular methods. However there may be severe limitations with some products (e.g. incomplete neutralization)

Although a head-to-head comparison between the NGS method and the *in vitro* adventitious virus tests is not strictly needed for all model viruses used in the spiking studies, it is recommended to compare both methods at least for a few relevant model viruses.

Confirmatory results from *in vitro* adventitious virus testing on 1 or a few crude harvests may be required/helpful as part of the NGS method qualification (?).

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Replacing *in vitro* testing (3)

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<u>Products with viral clearance (ICHQ5A products)</u>

Justify NGS sensitivity by a product-specific risk assessment....(see ICHQ5A Annex 5 for way of calculation of genomes per final vial)

e.g. having a NGS-detection limit of 10^3 genomes per ml at unprocessed harvest testing, needing 1000ml harvest fluid to produce a dose, and having an overall reduction capacity of 6 log results in not more than 1 virus particle (genome) per 10 000 doses. (a genome does not necessarily reflect an infectious virus particle).

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Replacing *in vitro* testing (4)

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<u>Products without viral clearance (e.g. live vaccines, certain viral vectors)</u>

Experience with NGS screening is still limited.

High sensitivity remains desirable and, currently, a cautious strategy is advised.

It is desired to supplement *in vitro* testing of cell banks with NGS

Carefully compare sensitivity with existing in vitro assays when replacing.

However, consider vaccines/ viral vectors that are difficult for in-vitro assays (e.g. cannot be neutralized)

no ICH regulation

Thank you for the attention!