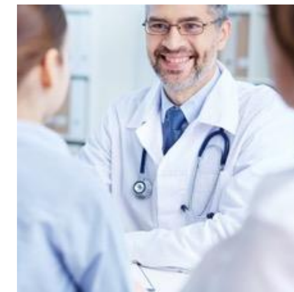
European Federation of Pharmaceutical
Industries and Associations

EFPIA's Perspectives on the Validation and Implementation of Next Generation Sequencing for Virus Safety Testing of Biological Products

Noémie Deneyer, GSK
on behalf of EFPIA

IABS 4th Conference on NGS for Adventitious Virus Detection in Biologics for Humans and Animal



Presentation Outline

1. EFPIA Biomanufacturing Supportive Group: Clonality, Characterisation and Viral Safety of Cell Lines
2. Objective of the Position Paper on Next Generation Sequencing for Viral Safety Testing of Biotechnology Products
3. Presentation of the Position Paper – Focus on validation and comparability sections
4. Case study and remaining needs from industries

https://www.efpia.eu/media/t22f5yoz/efpia-ngs-virus-detection-paper_finaljun2024.pdf

1. Clonality, Characterization and Viral Safety of Cell Lines

Project goal

- Facilitate the implementation of advanced analytical **NGS technologies** in relation with clonality, cell line characterization and viral safety for **biotechnological products** by sharing and leveraging industry expertise / experience and best practices.
- As a first priority, develop, promote and provide guidance for implementation of NGS as an alternative for current standards on **viral safety testing**
- Present industry position in perspective of shaping regulatory landscape for smoother implementation and acceptance by Heath Authorities

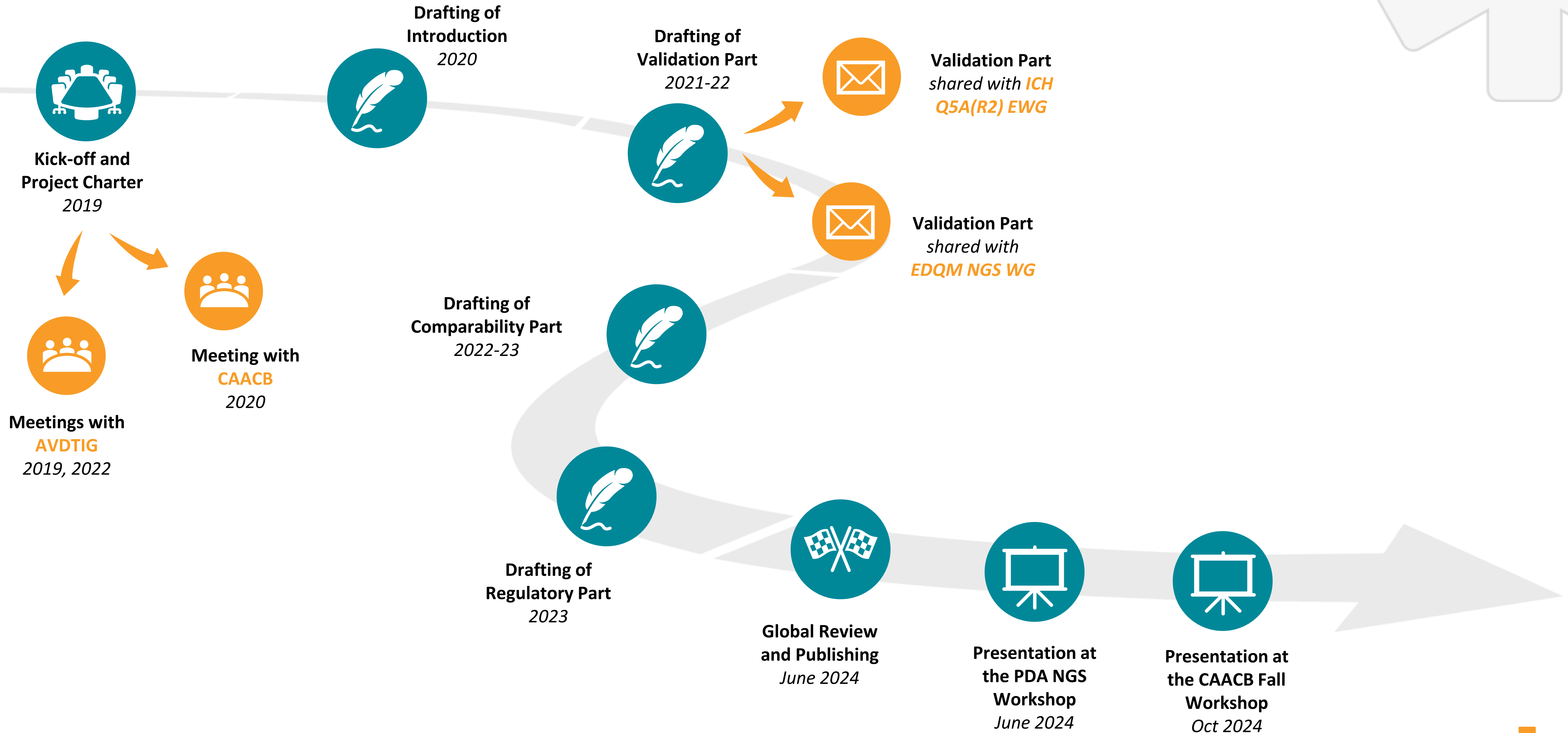
Scope

- Technology of interest: NGS
- Applicable for viral safety testing on biotechnology products (ATMPs out of scope)
- EU, US, ICH/WHO regulatory landscapes
- 16 Companies Participate to the NGS Supportive Group

Deliverables

- Publication of a **Position Paper**: acting as a practical implementation guide by providing an industry position for discussion with the Regulatory Authorities.
- Presentation of the Industry position in conferences

1. Journey of the EFPIA Position Paper on NGS for Virus Detection



2. Objectives of the Position Paper

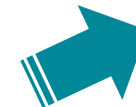
Envisaged as a practical implementation guide providing a position from Industry for dialogue with Regulatory Authorities.

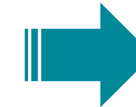
Introduction: Why NGS?
Characteristics of NGS technologies and comparison with conventional virus safety tests

Validation strategy for NGS methods

Assessing **Analytical Comparability** of NGS with *in vivo / in vitro* virus safety tests

Regulatory strategy for NGS **implementation**


Support revision of ICH Q5A


Support drafting of the Compendial General Chapter (PhEur 2.6.41)

Not achieved: Support potential creation of an ICH Q2(R2) Annex for NGS

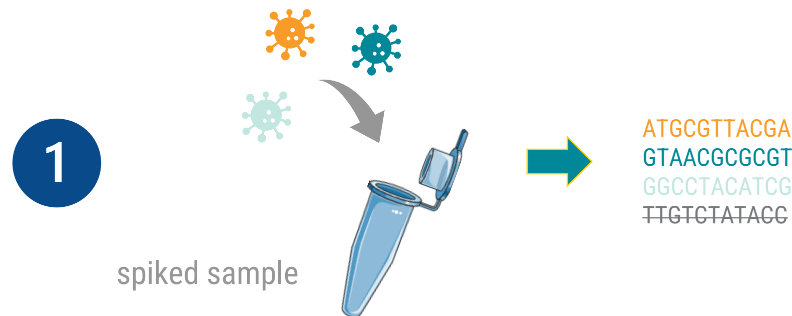
3. Validation of NGS-based Methods for adventitious virus detection

NGS AVD method is considered as a “**Limit test** for the control of impurities” → Specificity, Detection limit and Robustness

Specificity:

✱ *ICH Q2(R2): Specificity and selectivity are both terms to describe the extent to which other substances interfere with the determination of an analyte according to a given analytical procedure.*

✱ For NGS, this means demonstrating ❶ the method's ability to detect a viral contaminant in a complex matrix, ❷ discriminating viral nucleic acids from the background.



The method is specific if it identifies exclusively the viral sequences of the spiked virus or closely related viral species, belonging to the same taxonomic group.

The method is specific if the analysis of native samples does not generate signals.
This shows that background signals are properly identified and filtered out by the method through appropriate bioinformatic analysis.

Image credit © SMART-Servier Medical Art

3. Validation of NGS-based Methods for adventitious virus detection

Detection Limit:

ICH Q2(R2): The detection limit is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated as an exact value

- For NGS, DL determination is required to demonstrate the performance (analytical sensitivity) of the method to detect low levels of contaminant viruses present in the sample.

- To assess the DL, samples can be artificially spiked with different quantities of model contaminants to mimic different levels of contamination. The sample should be spiked prior to the initial steps of the method.

- While NGS can detect a broad range of viral species, it is not possible to determine the DL for all the detectable viral contaminants

Different DL can be obtained for different model viruses (e.g. DNA vs RNA viral genomes)

→ How to report results?

- Worst case DL (underestimation)
- List of virus-specific DL (more informative)
- Range of DL for best and worst sensitivity

3. Validation of NGS-based Methods for adventitious virus detection

Detection Limit:

* Two types of model contaminants can be spiked: viral particles (using representative and fully characterized viral stocks) or infected cells (fully characterized).

* Sample preparation can have a strong impact on method performance → Model contaminants should be spiked directly in the sample.

* Sequencing depth can also impact DL – method design should include validity criteria for the depth of sequencing to be reached

	Viral Particles Approach			Infected Cells Approach
DL Unit	Lowest number of viral genome copies that can be detected			Lowest number of infected cells into non-infected cells that can be detected
Analyte for DL determination	Viral nucleic acid			
Sample type examples	Viral Seed	Cell Line	Manufacturing harvest	Cell Line
DL experiments	Fixed amount of sample as background, spiked with different known quantities of model viruses			Non-infected cells as background spiked with different known quantities of model virus-infected cells
Advantages	Control on the number of genome copies at the time of DNA/RNA extraction Easy to test several model viruses			Mimic a sample where cells are infected and the virus is actively replicating Sensitivity is similar to a real-life contamination
Limitations	Does not mimic a test sample containing infected cells (possible underestimation of method sensitivity)			Cell type and growing conditions impact viral replication, thus influencing the DL estimation Demanding to test several model viruses for the cell model during validation

3. Validation of NGS-based Methods for adventitious virus detection

Robustness:

* *Robustness can be either included as part of the validation or evaluated during the development phase as described in ICH Q2(R2) and ICH Q14*

* Robustness should show the reliability of the analysis with respect to deliberate variations in method parameters.

* NGS-based methods are made up of different analytical steps, therefore a risk assessment could be applied to identify critical steps and consequently critical parameters. Then, the critical parameters identified should be deliberately variated to demonstrate the robustness of the analytical method.

* Robustness can be evaluated using artificially contaminated samples, such as the ones used for the DL determination.

→ Examples of critical steps: sample extraction, library preparation, sequencing

3. Comparability of NGS-based Methods with Existing Virus Safety Tests

Problem statement

Implementation of NGS-based method for virus detection in the existing safety panel :

- Addition of a test (supplement / complement): validation is sufficient.
- Replacement: Is a comparison with the current test required ? Is it meaningful ?

Terminology: Head-to-head comparison ? Equivalence ? **Analytical comparability** ?

An analytical comparability study should demonstrate that "the new method coupled with any additional control measures is equivalent or superior to the original method for the intended purpose"

FDA 2015 - Analytical Procedures and Methods Validation for Drugs and Biologics Guidance for Industry

Context:

As per ICH Q5A(R2): "*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.*"

3. Comparability: Generic methodology to address comparability between two analytical methods in case of replacement

Pre-assessment

- Compare the intended purpose and targeted quality attributes
- Define extent to which it is scientifically justified to compare the analytical results or decisions
- Guide the design of a potential comparability study

Experimental comparability study (when justified)

- Compare the performance characteristics and analytical results of the two procedures
- Determine their ability to reach the same pass/fail decision for a given attribute

Additional supportive elements

- In case the experimental comparability study is not scientifically justified or provides partial justification for the replacement
- Identified through risk assessment and provided to Health Authorities as justification

Is the **intended purpose** of the alternative method achieved by measuring the same type of **Product Quality Attribute** as the reference method, or minimally is there a relationship between them?

Do the two methods use the same **signals or read-outs**, or is there at least scientific evidence of a relationship or correlation between them?

Is the **test category** of the alternative method the same as the reference method?

3. Comparability: Replacement of *in vivo* Viral Screening Assays

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.
(…) Non-targeted NGS can be used to replace the in vivo assays (…) without a head-to-head comparison as long as the method is demonstrated to be suitable for its intended purpose.

ICH Q5A(R2)

Position aligned with ICH Q5A(R2) that analytical comparability is not useful.

In the absence of an analytical comparability study, what are the elements which justify the replacement of *in vivo* tests? **Suitability for the intended purpose**

- Specificity and sensitivity (as demonstrated by validation studies)
- Breadth of detection (validation studies with a broad range of model viruses, extent of viral genome database content, ability of the NGS-based method to detect novel viruses not included in the database)

How to **justify that detection limits are appropriate** without reference data ?

- Can we make use of the comparative data already published in the literature?
- How to account for the different performance of different NGS workflows?
- Suitability needs to be justified based on the intended use and additional virus detection assays included in the overall control strategy.

3. Comparability: Replacement of Cell-based Infectivity Assays

> *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).*
>
> **ICH Q5A(R2)**

More **conservative** position about the use of analytical comparability studies between NGS-based methods and *in vitro* cell-based infectivity assays than for *in vivo* assays. This is due to the **critical role of those assays in current viral control strategies** (Barone et al, 2020; Barone et al, 2023).

- Analytical comparability data can provide justification that analytical method performance is suitable for the intended purpose.
- But validation data alone may be sufficient to demonstrate extensive and sensitive virus detection in line with the needs outlined in the risk assessment.

Considerations on the **design and limitations of an analytical comparability study** :

- Limited comparison due to the use of model viruses not covering the entire scope of viruses
- Option to use worst-case viruses in the comparability study (viruses detected at a low level)
- Characterization of virus stocks in genome copies and infectivity (different quality attributes)
- In the end, **differences in terms of sensitivity and breadth of detection** may be acceptable and need to be discussed in terms of the overall control strategy.

4. Case Study Sharing

Ways to interact with Health Authorities:

- Innovation-dedicated pathways: US FDA Emerging Technology Program, US FDA CBER Advanced Technology Team, EMA Innovative Task Force, French ANSM Innovation and Orientation (GIO)
- Ad-hoc: scientific non-regulatory advice at US FDA CBER, non-official interaction during conference

Topics discussed with Authorities:

- Detailed NGS workflow
- Validation approach and results
- Nature and type of spiking material
- When conducted, comparison results with *in vivo* assays and/or *in vitro* cell-culture assays.

At least one application was submitted and **accepted** for replacement of *in vivo* assays (content of the submission described in the paper).

Feedbacks / questions received:

- Positive feedback for replacement of *in vivo* and *in vitro* tests, for cell banks and unprocessed bulks
- Data analysis and viral genome database
- Validation of the bioinformatic pipeline
- Criticality of specific steps
- Justification of model viruses
- Justification of detection limits

efpia

4. Points of discussion – Remaining needs from industries

- Replacement of cell-culture infectivity assays without analytical comparability with NGS ?

- Justification of detection limits ?

- Foreseen acceptance of ICH Q5A(R2) statements regarding test replacement without head-to-head comparison ?
  - In EU and US
  - In other international countries

- Perspective of moving from a panel of orthogonal virus safety tests to a unique NGS-based assay ?

European Federation of Pharmaceutical Industries and Associations

Group Lead

Lionel Randon (EMD Serono)

Lead Authors

Elodie Charbaut Taland (EMD Serono), Simone Olgiati (EMD Serono), Morgane Rochemont (EMD Serono)

Contributors

Alice Alston (AstraZeneca), Chakameh Azimpour (Capsida Biotherapeutics), Alessia Bachmann (EMD Serono), Sakthi Balaji (Abbvie), Maria Gilleece (Biogen), Amrut Bhogle (Merck), Kang Cai (AstraZeneca), Noémie Deneyer (GSK), Marc Eloit (Pathoquest), Lianchun Fan (Abbvie), Christopher Frye (Lilly), Luhong He (Lilly), Sandrine Lemaire (GSK), Carine Logvinoff (Sanofi), Sean Michael O'Donnell (Lilly), Nasrin Salehi (Pfizer), Bin Yang (Genentech), Yuan Zhu (GSK)

Thank you