

Next-Generation Sequencing-based Adventitious Viral Agent Detection in Challenge Agents and Cell Banks

Our Journey Bridging Science and Regulatory Compliance

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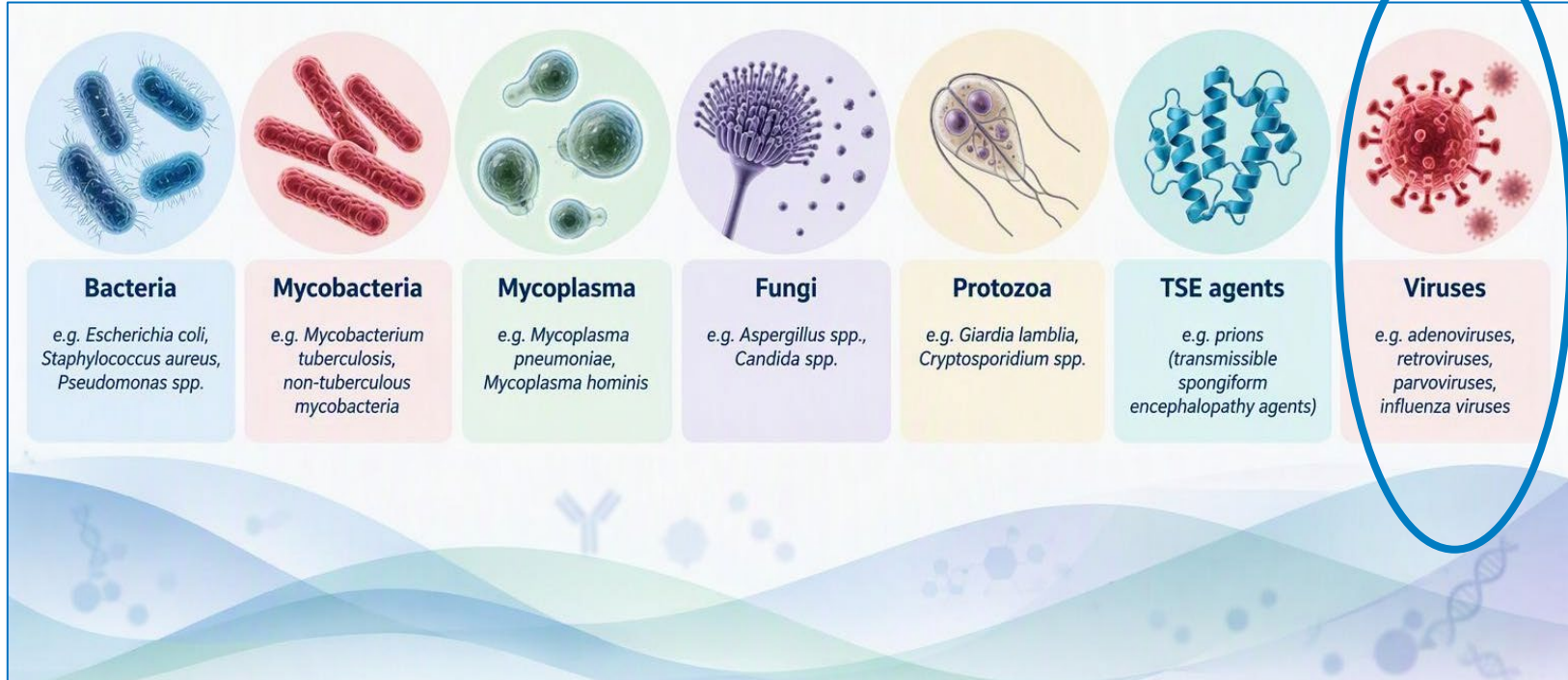


innovative
medicines
initiative



Adventitious Agents

Microorganisms unintended to be present in the biological product

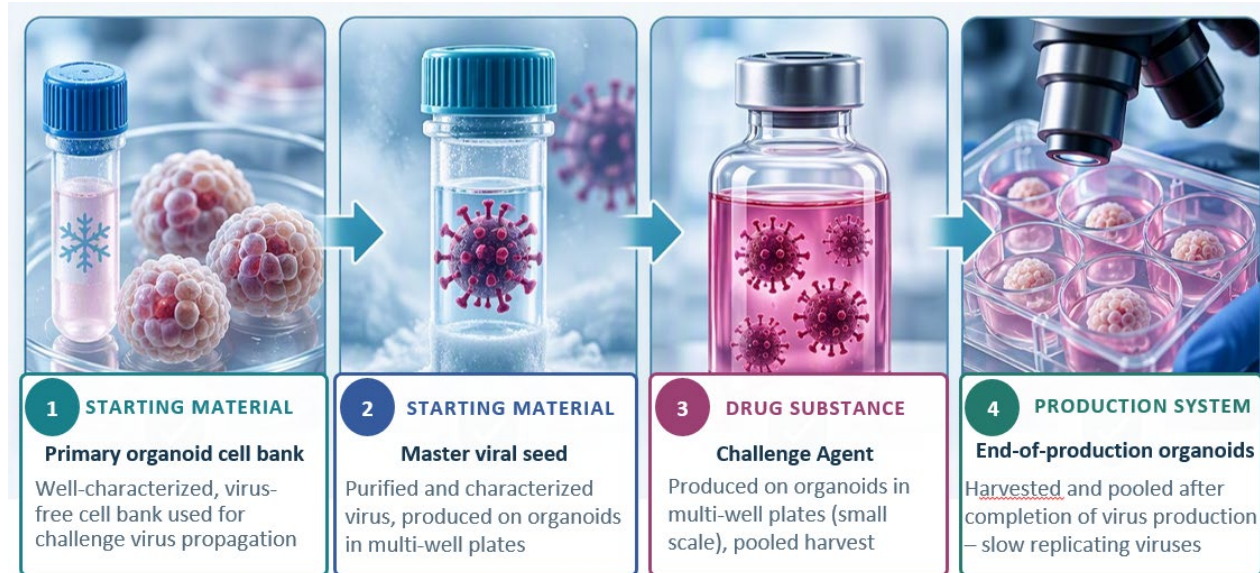


Adventitious Viral Agents (AVA) – in challenge agents

But what if your biological product **IS** a virus?

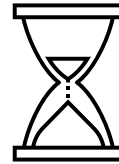
- That is cytotoxic to detector cell lines (creates cytopathic effect)
- ... produced at small-scale (organoid based production platform -> low volumes)
- manufactured under time pressure in the context of **pandemic preparedness**

NGS testing strategy:



Conventional *in vitro* culture methods: limitations for infectious challenge agents & small scale platforms

Article can be toxic to cells or induce aspecific CPE → additional cell lines + article inactivation necessary



+ TIME

Observed CPE: follow up investigations required for identification of cause; not always successful

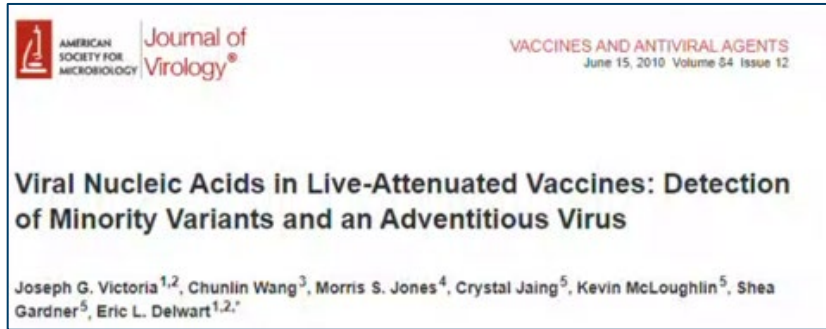
Some viruses do not infect or propagate efficiently in used cell lines: clearance



False-negative
Risk = release of AVA
in Drug Substance
/ Drug Product

→ Article volume!

NGS can pick up what conventional methods have missed



Identification of PCV1 in a licensed rotavirus vaccine using molecular methods



Discovery of a novel Rhabdovirus in an insect Sf9 cell line for baculovirus production

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

Key themes of ICH Q5A(R2)



Risk-based approach

Science- and risk-based strategy across the product lifecycle



Modern detection methods

Recognises NGS and other molecular methods for broad adventitious agent detection; allows replacement of *in vivo* assays when justified



Cell substrate & raw materials

Characterisation and control of cells, raw materials and other starting materials



Viral clearance

Demonstration and validation of effective clearance steps in the manufacturing process



Platform / prior knowledge

Use of relevant prior knowledge to streamline testing where applicable



Modern product modalities and manufacturing

Addresses new technologies and continuous manufacturing

Revised ICH Q5A(R2) on Molecular Methods:

*“Nontargeted 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 endpoints 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.”*

NGS for adventitious agents – Finding the needle in the haystack

‘Unbiased’

Breadth of detection

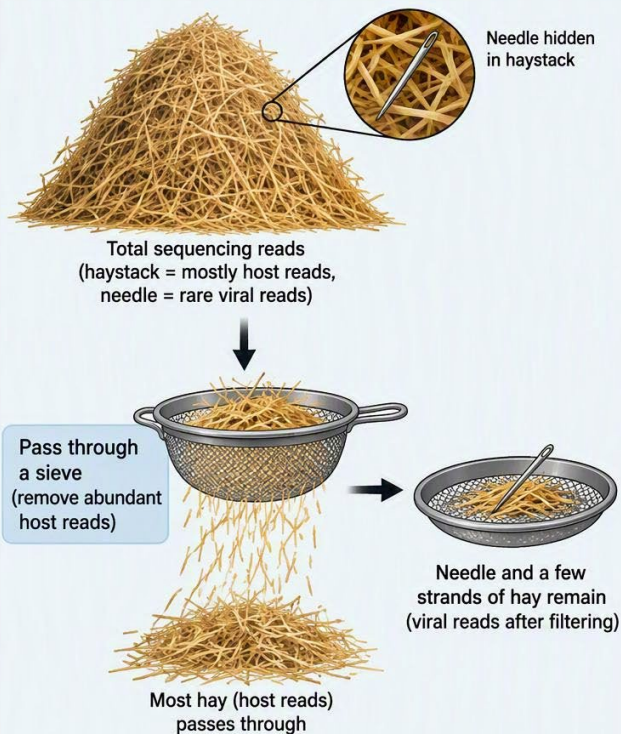
Novel pathogens

Many reads required

Challenging in complex backgrounds:
Host depletion

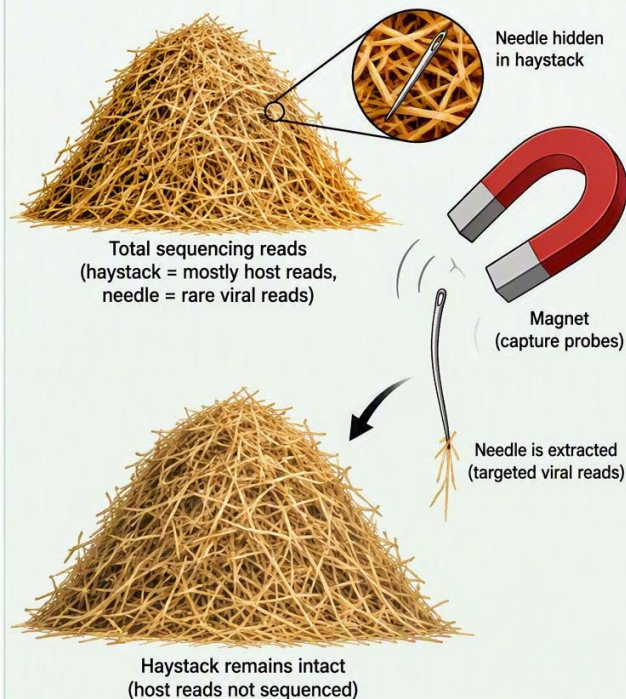
Untargeted (agnostic) NGS

Filter out the background, keep what remains



Targeted NGS (hybrid capture)

Use a magnet to specifically pull out what you want



Selective

High sensitivity

Known sequences /
sequence
homology

Specific panels
(e.g. 9 CFR)

Follow-up
investigations

From NGS signal to regulatory decision

What must be aligned before NGS can support routine AVA decisions?

01

Method standardisation & validation

Can the complete workflow be trusted?

- End-to-end GMP validation of wet lab + pipeline
- Matrix- and virus-specific limit of detection
- Sufficient read depth and run acceptance criteria
- Multiplex product identity + AVA testing?

02

Expertise & reference databases

Can viral sequences be identified reliably?

- Specialised bioinformatics personnel required
- No universally accepted reference library
- Consensus on database content and curation
- Requalification after database updates

03

Data governance & infrastructure

Can every result be reconstructed and defended?

- Large raw and processed data volumes
- Validated storage and secure transfer
- Data integrity, audit trail and retention
- Software, pipeline and version traceability

04

Interpretation & follow-up

When does a read become an adventitious agent?

- True contaminant vs background or artefact
- False-positive assignments in complex matrices
- Predefined “reportable signal” criteria
- Proportionate orthogonal follow-up investigations



Thank you for building confidence in NGS together!

