

Use of Bovine Serum and NGS-Based Testing of Biological Products

Marc Eloit, Prof. · Founder & Scientific Advisor

IABS Bovine Serum Conference · September 2026



Viral Contaminations | Sources and Downstream Impact

1. SOURCE: BOVINE SERUM



Collected from healthy cattle under controlled conditions and processed to ensure safety and quality.



2. VIRUSES THAT MAY BE PRESENT IN BOVINE SERUM



BVDV



Polyomaviruses



Parvoviruses



Orbiviruses

...

Others

3. PATHWAYS OF CONTAMINATION

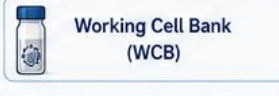
Serum used in:



Cell culture
(media supplement)



Master Cell Bank
(MCB)



Working Cell Bank
(WCB)



Upstream
fermentation



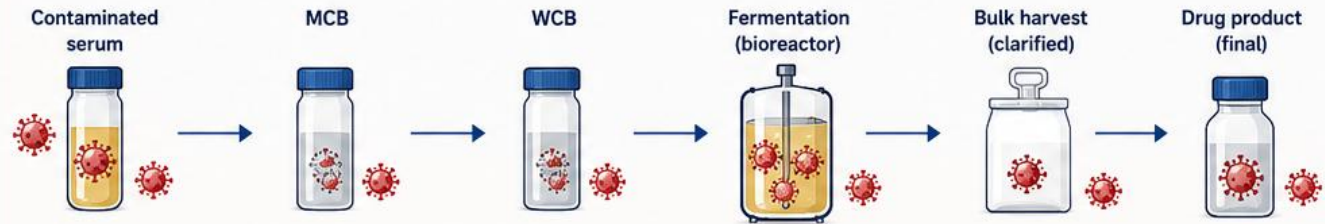
Production
(added to bioreactor)

→ Process flow

--- Risk of viral contamination

⚠ Potential viral presence

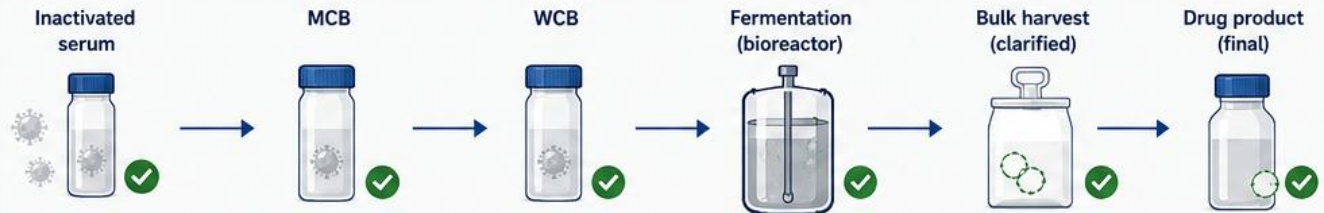
4A. DOWNSTREAM CONSEQUENCES – SERUM USED WITHOUT PROPER INACTIVATION



HIGH RISK OF VIRAL CONTAMINATION

- Contamination of MCB, WCB and bioreactor
 - Potential detection of adventitious viruses in bulk harvest and final drug product
- Possible impact on patient safety, product recall and regulatory compliance

4B. DOWNSTREAM CONSEQUENCES – SERUM CORRECTLY INACTIVATED



ANALYTICAL RISK: POSSIBLE FALSE POSITIVES (PCR/NGS)

- Residual viral nucleic acids may persist after inactivation
- May be detected by PCR or NGS in: MCB, WCB, bulk harvest and/or drug product

→ No infectious risk, but may lead to false-positive results and impact investigations, release decisions and regulatory filings



KEY MESSAGE

Proper inactivation of bovine serum is essential to prevent infectious viral contamination. However, it may result in detection of residual viral nucleic acids and false-positive PCR/NGS results.



Patient
Safety



Regulatory
Compliance



Product
Quality

Limitations of Current Serum Bioassays

Limited test sensitivity and breadth of detection

- Only two cell lines used: a bovine (BT or MDBK) and a primate (VERO) or target species cell line
- Only viruses that can grow in these cell lines with CPE or HAD/HA can be detected
- Only 7 viruses chosen for their pathogenicity for cattle tested specifically by 9CFR
- Assay LoD unknown
- Hard to grow/detect agents are readily missed (e.g., BPyV, retro, parvoviruses, adeno, picobirna, annelo, circo, calici-virus)

Long assay duration (technically > 28 days)

Agnostic NGS in Viral Safety

From broad detection to biologically meaningful viral-safety evidence

WHAT “AGNOSTIC” NGS ADDS

Targeted PCR
Is virus X present?

Agnostic NGS / HTS
What viral sequences
are present?

Unbiased sequencing → bioinformatics → broad viral identification

WHY IT MATTERS

- Broad detection beyond predefined virus panels
- Known, divergent and potentially novel viral sequences
- Potential complement / alternative to selected conventional assays

KEY POINT — Broad detection does not by itself demonstrate infectivity

REGULATORY FRAMEWORK

BOVINE SERUM / ANIMAL-DERIVED MATERIALS

Ph. Eur. 2262 — Bovine serum

Ph. Eur. 5.2.5 — Extraneous agents

EMA/CHMP/BWP/457920/2012 Rev.1 — bovine serum

NGS / HTS FOR VIRAL SAFETY

ICH Q5A(R2) — Viral safety

Ph. Eur. 2.6.41 — HTS / NGS

Validation, controls and meaningful interpretation

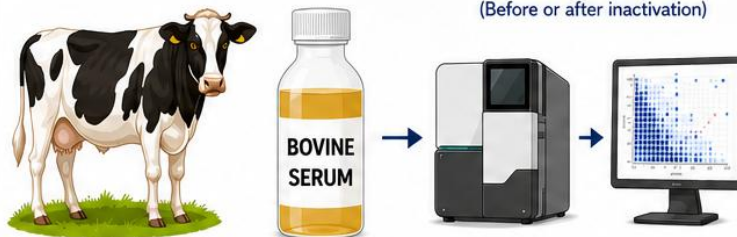
The regulatory logic is converging: control the serum risk, broaden detection, then interpret biological relevance.

How to Demonstrate Viral Safety Effectively

Why direct NGS testing is not discriminant and what should be done

1 DIRECT NGS TESTING OF BOVINE SERUM IS NOT DISCRIMINANT

Bovine serum contains a high background of viral nucleic acids (infectious or not)



Many viral sequences will be detected

- Not specific
- Not discriminant
- Does not inform on infectivity



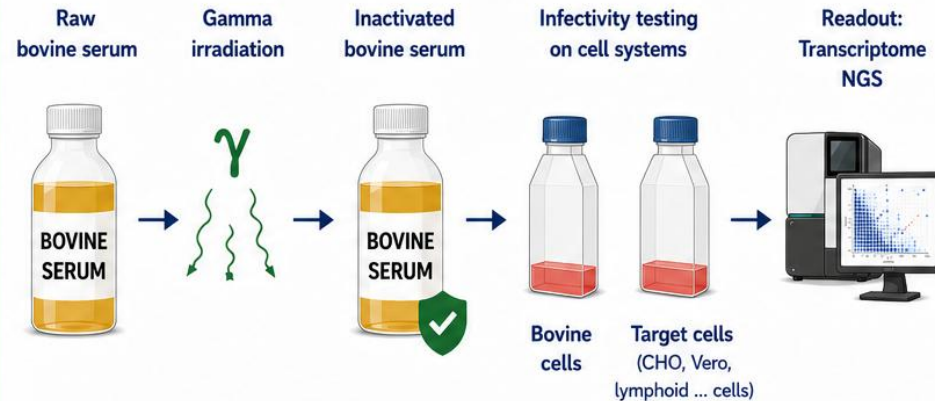
OPTION (if NGS must be performed):

Perform qPCR on viral indicators (e.g., BVDV) to confirm that the viral load is below the gamma irradiation inactivation capacity.



Viral titer < Inactivation capacity of gamma irradiation

2 THE RIGHT APPROACH: INACTIVATE THEN DEMONSTRATE ABSENCE OF INFECTIVITY



WHY THIS APPROACH IS RECOMMENDED

- ✓ Demonstrates absence of replication-competent (infectious) virus
- ✓ Uses relevant cell systems to cover a broad host range
- ✓ Transcriptome NGS detects active viral replication (mRNA), not just residual DNA/RNA
- ✓ Meets regulatory expectations for viral safety assessment



KEY TAKE-HOME MESSAGES



Direct NGS of bovine serum (before or after inactivation) is not discriminant.



Inactivate first, then demonstrate absence of infectious virus using infectivity assays with transcriptome NGS readout on bovine cells and target cells (CHO, Vero, lymphoid ...).



Patient Safety



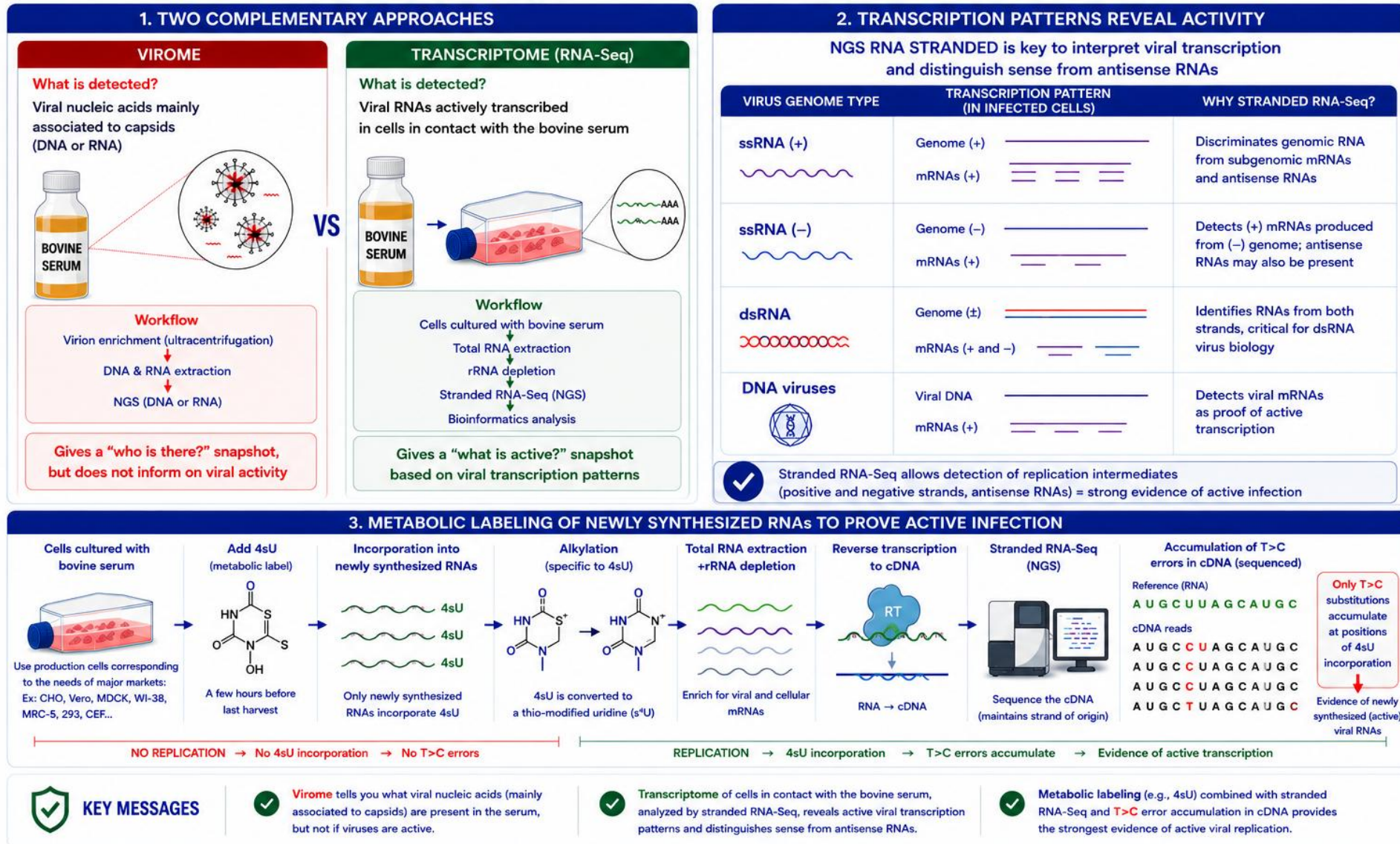
Regulatory Compliance



Product Quality

Virome vs Transcriptome: Why It Matters and How It Works

Capturing the biology of infection: transcription patterns reveal active viruses

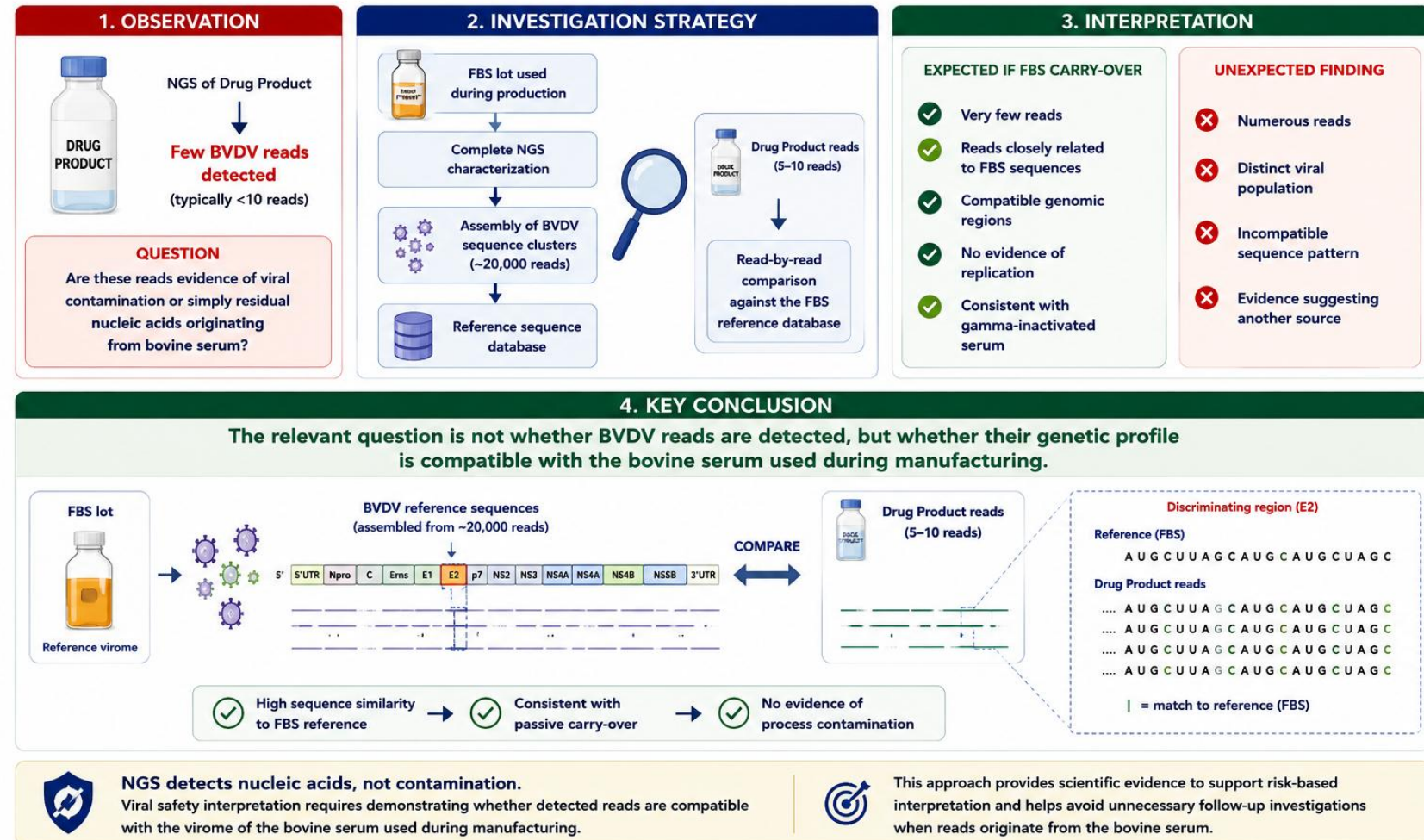


Benefits of Using Transcriptome NGS-based Assays

- **Cell culture phase with 2-3 passages, followed by Transcriptome NGS**
- **Only live (replicating) viruses are detected**
- **Equal or less assay duration than conventional tests**
- **Able to detect a very wide range of viruses (addresses both serum users & regulatory needs)**
- **Also detects emerging or novel viruses**
- **Detection and identification accomplished at the same time, minimizing a need for lengthy investigations**
- **Overall method can be validated and performed under the GMP requirements**

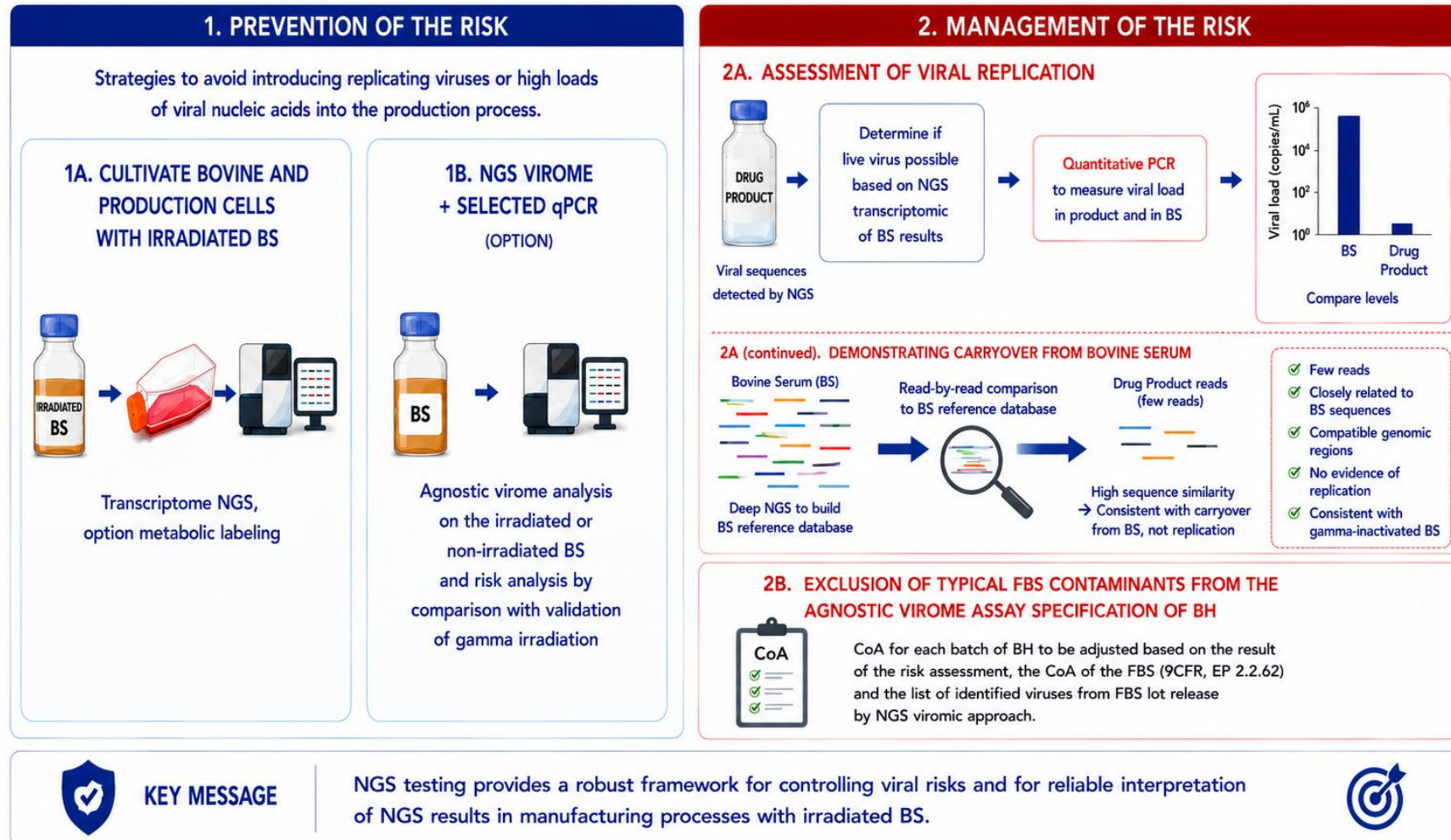
Demonstrating Carry-over from bovine serum if requested

Demonstrating Carry-over from Bovine Serum



Summary of Approaches

MITIGATION OF INERT VIRAL SEQUENCES WHILE CONTROLLING THE RISK OF VIRAL CONTAMINATION



Application of NGS for Bovine Serum Testing

Supporting Viral Safety from Early Development to Commercial

BS Production (FBS Manufacturer)

Phase 1:

slaughterhouse blood collection in individual bags → serum separation from each bag → small serum pools (~5L) → storage

Phase 2:

Thawing of 5L containers → final pool, batch size 1600-2000L → aseptic filtration → dispensing → labeling → Storage → QC testing → CoA → distribution



Final batch testing.

Likely point of transcriptome NGS introduction (bovine indicator cell line).

User of BS (Pharma)

Storage → QC testing for cell/virus growth potency → CoA → use



Additional test from cells sampled during BS potency testing on production cells.

Likely point of transcriptome NGS introduction.

Thank you for your attention

Questions & discussion

Marc Eloit, Prof. · Founder & Scientific Advisor

IABS Bovine Serum Conference · September 2026



Our Methods in Two Peer-Reviewed Publications



RESEARCH ARTICLE
Applied and Environmental Science

Adventitious Virus Detection in Cells by High-Throughput Sequencing of Newly Synthesized RNAs: Unambiguous Differentiation of Cell Infection from Carryover of Viral Nucleic Acids

Justine Cheval,^a Erika Muth,^a Gaëlle Gonzalez,^b Muriel Coulpier,^b Pascale Beurdeley,^a Stéphane Cruveiller,^a Marc Eloit^{a,c,d,*}

^aPathoQuest, Paris, France

^bUMR1161 Virologie, ANSES, INRA, Ecole Nationale Vétérinaire d'Alfort, Université Paris-12 Est, Maisons-Alfort, Paris, France

^cEcole Nationale Vétérinaire d'Alfort, Université Paris-Est, Maisons-Alfort, Paris, France

^dPathogen Discovery Laboratory, Biology of Infection Unit, Institut Pasteur, Paris, France

ABSTRACT The use of high-throughput sequencing (HTS) to identify viruses in biologicals differs from current molecular approaches, since its use enables an unbiased approach to detection without the need to design specific primers to preamplify target sequences. Its broad range of detection and analytical sensitivity make it an important tool to ensure that biologicals are free from adventitious viruses. Similar to other molecular methods, however, identification of viral sequences in cells by HTS does not prove viral infection, since this could reflect carryover of inert viral sequences from reagents or other sources or the presence of transcriptionally inactive cellular sequences. Due to the broad range of detection associated with HTS, the above can potentially be perceived as a drawback for the testing of pharmaceutical biological products using this method. In order to avoid the identification of inert viral sequences, we present a methodology based on metabolic RNA labeling and sequencing, which enables the specific identification of newly synthesized viral RNAs in infected cells, resulting in the ability to unambiguously distinguish active infection by DNA or RNA viruses from inert nucleic acids. In the present study, we report the ability to differentiate Vero cells acutely infected by a single-stranded positive-sense RNA virus (tick-borne encephalitis virus) from cells which have been in contact with nonreplicating virus particles. Additionally, we also found a laboratory contamination by the squirrel monkey retrovirus of our Vero cell line, which was derived from an Old World (African green) monkey, a type of contamination which until now has been identified only in cells derived from primates from the New World.

IMPORTANCE The use of high-throughput sequencing (HTS) to identify viral contamination of biological products is extremely sensitive and provides a broad range of detection. Nevertheless, viral sequences identified can also be inert. Examples include contamination resulting from reagents or the presence of inactivated viruses in animal-derived components of the cell culture medium. We therefore developed a method that relies on the sequencing of newly synthesized RNAs, an unequivocal sign of the presence of a transcriptionally active virus. This improvement in the specificity of viral testing increases the acceptability of HTS as a standard test for cells used in manufacturing biologicals and in biotherapies.

KEYWORDS 4-thiouridine, RNA-seq, Vero, biologicals, high-throughput sequencing, metagenomics, squirrel monkey retrovirus, tick-borne encephalitis virus, virus safety

May/June 2019 Volume 4 Issue 3 e0298-19

Citation Cheval J, Muth E, Gonzalez G, Coulpier M, Beurdeley P, Cruveiller S, Eloit M. 2019. Adventitious virus detection in cells by high-throughput sequencing of newly synthesized RNAs: unambiguous differentiation of cell infection from carryover of viral nucleic acids. *mSphere* 4:e0298-19. <https://doi.org/10.1128/mSphere.0298-19>

Editor Matthew B. Frieman, University of Maryland, College Park

Copyright © 2019 Cheval et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Marc Eloit, marc.eloit@pathoquest.com.

Received 29 April 2019

Accepted 13 May 2019

Published 5 June 2019

mSphere mSphere.asm.org 1

Biologicals 59 (2019) 29–36



Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals



Use of a new RNA next generation sequencing approach for the specific detection of virus infection in cells

Audrey Brussel^a, Kerstin Brack^b, Erika Muth^c, Rudolf Zirwes^c, Justine Cheval^a, Charles Hebert^a, Jean-Marie Charpin^c, Alice Marinaci^b, Benoit Flan^a, Horst Ruppach^b, Pascale Beurdeley^a, Marc Eloit^{a,c,d,*}

^aLFB, Courbevoie, France

^bCharles River Laboratories Germany GmbH, Erkrath, Germany

^cPathoQuest, Paris, France

^dNational Veterinary School of Alfort, Paris-Est University, Maisons-Alfort, France

^ePathogen Discovery Laboratory, Biology of Infection Unit, Institut Pasteur, Paris, France

ARTICLE INFO

Keywords:
Virus testing
Next generation sequencing
Metagenomics
Biologicals
Viral safety
Adventitious virus

ABSTRACT

The utilization of the current combination of *in vitro*, *in vivo* and PCR assays for the identification of adventitious viruses in production cells has a limited range of detection. While Next Generation Sequencing (NGS) has a broader breadth of detection, it is unable to differentiate sequences from replicating viruses versus background inert sequences. In order to improve NGS specificity, we have designed a new NGS approach which targets subsets of viral RNAs only synthesized during cell infection. In order to evaluate the performance of this approach for detecting low levels of adventitious viruses, we selected two difficult virus/cell systems. This included B95-8 cells persistently infected by *Human herpesvirus 4* (HHV-4) and serially diluted into HHV-4 negative Ramos cells and Madin-Darby bovine kidney cells with an early infection produced via a low dose of *Bovine viral diarrhoea virus*. We demonstrated that the sensitivity of our RNA NGS approach was equivalent to targeted PCR with an increased specificity for the detection of viral infection. We were also able to identify a previously undetected *Murine Leukemia Virus* contaminant in Ramos cells. Based on these results, we conclude that this new RNA NGS approach is suitable for conducting viral safety evaluations of cells.

1. Introduction

Insuring viral safety during the development and manufacture of biological and medicinal products is a critical quality parameter. All animal-derived materials used for the manufacture of biological products need to be free from adventitious viruses. Currently *in vivo* and *in vitro* assays are utilized for the detection of viruses in biological products. This includes the testing of starting materials such as cell substrates, virus seeds, and cell culture intermediates or ancillary materials such as porcine trypsin and animal serum.

Currently utilized tests were developed with known viruses discovered via isolation in cell culture or in animals. This results in a false assumption that all potential viral contaminants can be identified by *in vitro* and *in vivo* assays. With the advent of the use of molecular biology assays like PCR, the discovery of viruses is now primarily based on the detection of viral nucleic acids. This includes the identification of wild-type strains of human viral pathogens which cannot be isolated and

identified by current *in vitro* methods. Recent studies have demonstrated the viral detection and sensitivity limitations associated with current *in vivo* assays and recommended *in vitro* 9 CFR testing method [1,2]. This is especially important for biological products manufactured using bovine or porcine raw materials. While electron microscopy is used as an alternative method to screen for viral contaminants in biologic products since it is suitable for evaluating the presence of virus-like particles, it lacks sensitivity and is operator dependent. The above lead to both *in vitro* assays and electron microscopy frequently failing to clearly identify contaminants and the requirement for subsequent analyses.

Although targeted PCR is a useful testing alternative, it is limited to only detecting and identifying viruses known or suspected to be potential contaminants in the material being analyzed. When animal origin materials are used for the production of biologicals, the number of PCR tests is typically limited to a small list of animal viruses with known pathogenicity in the original host species, however, these may

* Corresponding author. PathoQuest, 11 rue Watt, 75013, Paris, France.
E-mail address: marc.eloit@pathoquest.com (M. Eloit).

<https://doi.org/10.1016/j.biologicals.2019.03.008>

Received 21 December 2018; Received in revised form 15 March 2019; Accepted 31 March 2019
Available online 13 April 2019

1045-1056/© 2019 International Alliance for Biological Standardization. Published by Elsevier Ltd. All rights reserved.



Inoculate serum and passage the cells

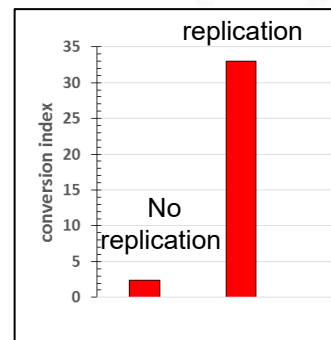
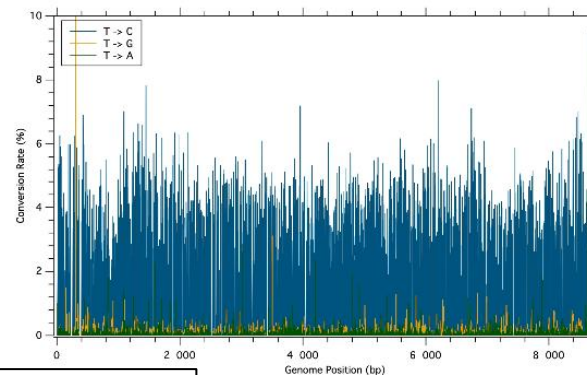
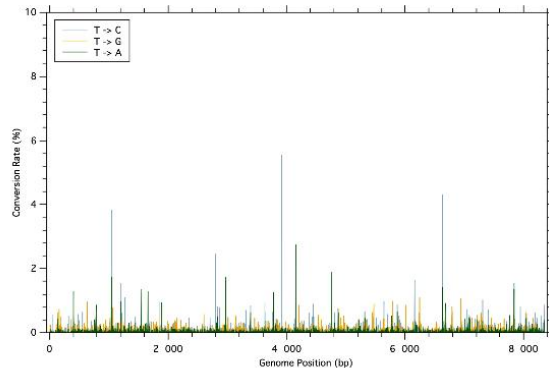
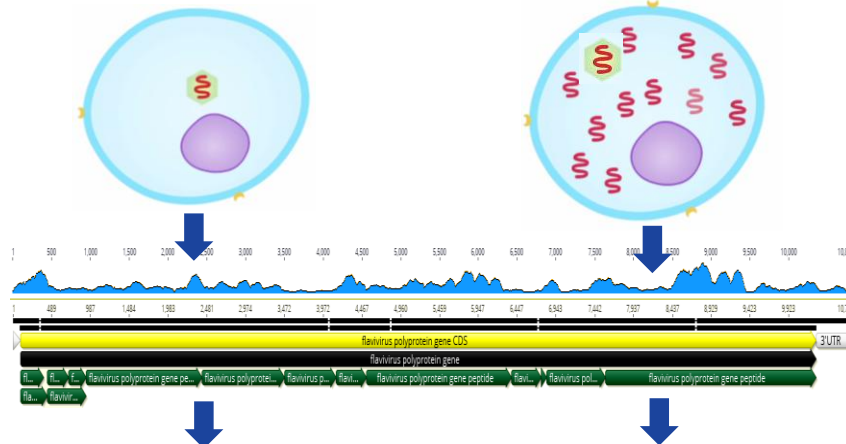
Add labelling compound
a few hours before last harvest



Use production cells corresponding to the usage of the serum
Ex : WI-38, MRC-5, Vero, 293, MDCK, CEF

No replication

Replication



Standard NGS (as PCR) is unable to distinguish
inert sequences from replicating viruses

**NGS targeted to newly synthesized RNAs
differentiates both situations**

Application of this testing method to BS presented April 2019
Bioprocess International (BPI) Viral safety and raw material meeting in
Vienna by Jean Pol Cassart from GSK vaccines – “An infectivity/NGS
combined assay to specifically detect live viruses and control the risk
of viral contamination”