

Regulatory Requirements in the light of risk assessment for Bovine Serum used in production of medicinal products

IABS EU-Meeting on bovine serum:
29-30 September 2026

Dr. Johannes Bluemel
Head of Department Infectious Diseases

Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines



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.

Paul-Ehrlich-Institut



Disclaimer

The views expressed in this presentation are the personal views of the presenter(s). They shall not be understood or cited as opinions of the Paul-Ehrlich-Institut.

It is difficult to exclude any virus contamination from pooled batches of bovine serum.

The viral safety is evaluated in the context of the medicinal product.

Risk from having no species barrier (1)

Example: 1940s: Human serum excipient in human yellow fever vaccine caused transmission of serum hepatitis (HBV)

<https://www.ncbi.nlm.nih.gov/books/NBK234464/>

Human vaccine Industry switched to (fetal) bovine serum for cell culture and virus- inactivated human serum albumin as excipient

Cave: During culture for human cell-based medicinal products, FBS is now often replaced by human serum.

Risk from having no species barrier (2)

In 1999 several herds with BVDV cases in NL and IT were associated with batches of a live vaccine against Infectious Bovine Rhinotracheitis (IBR)

No signal from calves used for routine safety testing

Ca 10^5 genomes/dose and infectivity (10^2 u) detected in vaccine bulk

No contamination of cell banks and virus seed (WVS)

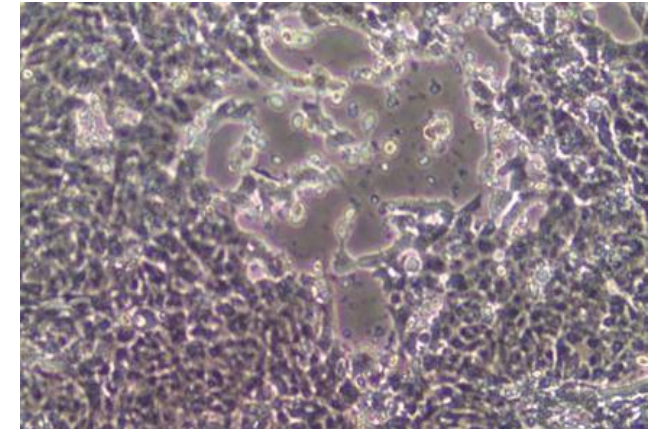
...Batch of (gamma irradiated, dose?) calf serum was formerly tested and certified as BVDV free according to the EU Guidelines and USA Federal Regulation. However, re-evaluation revealed that the presence of a rather high level of BVD antibodies in this serum masked the presence of contaminating BVD-virus and limited its multiplication. Using FPLC preparation and ultrafiltration to concentrate virus and to eliminate antibodies, viable BVD virus types 1 and 2 was detected in this batch

...In conclusion, the CVMP agrees that the safety problems in the field originated most probably from contaminated foetal calf serum used in the manufacture which may have arisen from the inadequacy of the applied inactivation used which therefore merit particular attention. The present quality control appears to be insufficient to detect BVD virus

https://www.ema.europa.eu/en/documents/referral/bayovac-ibr-marker-vivum-article-78-referral-annex-i-ii-iii_en.pdf

Isolation of Highly Pathogenic Avian Influenza A(H5N1) Virus from Fetal Bovine Serum, United States, 2025

- In February 2025, we detected highly pathogenic avian influenza virus A(H5N1) clade 2.3.4.4b virus in a fetal bovine serum lot during routine adventitious agent testing. (CPE in Vero cells 7d post inoculation, NGS performed for identification. probably <1 infectious particle/mL) in this pooled FBS lot.
- We classified a total of 13,599 reads as avian influenza A virus subtype H5N1. P ... closely related to dairy cow samples from California collected during December 2024
- The low frequency of HPAI H5N1 virus detection in the tested FBS lot (even when tested using rRT-PCR) and the lack of detection of the virus suggest low viral loads (probably <1 infectious particle/mL) in this pooled FBS lot.
- Heating at 56°C for 30 minutes completely inactivated the virus (Note: Heating at 56°C was 30min has been designed for inactivation of complement factors and not for inactivation of a wide range of viruses).



[Rebelo et al., EID Volume 32, Number 8—August 2026 https://wwwnc.cdc.gov/eid/article/32/8/26-0077_article]

Risk from Zoonotic viruses: Example: Polyomavirus SV40



SV40 in Rhesus Monkey: asymptomatic (or mild disease).

Virus (T-Antigen) activates cellular DNA replication (S-Phase)



Cellular DNA Replication enzymes replicate virus DNA



Virus growth (lysis of infected cells)

Two Functions of SV40 T-Antigen

1. Keep cells replicating
2. Viral DNA replication

SV40 in Hamster: Tumor formation

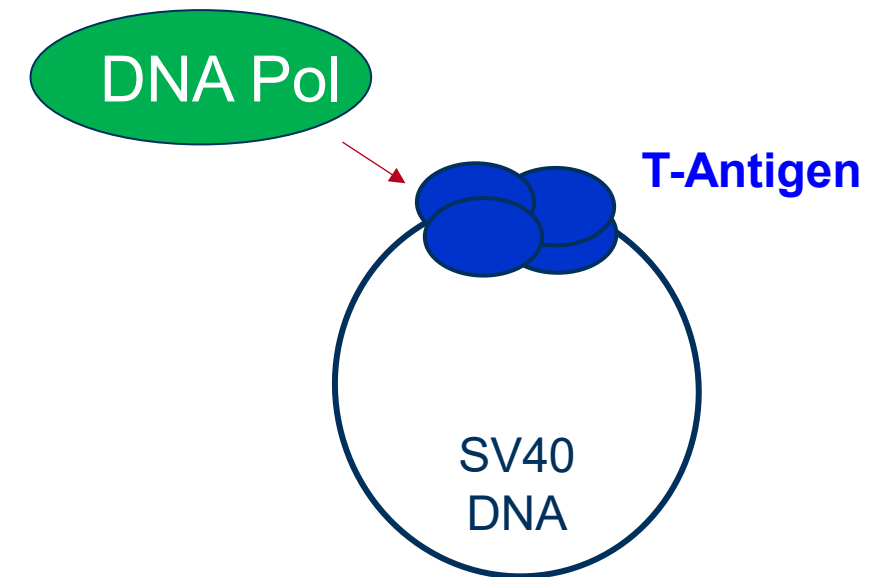
Virus (T-Antigen) activates cellular DNA replication (S-Phase)



Cellular DNA Replication enzymes do not replicate viral DNA



Cell division but no virus production (no lysis of cells)



SV40 and human infection

During 1955-1963, approximately 90% of children and 60% of adults (ca. 98 million children and adults) in the USA were inoculated for polio and possibly exposed to SV40-contaminated vaccines.

Despite SV40's ability to transform both rodent and human cells, the question of whether SV40 causes cancer in humans remained controversial

Occasional detection of SV40 DNA sequences in several rare human tumors, including ependymomas, osteosarcomas, and mesotheliomas

A meta-analysis of five published studies did not support the hypothesis that SV40 exposure increases the overall risk of cancer incidence or cancer mortality.

Dang-Tang et al., 2004 Aug 23;23(38):6535-40. doi: 10.1038/sj.onc.1207877

Bovine Polyomavirus (BPyV)

Originally reported by Rangan et al (1974) as a contaminant of Stump-tailed macaque (*Macaca arctoides*) kidney cell cultures and was originally designated as STMV.

Detected in rhesus monkey kidney cell lines (FRhK, Vero, MDBK)

Very slow growth in cell culture

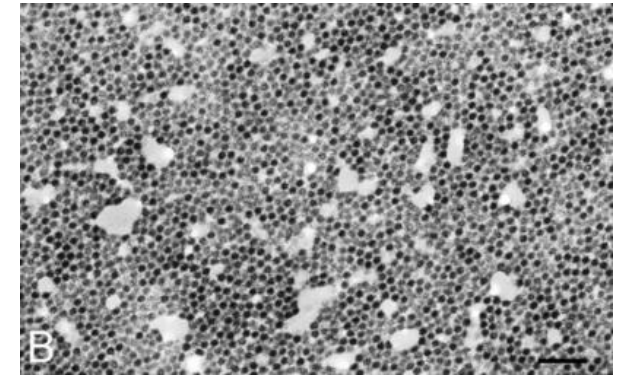
Antibodies in 16/27 FBS batches

[Parry et al., *Archives of Virology* 78, 3–4, 1983

<https://link.springer.com/article/10.1007/BF01311311>)

BoPyV-1 Virus has been isolated from aborted bovine fetus with nephropathy

[Giannitti et al. *Viruses* . 2022 Sep 14;14(9):2042. doi: [10.3390/v14092042](https://doi.org/10.3390/v14092042)]



Frequent contaminant of FBS batches. BPyV-DNA detected in

FBS: 70% (n=66)

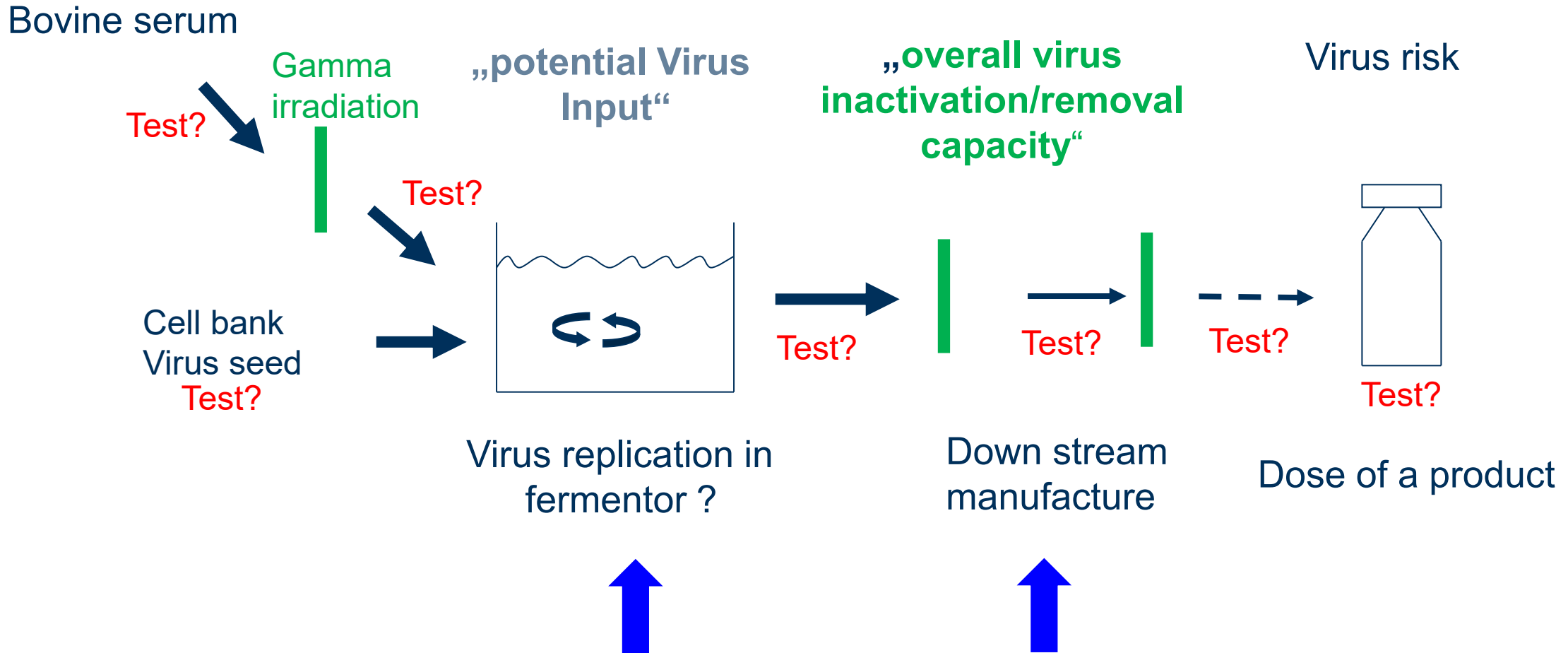
Calf serum 32% (n=34)

Adult serum 2% (n=30)

[Wang et al *New Zealand Veterinary Journal* 53(1), 26-30, 2004

<https://www.tandfonline.com/doi/full/10.1080/00480169.2005.36465>]

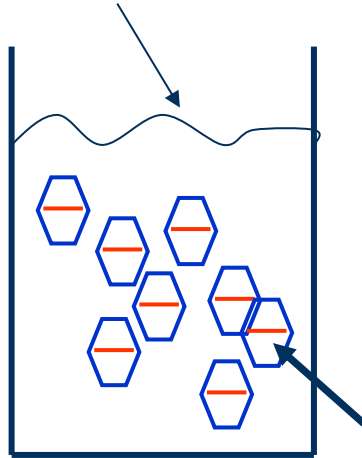
Risk assessment



- Weigh up „potential virus input“ against „overall virus inactivation/removal capacity“

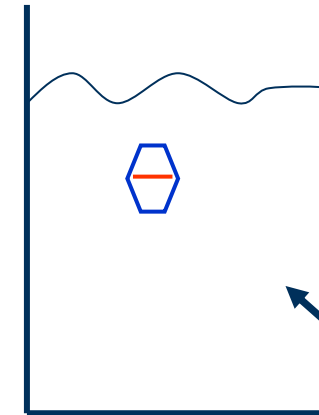
Testing before or/and after virus reduction?

Virus Spike



Test before inactivation

Inactivation

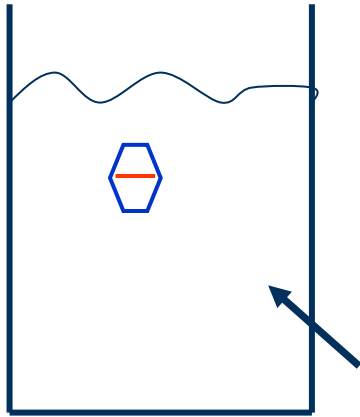


Sampling error?

Test after inactivation“

A very low number of infectious particles might contaminate a cell culture

$$p = ((V-v) / V)^n$$



Sampling error?

p: probability of negative sampling

V: volume of serum batch

v: volume applied in the test

n: absolute number of infectious particles statistically distributed in V

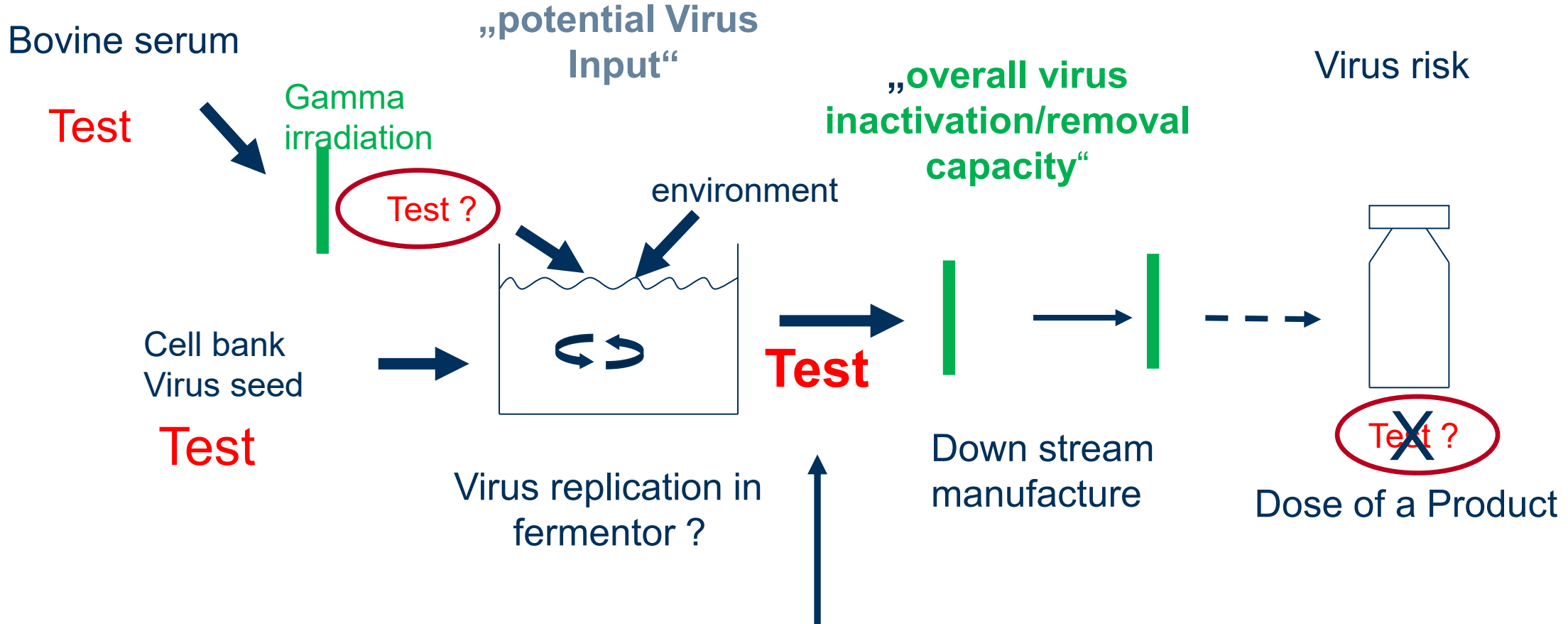
c: concentration of virus particles

or if $V \gg v$

$$c = \ln p / -v$$

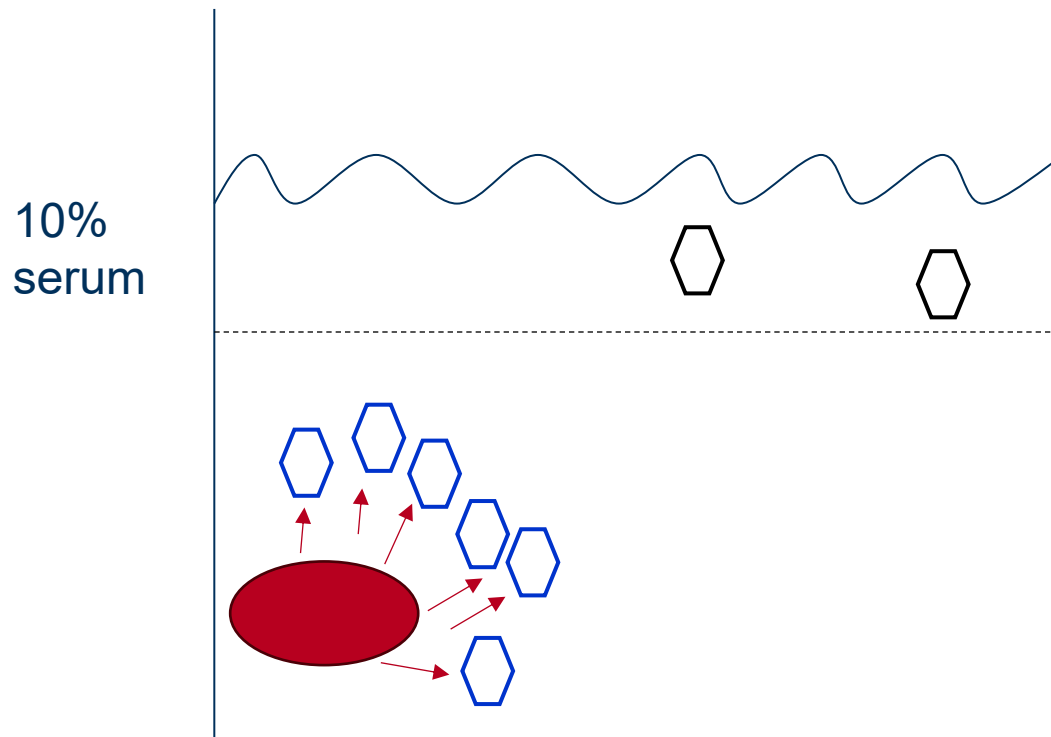
Example:

- Serum Pool of 1000L with 10^3 infectious particles per ml and >6log inactivation
- The expected concentration (c) of virus after inactivation is $c = 10^3 : 10^6$ $c = 0.001/\text{ml}$
- $P=0.05$ for a 95% probability of positive sampling.
- $v = \ln(0.05) / -0.0001 \text{ IU/ml} = \mathbf{2996 \text{ ml}}$ of inactivated serum needed for cell culture in order to verify the >6 log inactivation testing with 95% probability of detection



Testing at the harvest stage is most relevant for the risk assessment

Testing of harvest/unprocessed bulk



1. Viruses particles from 10% serum in fermentor floating around in fermenter/cell culture and not replicating

Risk:

- if present in high amount in pooled serum
- if not detected and excluded by testing of serum batches
- If not neutralized by antibodies in serum pool
- if not inactivated by gamma irradiation
- If not removed during down stream processing
- If pathogenic to patient/animal

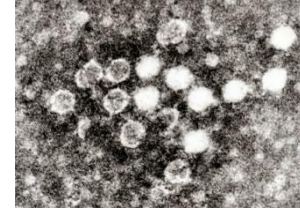
2. Viruses particles produced from cultured cell

Risk

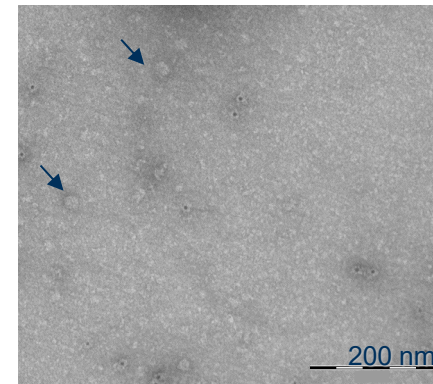
- If not removed during down stream processing
- If pathogenic to patient/animal

Viruses Resistant to Gamma Irradiation

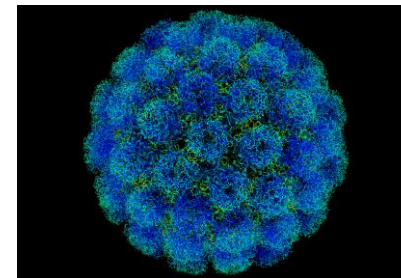
(Parvoviruses)



Circoviruses



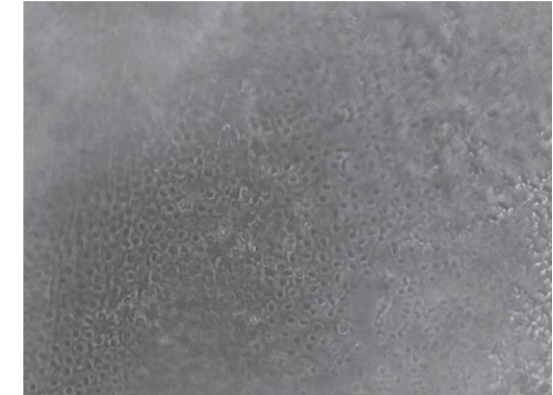
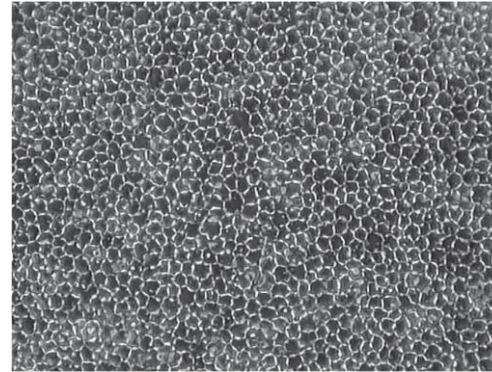
Polyomaviruses



Bovine Serum used during transplantation of human cornea

Human Donor
Cornea

Bovine
Serum



Human Cornea is cultivated in medium containing pooled fetal bovine serum in order to preserve the endothelium.

Bovine Serum should be virus-inactivated as good as possible (e.g. by $\geq 30\text{kGy}$)
Bovine Serum should not be replaced with (pooled) human serum!

Guideline ICH Q5A: for human medicinal products from cell culture

- *Manufacturers should avoid using human- and animal-derived raw materials (e.g., human serum, bovine serum, porcine trypsin) in their manufacturing processes when possible.*
- *When possible, biological materials with a high risk of introducing adventitious virus such as animal or human sera or porcine trypsin should be subjected to viral inactivation such as gamma irradiation.*
- *....this testing should be performed before inactivation, when possible*
- *Additional virus risk-mitigation strategies can include, for example, cell culture media or media supplement treatments such as virus filtration, high temperature short time processing, or ultraviolet C irradiation, where appropriate*
- *For cell lines with exposure to human or animal derived raw materials, (e.g. bovine serum and porcine trypsin), tests for human, bovine and porcine viruses should be performed*
- **Testing of unprocessed bulk:** *..This may include testing for species-specific viruses, e.g. arboviruses in insect cells, and bovine or porcine viruses if serum or trypsin are used, respectively.*

The new approach to extraneous agent testing in immunological veterinary medicinal products (IVMPs) in the European Pharmacopoeia as of 1 July 2020

What has changed and why

Prior to this revision, information on managing the risk of extraneous agents was spread across several chapters as well as general and individual monographs and required a prescriptive approach focusing on starting materials and extensive testing of the final product with a limited risk management approach to handling substances of animal origin in chapter **5.2.5. Substances of animal origin for the production of IVMPs**. There were also different levels of information for different types of products (e.g. avian and mammalian products).

All of the existing requirements have been collated, reorganised and harmonised by this revision, providing greater clarity for users.

This revision extends this approach to all materials of animal origin (including master seeds and substrates) **and the entire production process** allowing a more focused approach to the management of extraneous agents **which is based on risk identification and assessment**.

This approach, as well as the adoption of newer technologies for testing such as PCR, will lead to a reduction in the number of animals used for routine testing.

Ph. Eur. 5.1.7 VIRAL SAFETY

A risk assessment is carried out where materials of human or animal origin are used as ingredients of medicinal product or in the manufacture of active substances, excipients or medicinal products.

The risk assessment takes into consideration relevant factors, for example...

Ph. Eur. 5.2.5 MANAGEMENT OF EXTRANEIOUS AGENTS IN IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

No single measure or combination of measures can ensure the safety of use of materials of animal and human origin, but they can reduce the risks associated with such use. It is therefore necessary for manufacturers of IVMPs to take this into account when choosing a material for use in product manufacture, and to conduct a risk assessment taking into account the origin and nature of the material and the manufacturing steps to which it is subjected.

It is difficult to exclude any virus contamination from pooled batches of bovine serum.

The viral safety is evaluated in the context of the medicinal product.

Thank you for the attention!