

Risk Assessment of Bovine Viruses Unable to Replicate in Production Cell Culture

A scientific approach
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HTS Analysis of Bovine Serum Described in Scientific Publications

How to deal with viral sequences detected by HTS in Bovine (Fetal) Serum?

- 19 virus sequences detected in serum originating from AUS/NZ
- 45 virus sequences detected in Serum originating from US/North America
- Different sequencing technologies and bioinformatic analysis used
- Large range of number of reads



- New Parvoviruses described e.g Bosavirus
- Identified viral sequences currently does **not** fulfil Kochs postulates
 - No infectious virus isolation in cell culture
 - Not related to a specific disease
 - Detected also in healthy animals



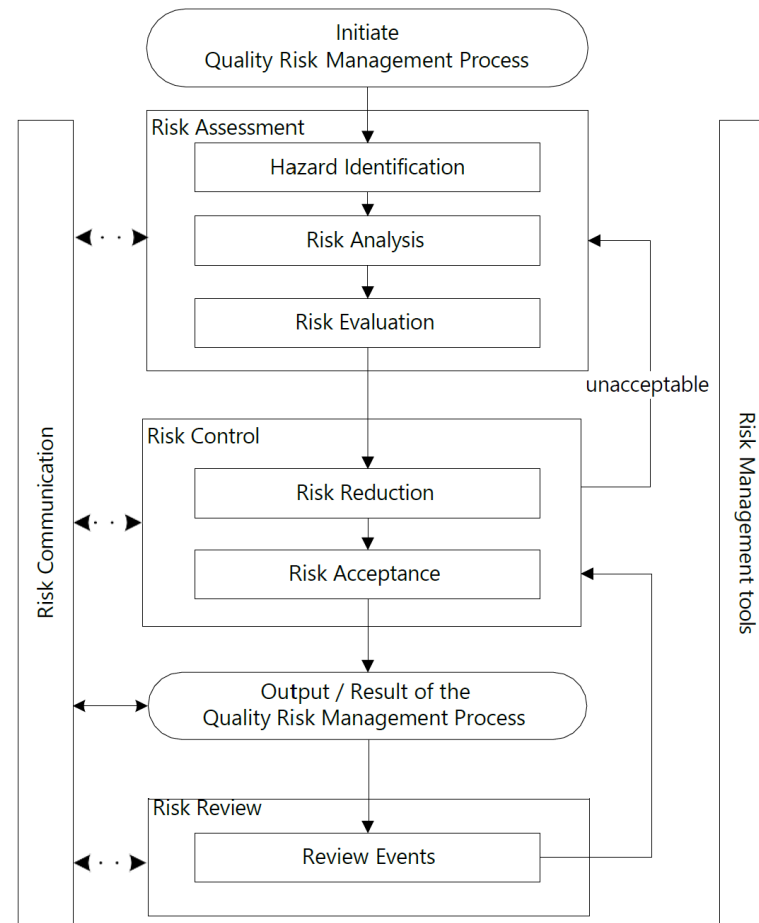
Not every virus might be a pathogen



Viral safety risk assessment required !

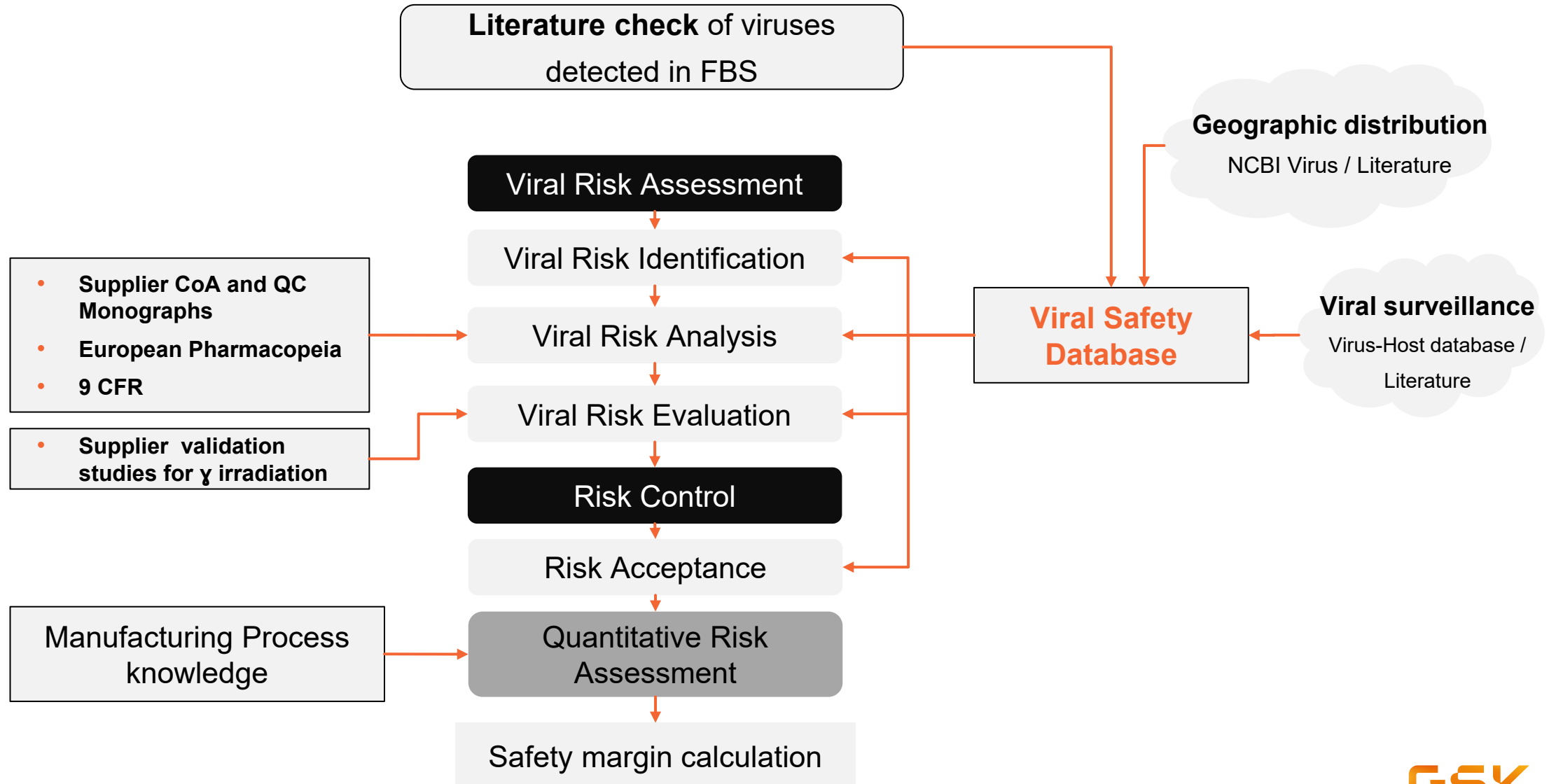
ICH Guideline Q9 (R1) on Quality Risk Management

Overview of a typical quality risk management process

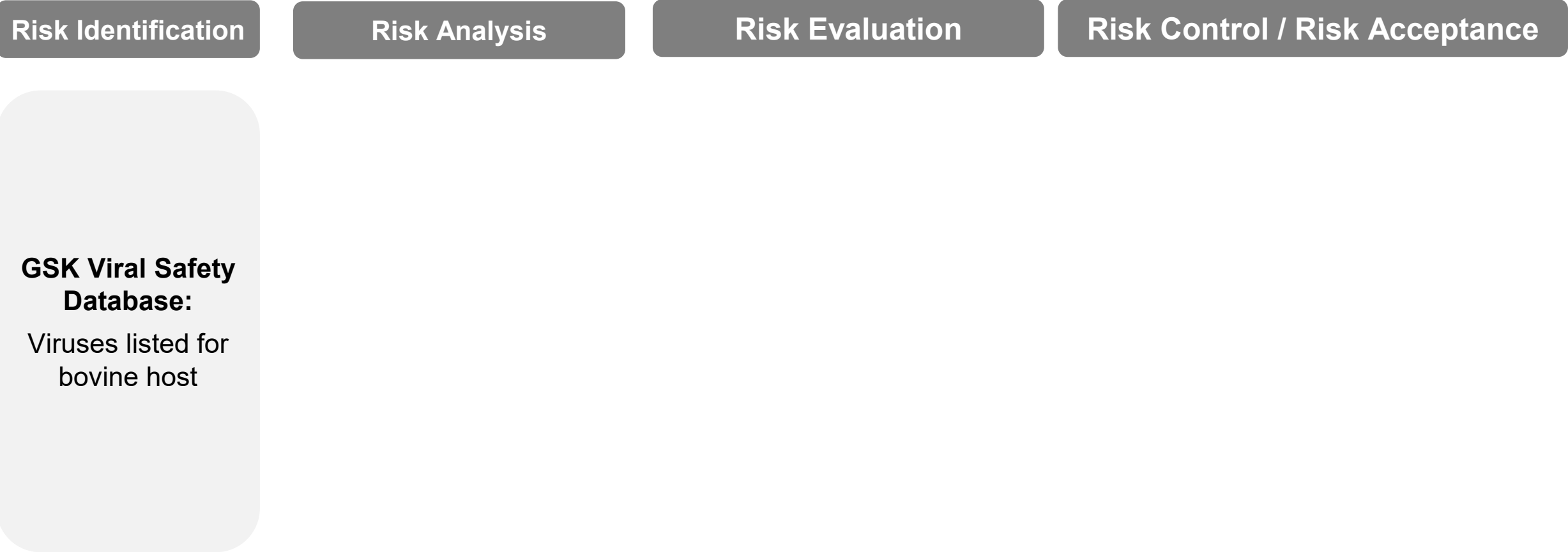


Introduction

Knowledge management & Risk assessment process summary



Overview of Viral Risk Assessment for FBS



Risk Identification

Virus families recognized for *Bos taurus* host

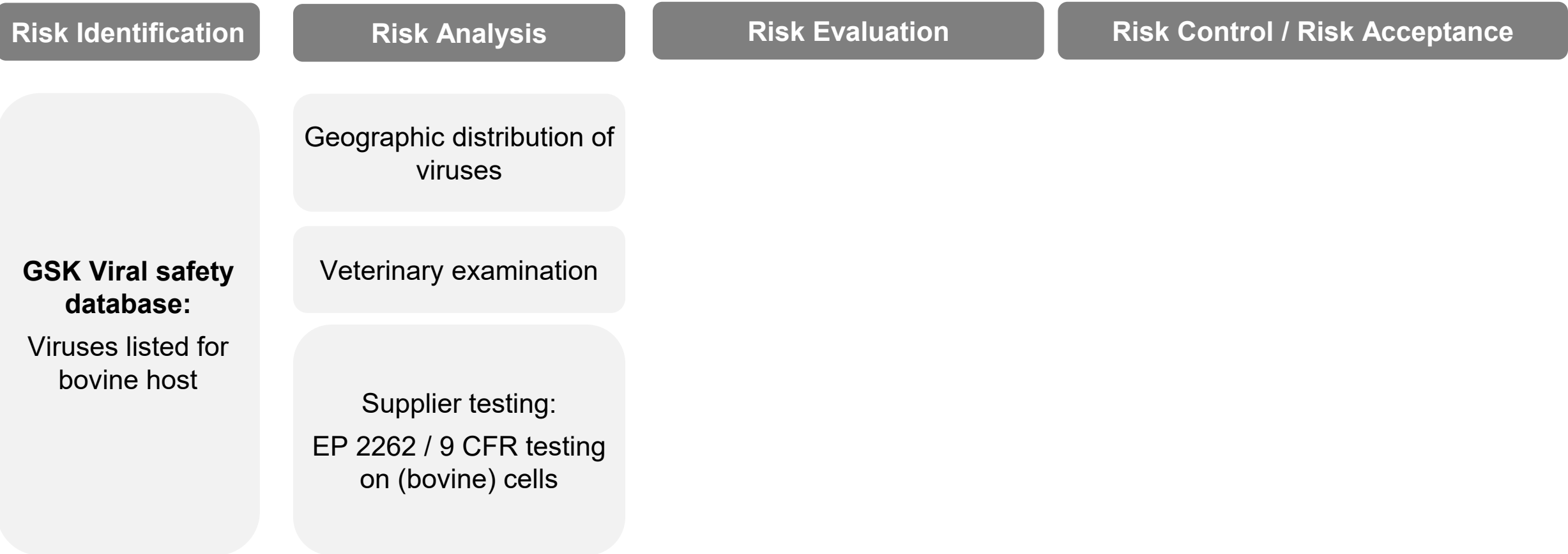
Enveloped virus families

- Bornaviridae
- Coronaviridae
- Flaviviridae
- Herpesviridae
- Nairoviridae
- Orthomyxoviridae
- Paramyxoviridae
- Peribunyaviridae
- Phenuiviridae
- Pneumoviridae
- Poxviridae
- Retroviridae
- Rhabdoviridae
- Tobaniviridae

Non enveloped virus families

- Togaviridae
- Adenoviridae
- Anelloviridae
- Astroviridae
- Caliciviridae
- Circoviridae
- Hepeviridae
- Papillomaviridae
- Parvoviridae
- Picornaviridae
- Polyomaviridae
- Reoviridae

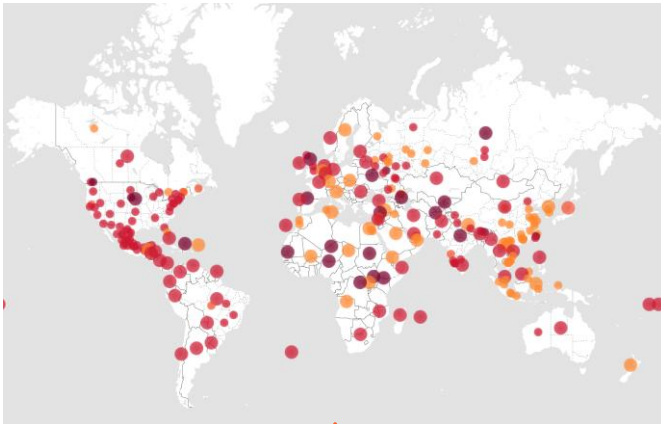
Overview of Viral Risk Assessment for FBS



Risk Analysis

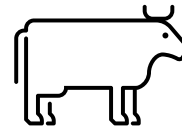
Removal of viruses from the risk based on scientific information

Geographic distribution



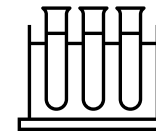
Is the virus circulating in the country of origin ?

Veterinary examination



Is the virus causing a disease with obvious symptoms?

EP2262 / 9-CFR testing

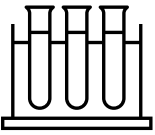


Is the virus detectable by CPE , HAD or specific Antibodies on *in vitro* cell culture ?

Disclaimer: Limitations of risk analysis : Not every virus variant can be individually assessed

Risk Analysis

EP 2262 / 9CFR



EP2262
9-CFR
testing

In vitro testing on Vero and bovine cells

The cell lines used are suitable for detecting hemadsorbing viruses such as bovine parainfluenza virus 3 and cytopathic agents such as bovine herpesvirus 1.

Antibody test

Specific tests (fluorescent antibody technique) for viral contaminants (if not detected by general tests), where relevant in view of the country of origin of the serum:

- Bluetongue virus
- Bovine adenovirus
- Bovine parvovirus
- Bovine respiratory syncytial virus
- Bovine viral diarrhoea virus
- Rabies virus
- Reovirus

Diversity of bovine parvovirus

#1	Family: <i>Parvoviridae</i> > Subfamily: <i>Parvovirinae</i> > Genus: <i>Bocaparvovirus</i> Species: Bocaparvovirus ungulate1 Exemplar virus: bovine parvovirus 1 (DQ335247)
#2	Family: <i>Parvoviridae</i> > Subfamily: <i>Parvovirinae</i> > Genus: <i>Copiparvovirus</i> Species: Copiparvovirus ungulate1 Exemplar virus: bovine parvovirus 2 (AF406966)
#3	Family: <i>Parvoviridae</i> > Subfamily: <i>Parvovirinae</i> > Genus: <i>Erythroparvovirus</i> Species: Erythroparvovirus ungulate1 Exemplar virus: bovine parvovirus 3 (AF406967)
#4	Family: <i>Parvoviridae</i> > Subfamily: <i>Parvovirinae</i> > Genus: <i>Tetraparvovirus</i> Species: Tetraparvovirus ungulate1 Exemplar virus: bovine hokovirus 1 (EU200669)

Do you consider Bosavirus:
Ungulate copiparvovirus 5
To be detected by the antibody
test?

Overview of Viral risk Assessment for FBS

Risk Identification

GSK Viral safety database:

Viruses listed for bovine host

Risk Analysis

Geographic distribution of viruses

Veterinary examination

Supplier testing:
EP 2262 / 9 CFR testing
on bovine cells

Risk Evaluation

**Sensitivity of virus families
to γ - irradiation ≥ 4 logs :**

Virus scoring:
Low/Medium/High
Attention

Risk Control / Risk Acceptance

Gamma irradiation

Sensitivity of bovine viruses to γ - irradiation.

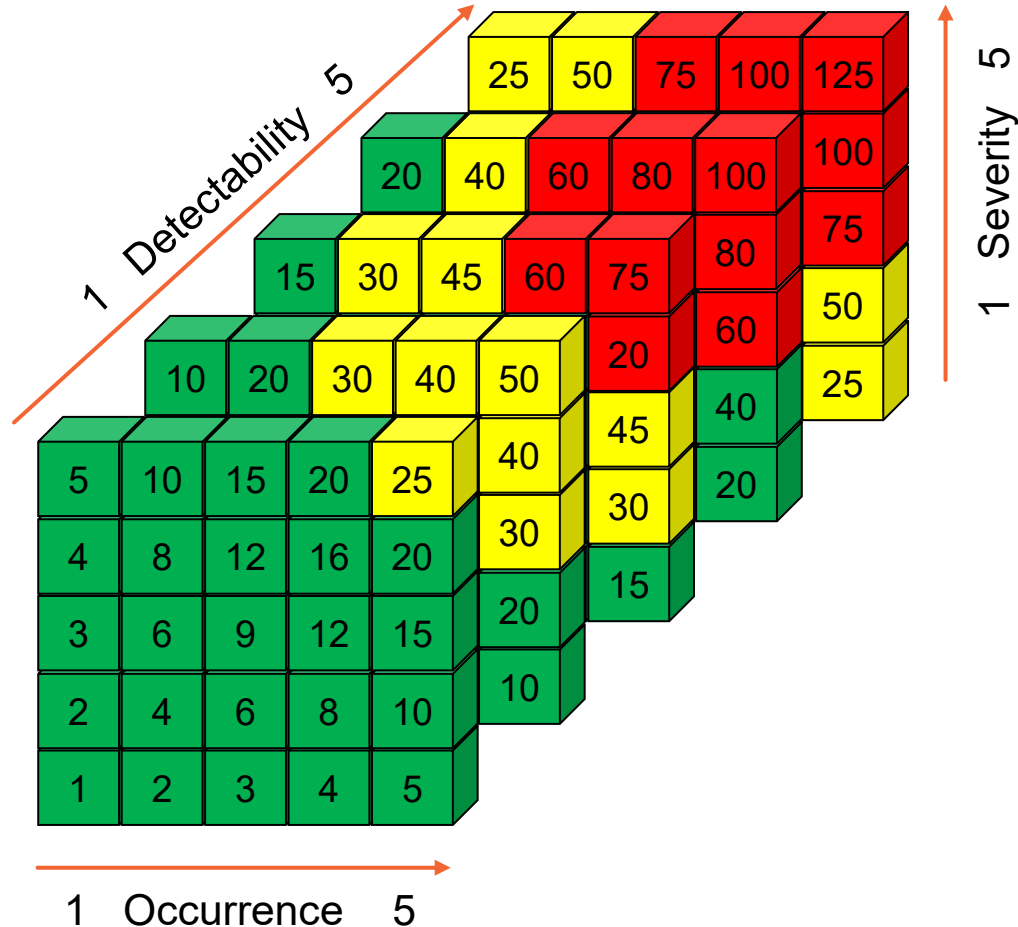
ICHQ5A R2 *When possible, biological materials with a high risk of introducing adventitious viruses such as animal or human serum or porcine trypsin should be subjected to viral inactivation such as ionizing irradiation.*

- Target at least 4 logs of reduction using minimum 25 kGy
 - ICHQ5A R2 *An effective viral clearance step generally gives reproducible reduction of virus load in the order of 4 log₁₀ or more shown by at least two independent studies.*
- Critical to obtain validation data from the individual bovine serum supplier demonstrating a ≥ 4 log reduction in the respective container.
- Critical parameter:
 - Selection of model viruses
 - Sufficient high titre stock virus
 - Non-treated control (transport)
 - Read out assay sensitivity / LOD

Risk Evaluation in the Context of the Manufacturing Process

RPN scoring to prioritize the risk control activities

Risk priority Number (RPN) :



RPN= Occurrence X Detectability X Severity

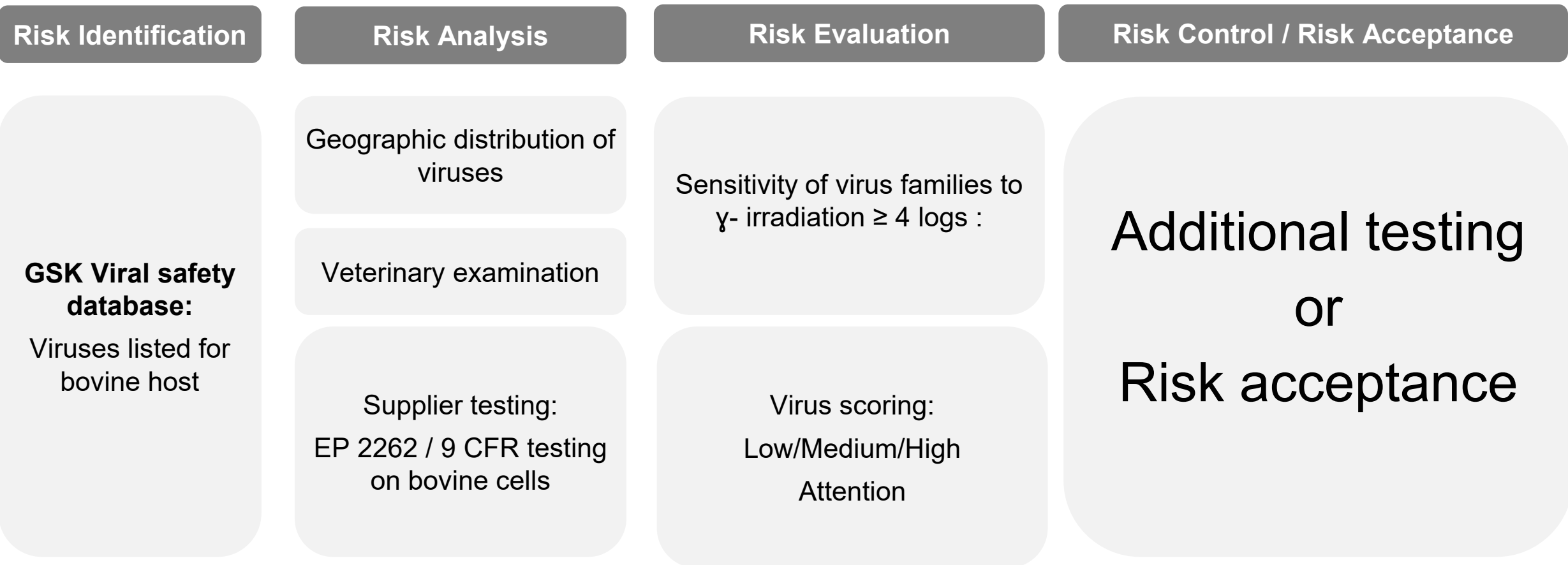
1 to 24 = low-attention category

25 to 59 = medium-attention category

60 to 125 = high-attention category

The highest risk levels need to be addressed as priorities.

Overview of Viral Risk Assessment for FBS



Risk Control

Risk control:

- Implementation of additional Testing (e.g. PCR or HTS) for human pathogenic viruses or viruses able to propagate on your product cell substrate if required

Risk acceptance for bovine viruses

- which are NOT human pathogenic,
- which CANNOT propagate in production cell culture

Perform a Quantitative Risk Assessment considering process dilutions to calculate additional safety margin if required

- Monitoring of scientific Literature : Surveillance of new emerging bovine viruses

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➤ Monitoring of scientific Literature :
Surveillance of new emerging bovine viruses

Quantitative Risk Assessment

Risk Control vs Risk Acceptance

Risk identification		BVDV	Bosavirus
Risk Analysis	Geographic distribution	Worldwide	Worldwide?
	Detectable by Veterinary exam.	No	No
	Detectable by EP 2262/ 9-CFR	Yes	No ?
Kochs postulate	Causing Disease	Yes	No
	Infectious virus isolated in vitro?	Yes MDBK cells	No Unknown
Inactivation	Resistant to γ -irradiation	No	Yes*
	Considered as Pathogen	Yes	No
	Replication on production cell substrate	Yes for Bovine cells	No <i>in vitro</i> replication
Risk		Controlled	Accepted
Additional safety margin			Quantitative Risk assessment

*Resistance extrapolated from inactivation data using other related Parvoviruses