



PCV-1 detection in Rotavirus vaccine:
a case study in the risk assessment for adventitious
agent testing

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Control of adventitious agents in vaccines: how to deal with? (1)

- Control of starting materials
 - In-depth assessment of cell lines and raw materials history
 - In-depth knowledge of starting materials origin, manufacturing process and risk reduction strategies
 - Appropriate testing of cell line and raw materials (including cell culture media and animal-derived raw materials) for the absence of undesirable viruses/agents which may be infectious and/or pathogenic for humans

- Process knowledge and validation
 - Complete assessment of the manufacturing process with respect to the capacity to inactivate or remove infectious viruses

Control of adventitious agents in vaccines: how to deal with? (2)

■ QC Testing program

- The product is tested at appropriate stages during production for the absence of contaminating infectious viruses by specific and non specific tests
- Testing program based on a risk analysis and in accordance to regulatory requirements

■ Risk management process

- Communication and transparency with Regulatory Authorities
- Share issue, if any, that may arise
- Pro-actively discuss implementation of new methods and technologies
- Regularly review the management process based upon new scientific findings or technology development

Control testing of live attenuated viral vaccines

Control of starting materials

- cell line and raw materials history
- comprehensive and specific testing program

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Process knowledge and validation

- limited downstream process
- no or few steps for virus inactivation

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QC testing program

- at appropriate stages during production
- based on risk assessment analysis

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Risk management process

- communication and transparency
- retesting/reevaluation needed based on new scientific finding or technology development

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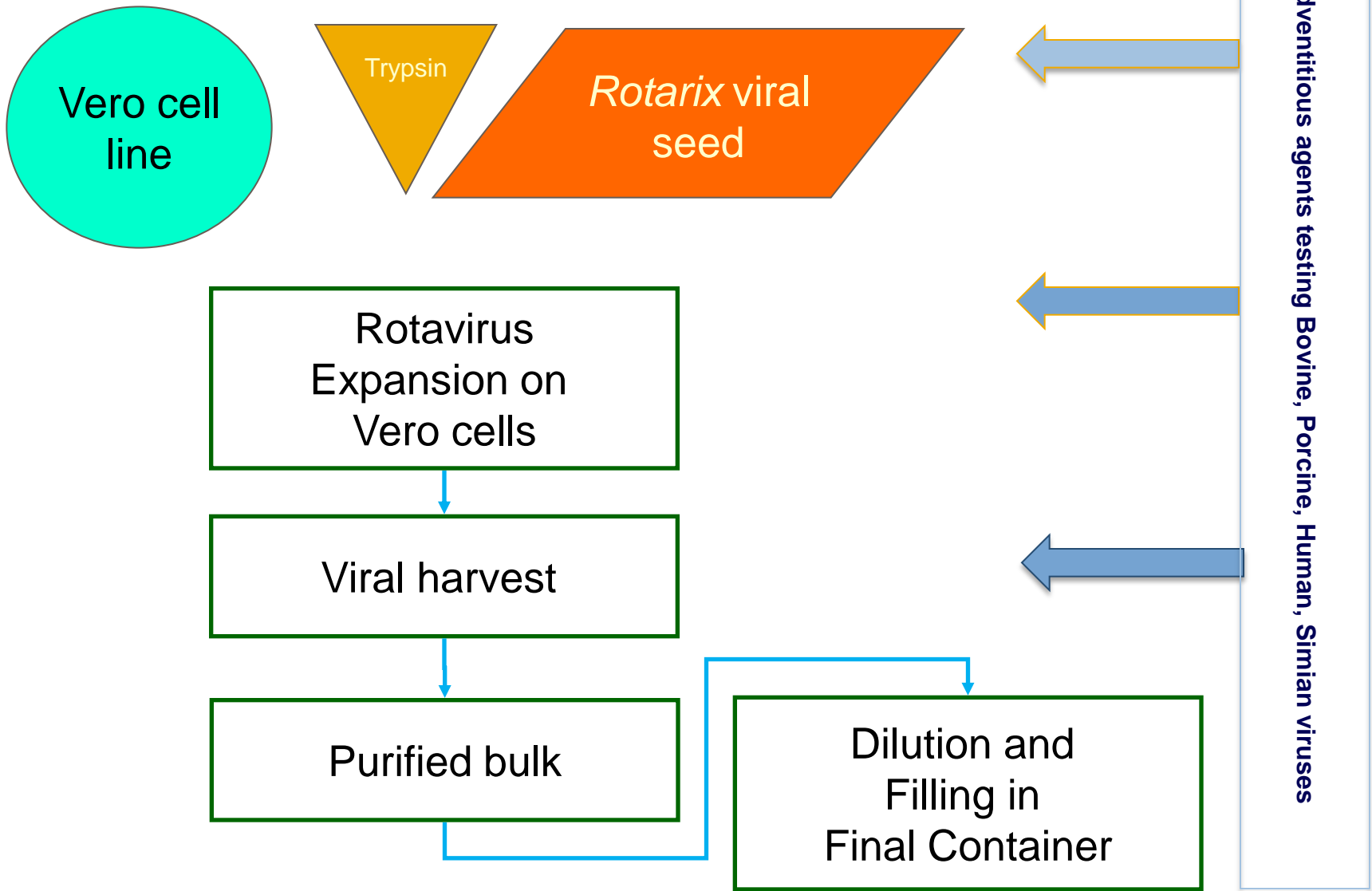
Rotarix[®] (Rotavirus Vaccine, Live Oral):

- Rotarix[®] is a live attenuated human rotavirus (HRV) vaccine for oral administration
- Rotarix[®] is indicated for prevention of gastro-enteritis (GE) due to rotavirus infection in infants from the age of 6 weeks
- The first licence for Rotarix[®] was obtained in July 2004
- Rotarix[®] is currently registered in 121 countries , with two coexisting formulations: lyophilised (oral applicator) and liquid (tube)
- Rotarix[®] is produced on Vero cell substrate

N.B. Rotarix[®] is a registered trademark of the GlaxoSmithKline group Companies



Routine manufacturing process



PCV-1 DNA detected in *Rotarix* the facts

Jan

- GSK notified of research data generated at UCSF (high tech method not routinely used for the testing of adventitious agents)
- DNA sequences originating from porcine circovirus (PCV-1) were detected in two batches of Rotarix®

March

- March 11th : confirmation by a validated assay
- March 15th : communication of results to Regulatory Authorities

April

- Investigations and immediate actions

May

- Joint VWP/BWP meeting
- VRBPAC

June

- Long term action plan:
 - ✓ Manufacture and validation of PCV-free starting materials and derived vaccine
 - ✓ Re-assessment of testing program for absence of adventitious agents

Summary of investigation strategy

1

1. Extent of the PCV-1 DNA presence?
 - At what manufacturing step is detected PCV-1 DNA?
 - What is the possible root cause?

2

2. Are infectious particles present or not?
 - Are infectious particles present or only PCV-1 DNA?
 - At which level? Viral titre of PCV-1 in the vaccine?

3

3. Is there any safety risk?
 - Evaluate whether PCV-1 viral particles are able to undergo productive infection in human cells?
 - Evaluate whether PCV-1 viral particles may cause infection in human infants?

Key success factors

1. Develop appropriate analytical tools
2. Build up strong PCV-1 expertise
3. Interaction with Authorities

GOAL

Extent of PCV-1 DNA presence?

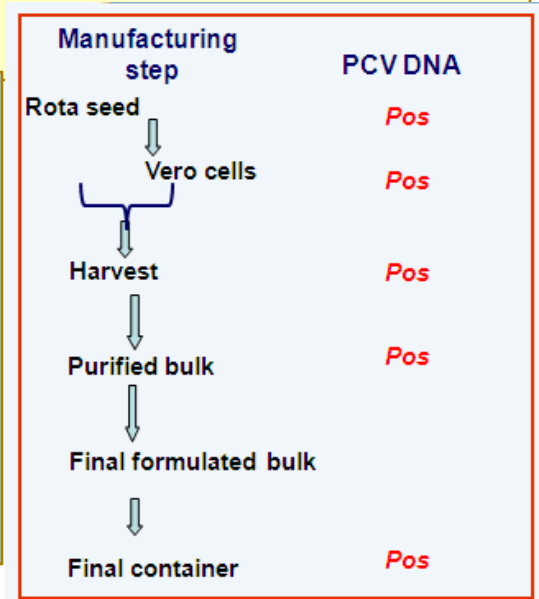
- At what manufacturing step is detected PCV-1 DNA?
- What is the possible root cause?

METHOD

- Generic PCR covering porcine and bovine circoviruses (PCV-1/2, BCV)
- Specific PCV-1 or 2 PCRs
- Full-length genome PCR determination

RESULTS

- PCV-1 DNA detected in Vero cell bank and at each intermediate steps and in the final vaccine of *Rotarix*
- Possible root cause: contaminated lot of trypsin used for Master Cell Bank preparation
- Same level of PCV-1 DNA found in commercial vaccine lots and in the 3 clinical lots used in the large Phase III safety trials



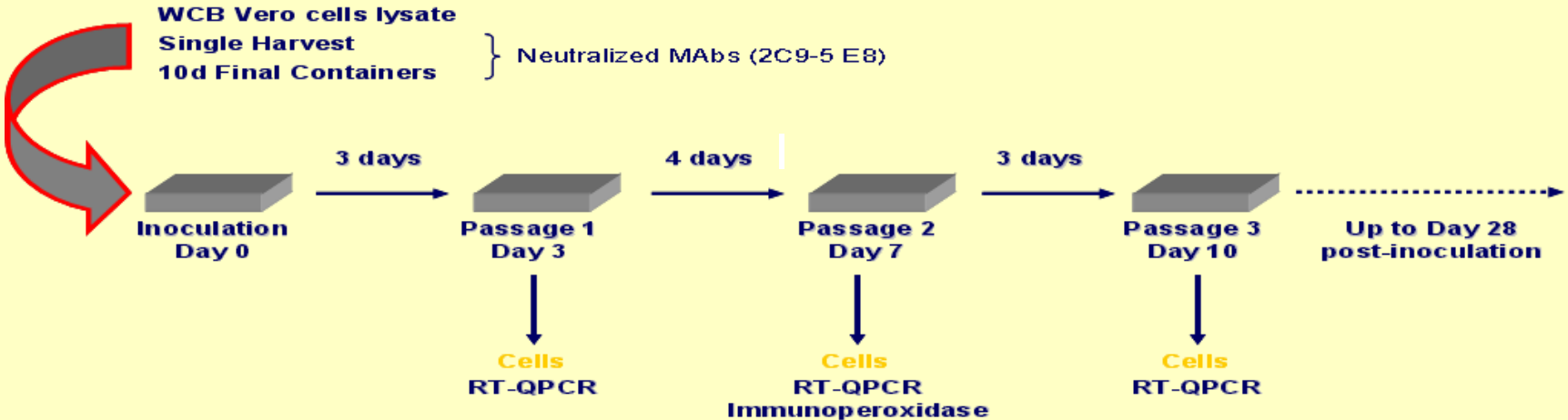
GOAL

Are infectious particles present or not?

- Are infectious particles present or only PCV-1 DNA?
- At which level? Viral titre of PCV-1 in the vaccine?

METHOD

Infectivity studies on permissive cells (Vero/PK15): sensitivity 1 CCID₅₀



RESULTS

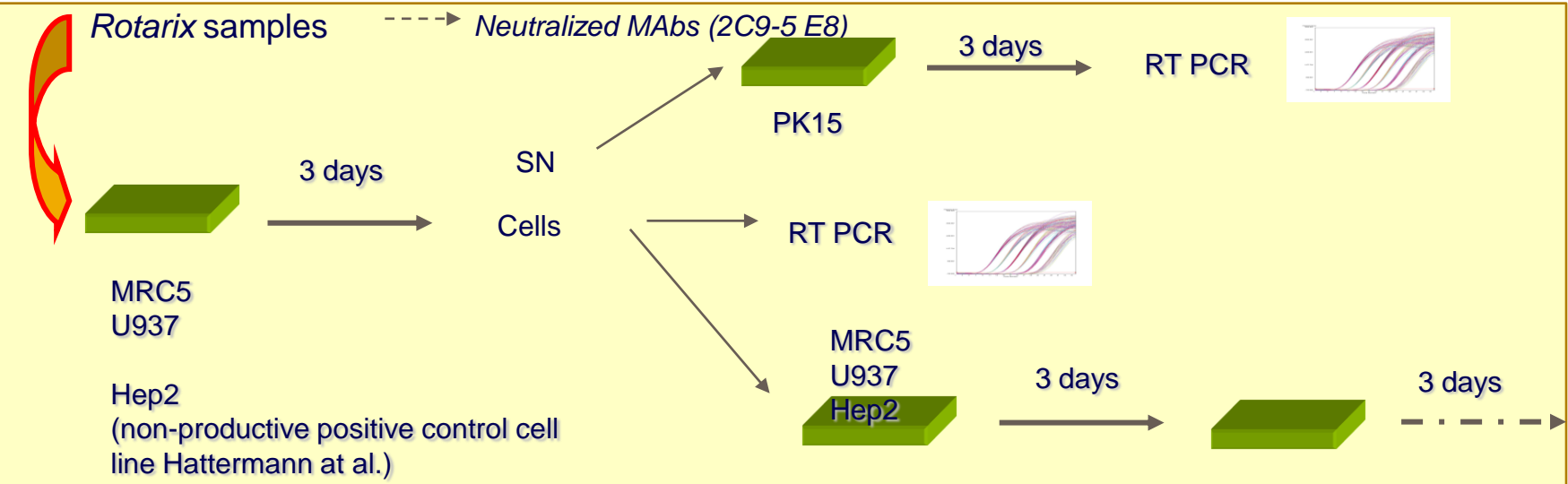
- Vero cell bank: positive
- Rotavirus: single harvest and final vaccine → positive
- PCV-1 titration in final vaccine => between 3 and 100 CCID50 per dose

GOAL

Is there a safety risk?

- Are PCV-1 viral particles able to undergo productive infection in human cells?

METHOD



RESULTS

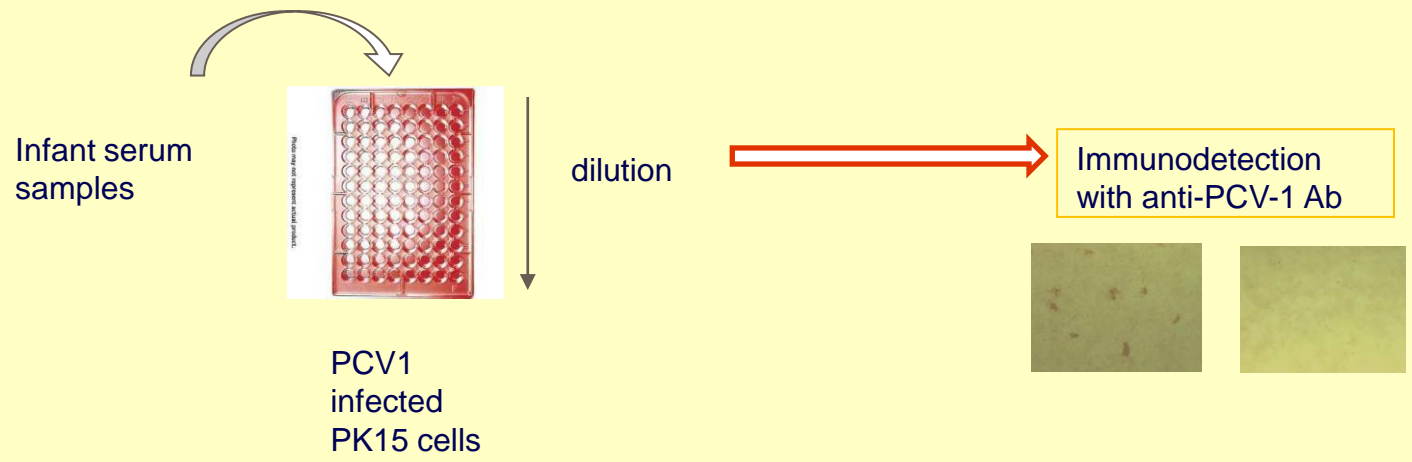
No evidence of productive PCV-1 infection in human cell lines

GOAL

Is there a safety risk ?

- Are PCV-1 viral particles able to cause infection in human infants?

METHOD



RESULTS

Absence of PCV-1 seroconversion as assessed in infants receiving *Rotarix* vaccine

***Rotarix* PCV-1 investigations - Conclusions**

1. Low level of infective virus in production process and in the final container
 2. No evidence of productive infection in human cells
 3. No suggestion of occurrence of PCV-1 infection in infants who received *Rotarix*
 4. All the safety data from clinical trials and postmarketing reflects presence of PCV1 and there is no evidence that PCV-1 material in *Rotarix* poses a safety risk
- The benefits of *Rotarix* together with safety data and laboratory findings of lack of infectivity in human cell lines led to agreement with Regulatory Authorities that the license should remain in effect.

Learnings from PCV-1... Key success factors

- To build up internal PCV-1 expertise
 - Small non enveloped virus : 17 nm
 - Single-strand, circular DNA (1.7 kb genome)
 - Resistant to inactivation (pH, T , disinfectant)
 - PCV-1 infection is widespread in swine
 - PCV-1 does not cause any disease in pig or other animal species including humans
 - There is no concrete evidence of human infection by PCV-1
- To develop analytical tools
 - PCV molecular biology assays (PCRs)
 - Infectivity assay on permissive cells (VERO & PK15) – read-outs = RT-PCR , immunostaining
 - Titration assays
- To keep open and transparent discussion with Regulatory Agencies WW



Learnings from PCV-1... Long-term action plan

- Manufacture and validation of PCV-free starting materials and derived vaccine
 - ✓ preparation of PCV-free Vero cell bank from ATCC cell bank
 - ✓ preparation of new Rotavirus seed starting from the pre - master seed of the current vaccine.
 - ✓ in-depth genotypic and phenotypic characterization of *Rotarix* manufactured from PCV-free cell bank and seeds in order to demonstrate of its comparability to the currently licensed rotavirus vaccine

- Re-assessment of testing program for specific adventitious agents
 - ✓ Test/screen all future lots of trypsin for porcine circoviruses. Only use PCV-free trypsin in all future manufacturing processes
 - ✓ Review and extend the testing program for starting materials including trypsin, cell banks and viral seeds according to a risk assessment approach
 - ✓ Investigate on the availability of infectivity assays to be used in combination with PCR-based assays

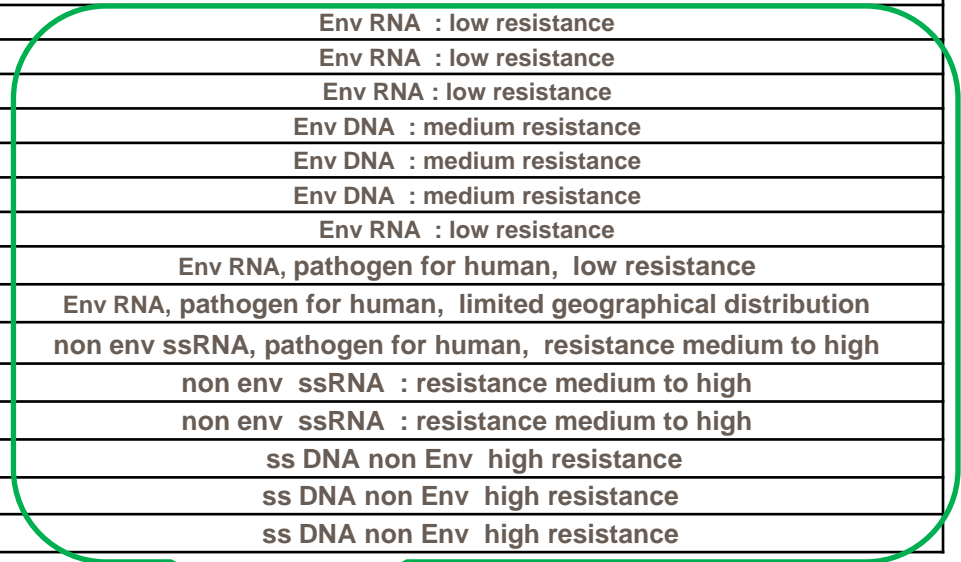
Testing program for specific adventitious agents

- Risk assessment approach to identify the most relevant list of viruses to be tested
- Step-wise implementation process
 - ✓ Review and extend the testing program for starting materials such as trypsin of porcine origin
 - ✓ Extend the selected adventitious agent screening to the testing of cell banks and seeds, as appropriate
- Investigation on the availability of infectivity assays to be used in combination with PCR-based assays

Porcine viruses - Risk assessment approach

9 CFR 113.47	FDA guidance on cell substrates Feb 2010	WHO draft on cell substrates Sep 2010	GSK BIO position
PPV	PPV	PPV	PPV
BVDV	BVDV	BVDV	BVDV
REO	REO	REO	REO
RABIES	RABIES	RABIES	RABIES
ADENO	ADENO	ADENO	ADENO
TGE	TGE	TGE	TGE
HEV	HEV	HEV	HEV
	PCV	PCV	PCV
	INFLUENZA		Env RNA : low resistance
	PRRS	PRRS	Env RNA : low resistance
	Hog Cholera		Env RNA : low resistance
	CMV		Env DNA : medium resistance
	PSEUDORABIES		Env DNA : medium resistance
	POX		Env DNA : medium resistance
	Retrovirus		Env RNA : low resistance
	VSV		Env RNA, pathogen for human, low resistance
	NIPAH		Env RNA, pathogen for human, limited geographical distribution
		HEPATITIS E	non env ssRNA, pathogen for human, resistance medium to high
	ENTERO		non env ssRNA : resistance medium to high
		EMCV	non env ssRNA : resistance medium to high
		TTV	ss DNA non Env high resistance
		HOKO	ss DNA non Env high resistance
		BOCA	ss DNA non Env high resistance

Current testing



RISK ASSESSMENT ANALYSIS

Porcine viruses - Risk assessment approach

Selection of the viruses to be tested is based on:

- ✓ the known exposure (e.g. PCV-1)
- ✓ the pathogenicity of the virus to human (e.g. Hepatitis E, Nipah virus)
- ✓ the resistance to the two viral inactivating processes used in the trypsin production (irradiation and low pH for extended period of time) (e.g. non-enveloped ssDNA viruses such PCV, Parvovirus, Torque Teno virus, Hokovirus, Bocavirus)
- ✓ the viral clearance results generated so far on model viruses
- ✓ the geographical distribution (e.g. Nipah virus)
- ✓ the ability of the cell culture-based assay to detect the virus
- ✓ the ability of the process cell substrate to sustain the growth of the virus
- ✓ the availability of a validated PCR or combined infectivity/PCR assay for the target virus

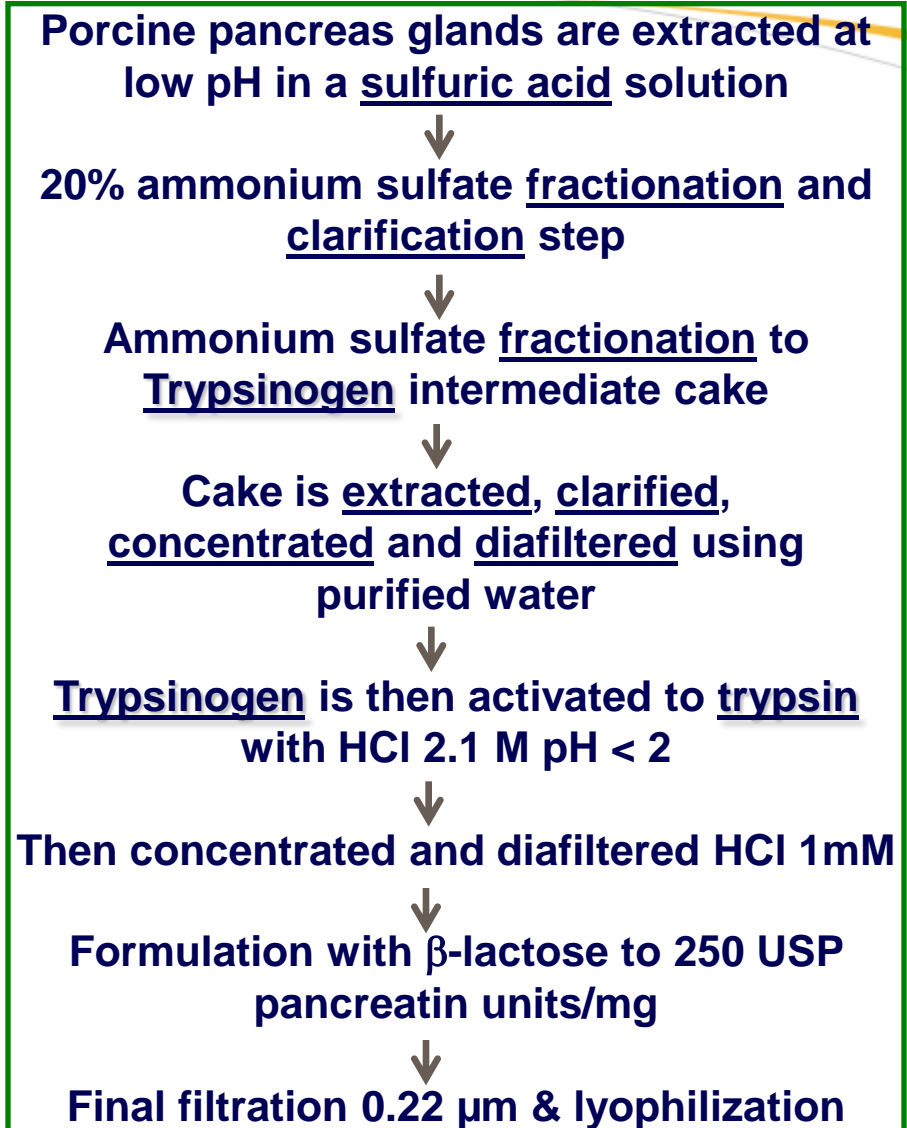
How is trypsin prepared ?

- Porcine pancreatic extracts with sulfuric acid solution and treated at extremic acid conditions (pH <2) and maintained for about 12 hours to inactivate a wide variety of potential adventitious viruses* originated from porcine pancreas.

* Bovine viral diarrhea (BVD), Porcine Parvovirus (PPV), Porcine Adenovirus (PAV), Transmissible gastroenteritis Virus (TGE), Rabies, Porcine Hemmagglutinating Encephalitis Virus (HEV), Reovirus.

* Information obtained from the supplier

Aliquoted and irradiated



Proposal for trypsin testing

- In addition to 9CFR viruses, additional viruses to be tested:
 - **Porcine Circovirus** (known exposure and high resistance)
 - **Hepatitis E virus** (high pathogenicity to human)
- Treatments applied during trypsin preparation are considered as supporting the effective inactivation/clearance of other viruses selected according to the risk assessment approach (e.g. ssRNA viruses such as EMCV and Enteroviruses)
- New clearance data are being generated using PCV-1 as a model of non-enveloped ssDNA viruses such as swine Torque Teno Virus, Hokoviruses, and Bocaviruses
- Current batches of trypsin have been tested by Q-PCR for the absence of sTTV upon request from different Regulatory Authorities

Proposal for cell banks and seeds testing

- In addition to 9CFR viruses, additional viruses to be tested:
 - **Porcine Circovirus**
 - **Hepatitis E virus**

- Provided that validated infectivity assays are available and could be used in combination with Q-PCR assays
 - **Non-enveloped ssRNA (Enteroviruses, Encephalomyocarditis virus or EMCV)**
 - **Non-enveloped ssDNA viruses (TTV, Hokovirus, Bocavirus)**

Available assays for detection of porcine viruses

9 CFR viruses	
Host	Virus
Bovine	Viral Diarrhoea Virus
Porcine	Parvovirus
Porcine	Reovirus
Porcine	Rabies Virus
Porcine	Adenovirus
Porcine	Transmissible Gastroenteritis Virus
Porcine	Haemagglutinating Encephalomyelitis Virus

Proposed Porcine viruses			
Host	Virus	Q-(RT-)PCR	Infectivity
Porcine	Hepatitis E Virus	YES	No assay
Porcine	Encephalomyocarditis Virus	YES	CPE
Porcine	Enterovirus	YES	CPE (Teschovirus)
Porcine	Circovirus	YES	IPMA/RT-PCR (PCV1)
Porcine	Torque Teno Virus	YES	No assay
Porcine	Hokovirus	YES	No assay
Porcine	Bocavirus	Being developed	No assay

Proposal for cell banks and seeds testing (cont'd)

- 9CFR viruses
- Porcine Circovirus + Hepatitis E virus
- Non-enveloped ssRNA (Enteroviruses, Encephalomyocarditis virus or EMCV) will be addressed through « extended » 9CFR testing
 - Use of different cell substrates for swine virus detection : Porcine Testicle cells (St-NEB, PT-1), Porcine Kidney cells (PK-15), Bovine Turbinate cells (BT), Vero and MA-104 cells
 - Use of additional control viruses such as EMCV (ssRNA non-env)
- Non-enveloped ssDNA viruses are proposed to be tested by Q-PCR assays (sTTV, Hokovirus, Bocavirus test under development)

Implementation of new technologies

GSK is currently reviewing the potential role of deep sequencing technology as complementary approach for adventitious agent detection.

- **First objective:**

- To investigate how a “Metagenomics” approach can detect viral adventitious agents (encapsidated) from a cell substrate.

- **Strategy:**

- To define and validate an appropriate sample preparation method (nuclease treatment)
- To evaluate the viral sequence recovery by “Massive Parallel Sequencing”
- To evaluate the Limit Of Detection of the entire “Metagenomics” approach

- **Need for strong collaboration with academics and regulatory authorities**

- To develop new technologies and share expertises and results
- To set a common approach for the use of such additional tool to the current standard testing methods for vaccines, considering its novelty and the absence of standardisation

Conclusions

- GSK is currently manufacturing PCV-free starting materials and derived vaccine
- In-depth genotypic and phenotypic characterization will be needed in order to demonstrate of its comparability to the currently licensed rotavirus vaccine
- Overall testing program have been reviewed and extended according to a risk assessment approach for adventitious agent selection
- Developing infectivity assays to be used in combination with specific PCR-based assay is key
- GSK is also assessing new technologies such as deep sequencing as complementary approach for adventitious agent detection



GlaxoSmithKline