Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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Methods for the Detection of Infectious Diseases

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US FDA Cellular, Tissue, and Gene Therapies Advisory Committee

June 29th and 30th, 2022

Sensitive detection systems for infectious agents in xenotransplantation

Infection after allotransplantations

- Herpesviruses: CMV, EBV, HSV
- HIV-1
- Rabies virus
- Hepatitis B
- Jacob Creutzfeld disease after transplantation of dura mater
- …

Eventually xenotransplantation may be safer compared with allotransplantation

Porcine viruses: our publications on methods and risk

Virus	PCR-based detection methods PCR, RT-PCR, real-time PCR, ddPCR	Immunological methods Western blot analysis, ELISA, immunoperoxidase assay (IPA), immunohistochemistry
PERV	Tacke et al., 2000; Denner et al., 2002; Karlas et al., 2010; Kaulitz et al., 2011a; Kaulitz et al., 2013; Morozov et al., 2016; Fiebig et al., 2018, Krüger et al., 2019a, b, 2020, 2021a, b, Halecker et al., 2021, 2022a, b	Stephan et al., 2001; Tacke et al., 2001; Irgang et al., 2003; Kaulitz et al, 2011b; Bittmann et al., 2012; Denner et al., 2015; Morozov et al., 2016;
PCMV/PRV	Plotzki et al, 2015; Morozov et al., 2016; Morozov et al., 2016¸Morozov et al., 2017; Fiebig et al., 2018, Egerer et al., 2018, Denner et al., 2020	Plotzki et al., 2016a; Fiebig et al., 2018
HEV	Morozov et al., 2015; Abicht et al., 2016;	Morozov et al., 2015
PLHV-1, -2, -3	Morozov et al., 2016;	Plotzki et al, 2016b
PCV1, PCV2	Heinze et al., 2016	
PCV3	Prinz et al., 2019; Krüger 2019	

Virus	Reviews analysing potential risk posed by these viruses
PERVs	Denner, 2008; Denner and Tönjes, 2012; Denner, 2016; Denner 2017; Scobie, Denner, Schuurman, 2017; Denner, 2018, Denner, 2021, Denner and Schuurman, 2021, Denner 2022,
HEV	Denner, 2015
PCMV/PRV	Denner, 2015; Denner, 2018, Denner et al, 2019
PCV1,2,3,4	Denner and Mankertz, 2017
PLHV-1,-2,-3	Denner, 2021
Virome	Denner, 2017

Lessons from the first pig heart transplantation

Transmission of a pig virus: porcine cytomegalovirus

- Involvement of competent virologists required
- Sensitive and specific detection systems
- Knowledge how, when and where to test

First clinical trials: Islet cells from Auckland Island pigs

- Donor pigs negative for 26 microorganisms

  PCV2, Porcine Circovirus Type 2; PCV1, Porcine Circovirus Type 1; PLHV, Porcine Lymphotrophic Herpesvirus; PCMV, Porcine Cytomegalovirus; RV, Rotavirus; PEV1, Porcine Enterovirus Type 1; PEV3, Porcine Enterovirus Type 3; PHEV, Porcine Hemagglutinating Encephalomyelitis Virus; HEV, Hepatitis E Virus; BVD, Bovine Virus Diarrhea; AujD, Aujesky's Disease; PPV, Porcine Parvovirus; PRRSV, Porcine Reproductive and Respiratory Syndrome Virus; EMCV, Porcine Encephalomyocarditis Virus

- Encapsulated islet cells
- New Zealand (14 patients) and Argentina (40 patients)
- No transmission of porcine viruses

Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. Xenotransplantation. 2014;21(4):309-323.

Morozov VA, Wynyard S, Matsumoto S, Abalovich A, Denner J, Elliott R. No PERV transmission during a clinical trial of pig islet cell transplantation. Virus Res. 2017;227:34-40.

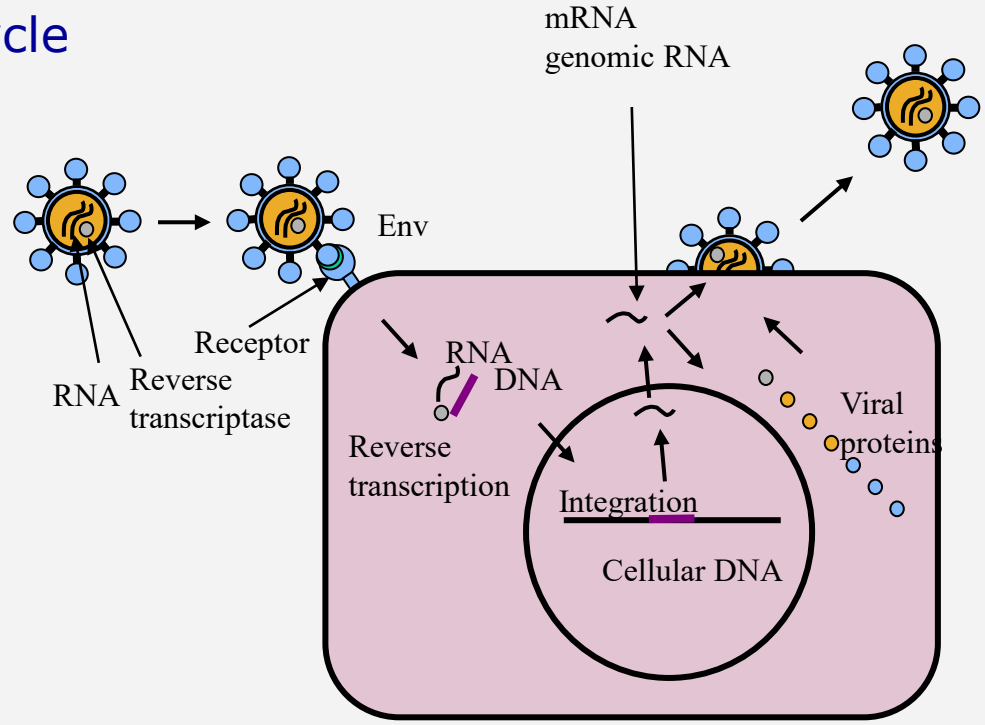
Detection systems

Complex of

- sample generation
- sample preparation
- sample origin
- time of sampling
- negative and positive controls
- along with the specific detection methods
  - PCR-based
  - cell-based
  - or immunological methods.

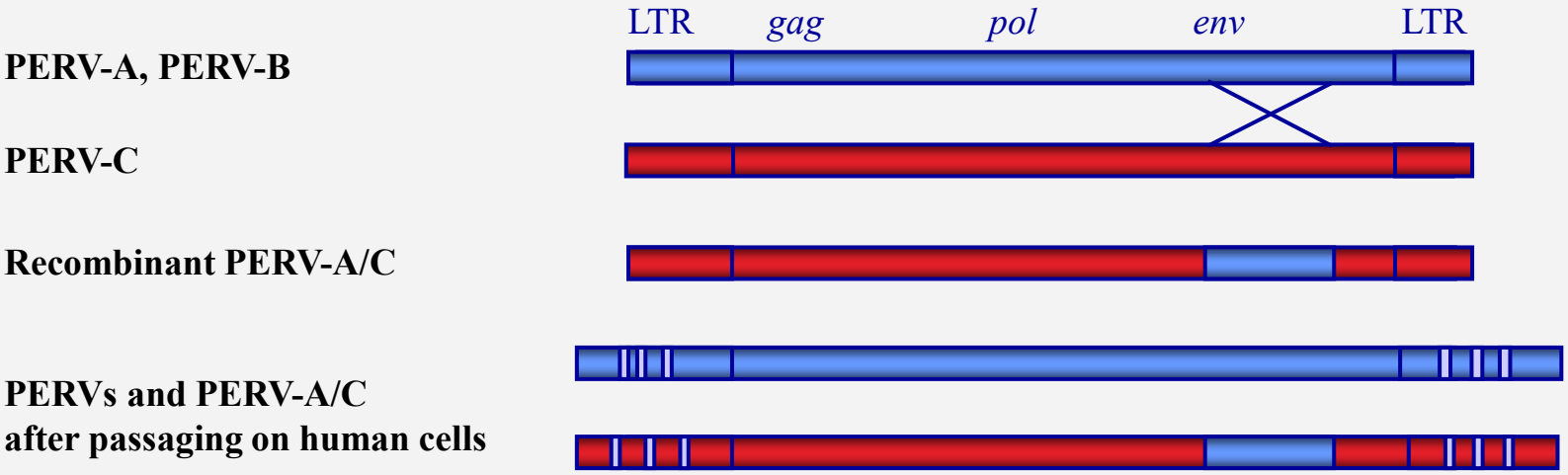
Porcine endogenous retroviruses (PERVs)

Life cycle



Denner J, Tönjes RR. Infection barriers to successful xenotransplantation focusing on porcine endogenous retroviruses. Clin Microbiol Rev. 2012;25(2):318-43.

PERVs: Recombination and adaptation



PERV-A/C - increased titres, not integrated in germ line
LTR - multimerisation of NF-Y binding sites in the LTR

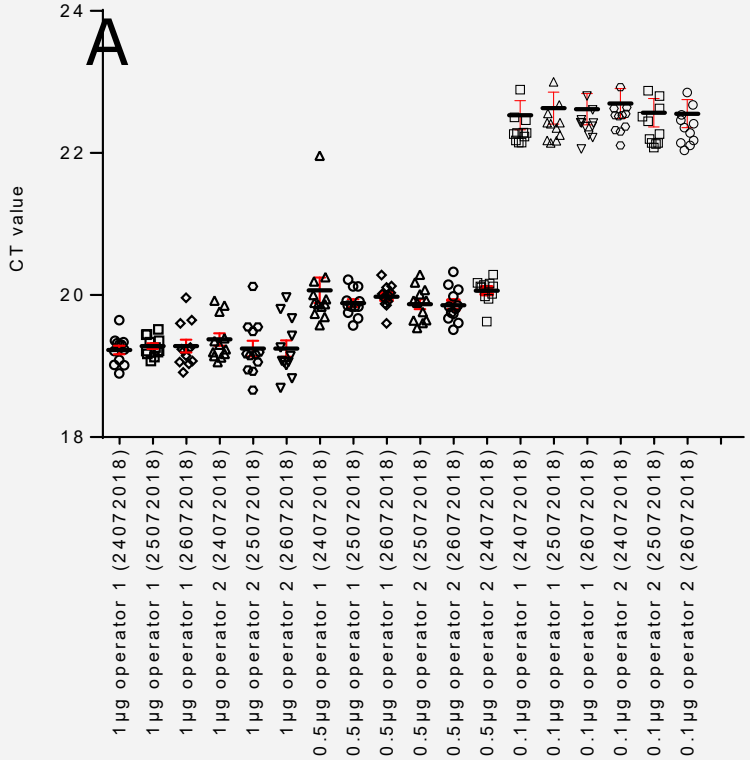
Denner J, Specke V, Thiesen U, Karlas A, Kurth R. Genetic alterations of the long terminal repeat of an ecotropic porcine endogenous retrovirus during passage in human cells. Virology. 2003;314(1):125-33.

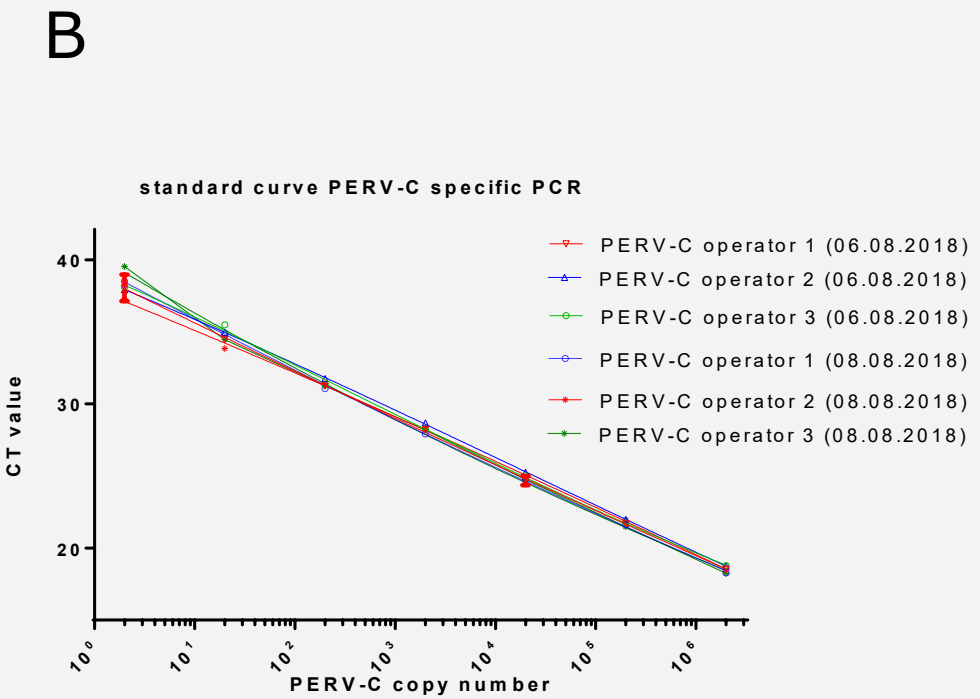
Direct detection of PERVs

Detection method	Viral target, goal	Required tools
PCR using pol primers	Detection of all integrated proviruses	Primers
PCR using primers specific for envA, envB, envC	Detection of the specific class of PERV	Primers
Real-time PCR, ddPCR	Quantification of proviruses	Primers, probes
RT-PCR, real-time RT-PCR	Detection and quantification of viral RNA	Primers, probes
Western blot of cell or virus lysates, immunohistochemistry, immunofluorescence, immunoperoxidase assay	Detection of virus protein expression	Specific anti-viral control sera, purified viral or recombinant proteins, virus-producing cells
RT assay	Detection of reverse transcriptase activity	Specific kits
Electron microscopy	Detection of viral particles	Embedding material
Infection assay	Detection of infectious virus	Target cells (human cells - human-tropic viruses, pig cells - ecotropic viruses)

Validation of detection methods

Real-time PCR

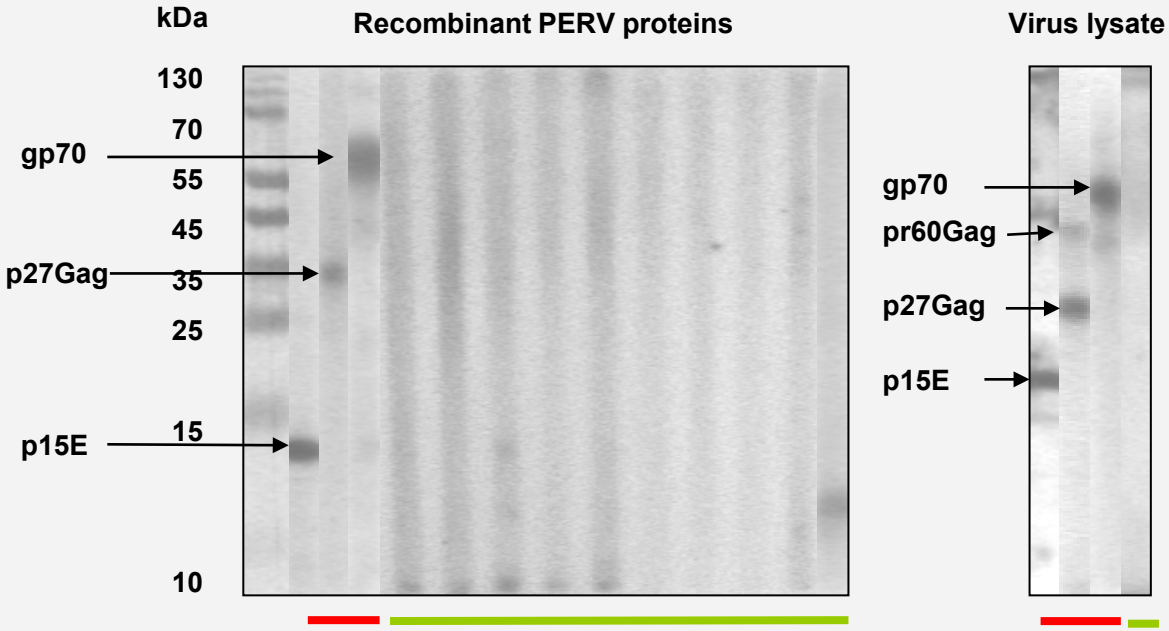




A: 0.1, 0.5, 1 µg PERV DNA in 100 µl human blood, two operators, three times each
B: titration of a PERV-C plasmid from 2 to $2x10^6$ to three operators, two different days

Indirect detection of PERVs

Detection method	Viral target, goal	Required tools
Western blot, ELISA	Detection of antiviral antibodies	Viral proteins (purified virus, recombinant viral proteins), positive control sera

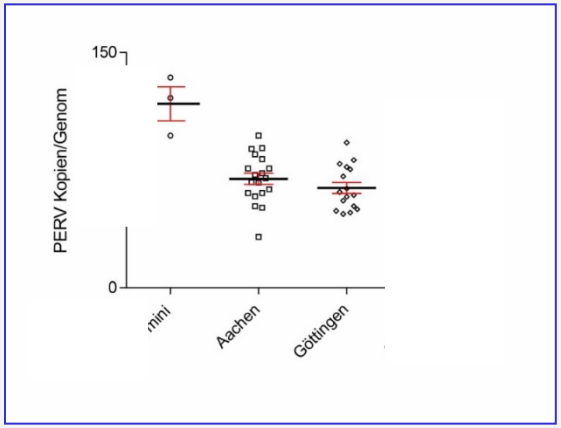


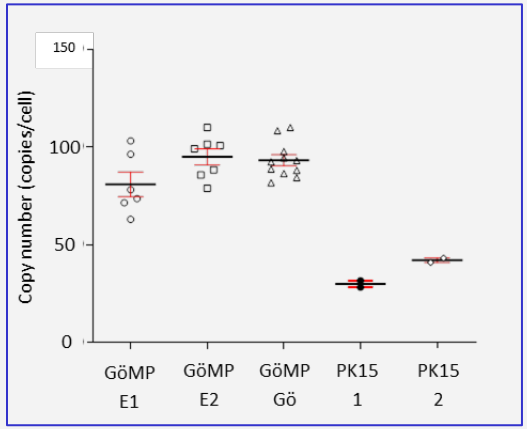
Morozov VA, Wynyard S, Matsumoto S, Abalovich A, Denner J, Elliott R. No PERV transmission during a clinical trial of pig islet cell transplantation. Virus Res. 2017;227:34-40.

Copy number of PERVs

Droplet digital PCR

QX200 Droplet Digital PCR System. QX200 Droplet Reader (left) and the QX200 Droplet Generator (right).
BioRad





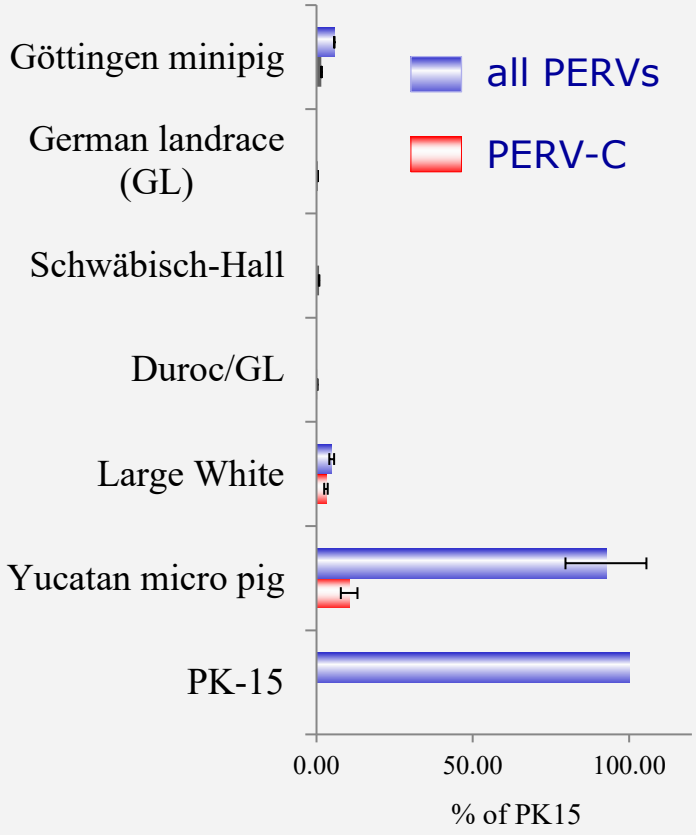
Fiebig U, Fischer K, Bähr A, Runge C, Schnieke A, Wolf E, Denner J. Porcine endogenous retroviruses: Quantification of the copy number in cell lines, pig breeds, and organs. Xenotransplantation. 2018;25(4):e12445

Krüger L, Stillfried M, Prinz C, Schröder V, Neubert LK, Denner J. Copy Number and Prevalence of Porcine Endogenous Retroviruses (PERVs) in German Wild Boars. Viruses. 2020 Apr 8;12(4):419.

Denner J. What does the PERV copy number tell us? Xenotransplantation. 2022 Mar;29(2):e12732.

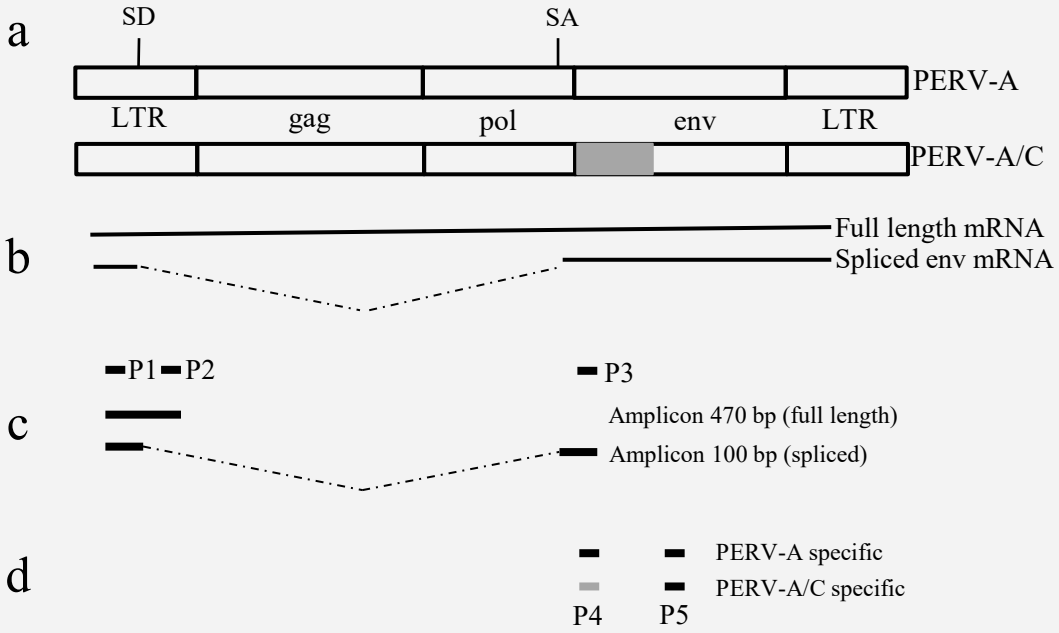
Expression of PERVs: mRNA and genomic RNA

RT-PCR



Dieckhoff B, Kessler B, Jobst D, Kues W, Petersen B, Pfeifer A, Kurth R, Niemann H, Wolf E, Denner J. Distribution and expression of porcine endogenous retroviruses in multi-transgenic pigs generated for xenotransplantation. Xenotransplantation. 2009;16(2):64-73.

Detection of spliced mRNA and PERV-A/C



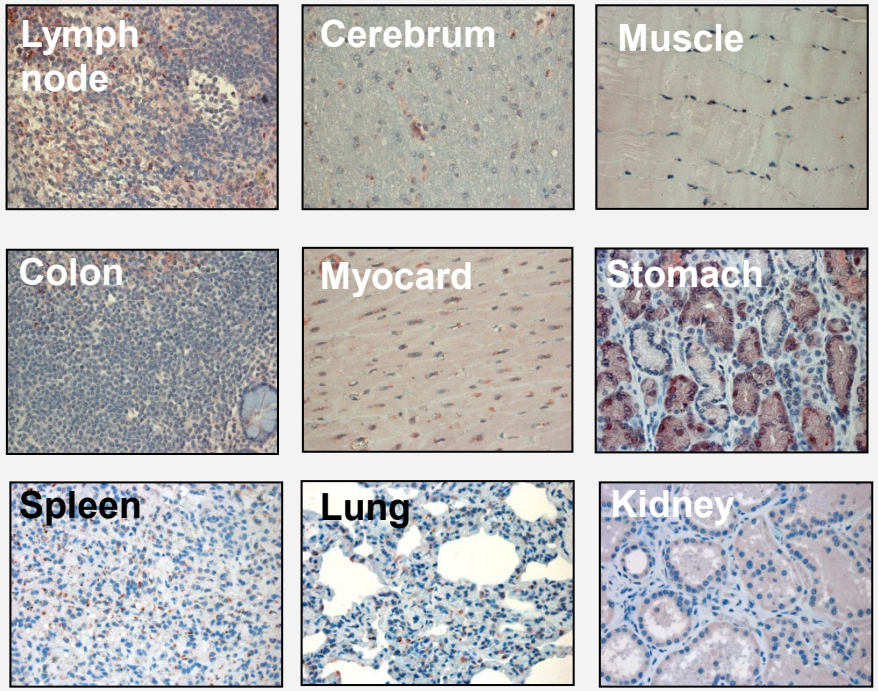
Denner J, Scobie L. Are there better assays to evaluate the risk of transmission of porcine endogenous retroviruses (PERVs) to human cells? Xenotransplantation. 2019;26(4):e12510

Expression of PERV proteins

Immunohistochemistry

Yucatan minipig

Antibodies against
PERV Gag



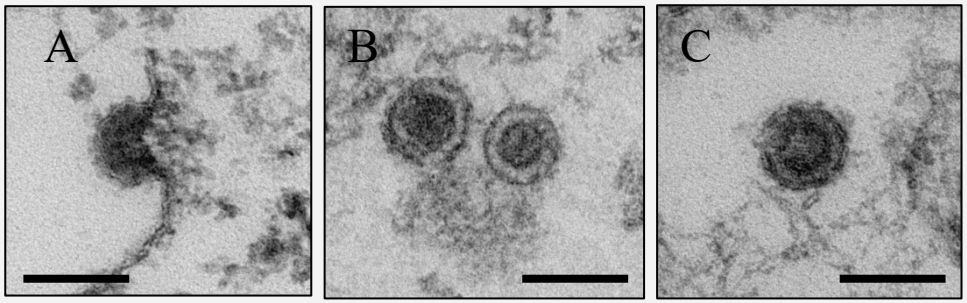
Bittmann I, Mihica D, Plesker R, Denner J. Expression of porcine endogenous retroviruses (PERV) in different organs of a pig. Virology. 2012 Nov 25;433(2):329-36.

Infection assays and PERV detection by electron microscopy

Detection of human-tropic PERV:

Using highly susceptible human 293 cells, PCR
Rare, mostly with minipigs, mostly PERV-A/C

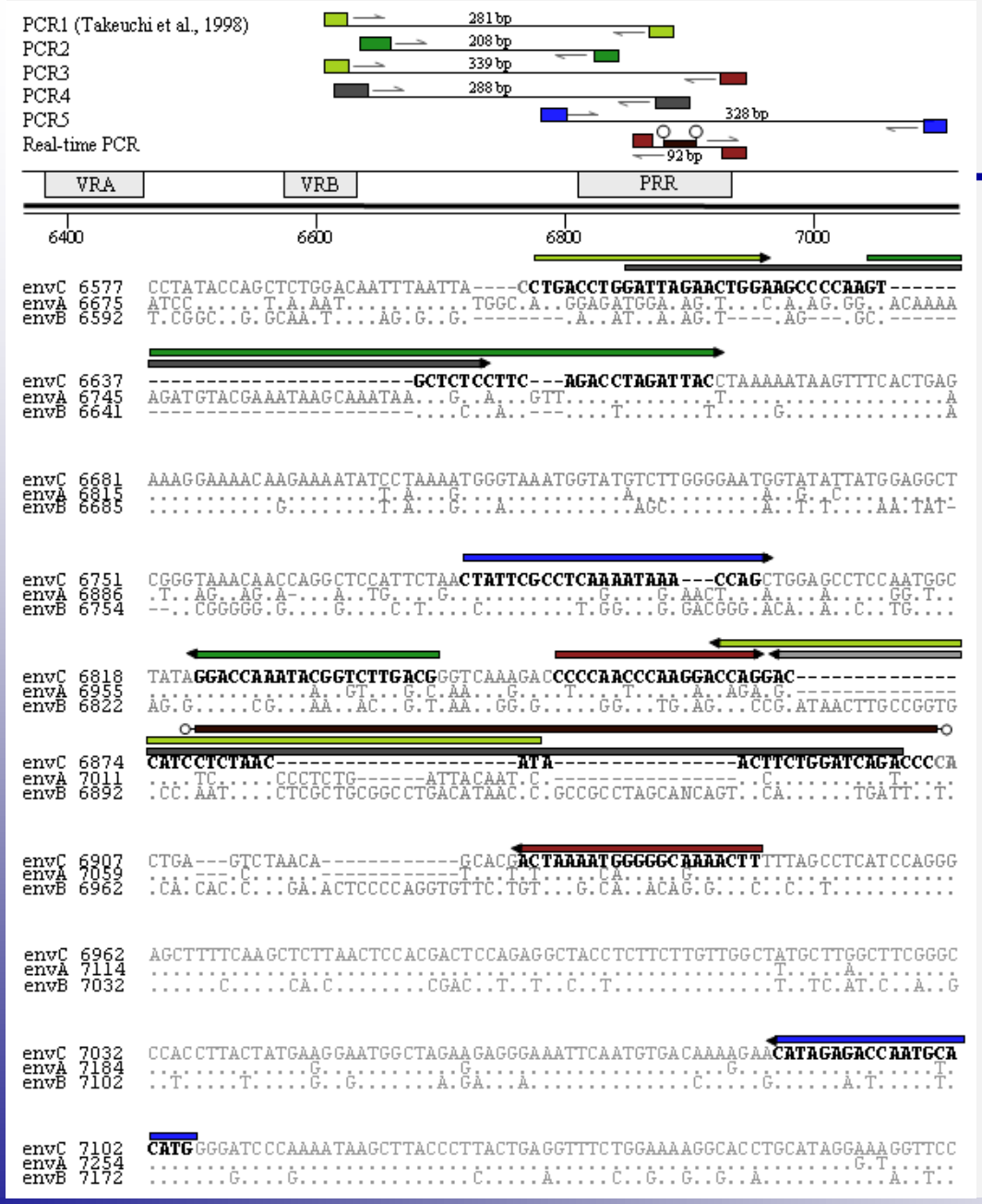
Halecker S, Krabben L, Kristiansen Y, Krüger L, Möller L, Becher D, Laue M, Kaufer B, Reimer C, Denner J. Rare isolation of human-tropic recombinant porcine endogenous retroviruses PERV-A/C from Göttingen minipigs. Virol J. 2022 Feb 21;19(1):30.
Denner J, Schuurman HJ. High Prevalence of Recombinant Porcine Endogenous Retroviruses (PERV-A/Cs) in Minipigs: A Review on Origin and Presence. Viruses. 2021 Sep 18;13(9):1869. d



A: Budding virus
B: Virus particles in the process of maturation
C: Mature virus

Denner J. Sensitive detection systems for infectious agents in xenotransplantation. Xenotransplantation. 2020;18:e12594.

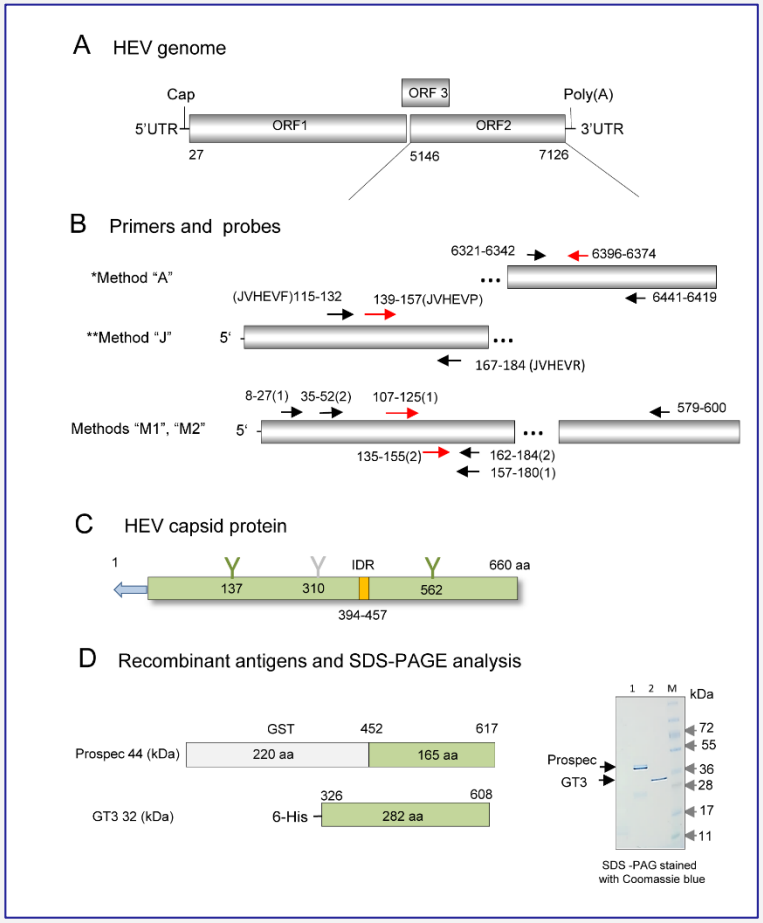
Detection of PERV-C



Use PERV-C free animals to avoid PERV-A/C recombination !

Kaulitz, D., Mihica, D., Dorna, J., Costa, M.R., Petersen, B., Niemann, H., Tönjes, R., Denner, J. Development of sensitive methods to detect porcine endogenous retrovirus-C (PERV-C) in the genome of pigs. J. Virol. Methods, 2011;175(1):60-65.

Detection of HEV



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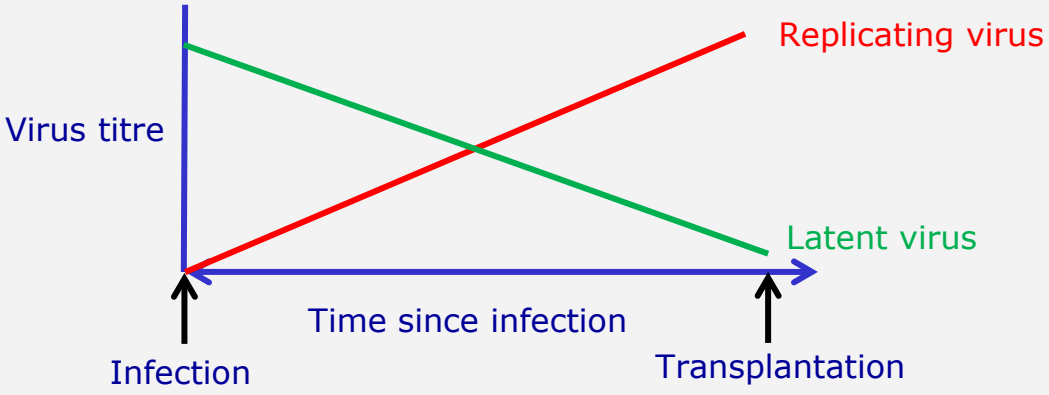
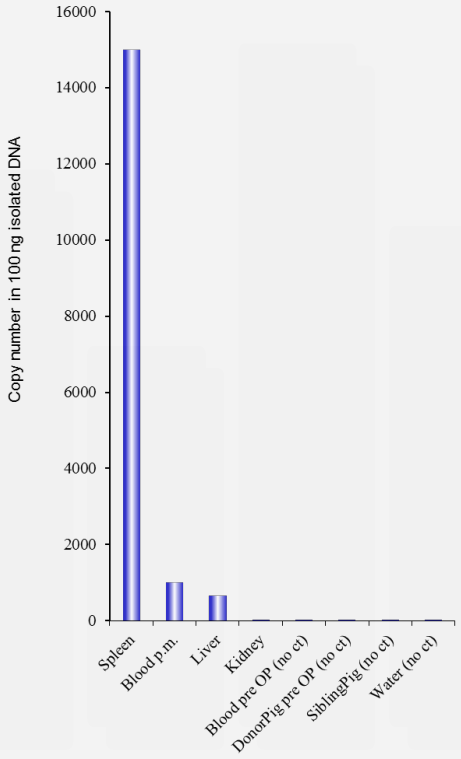
Differences in the detection of replicating and latent viruses



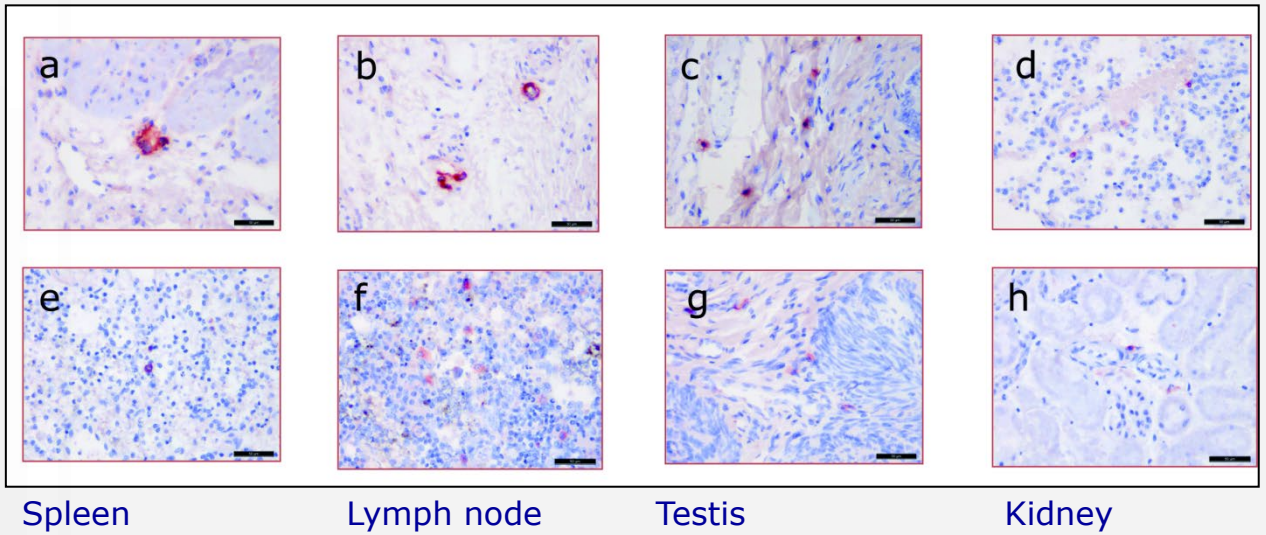
Herpes viruses are latent viruses
Example:
Porcine cytomegalovirus (PCMV) PCMV is a porcine roseolo virus (PRV), closely related to the human herpes viruses 6A, 6B and 7,
only distantly related to HCMV (HHV-5)!!
Call it PCMV/PRV or SuHV-2

PCMV/PRV detection by real-time PCR and immunohistochemistry

Orthotopic pig heart transplantation into baboons

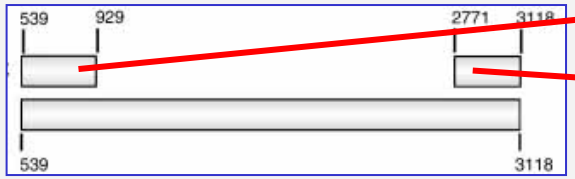


Antibodies against PCMV



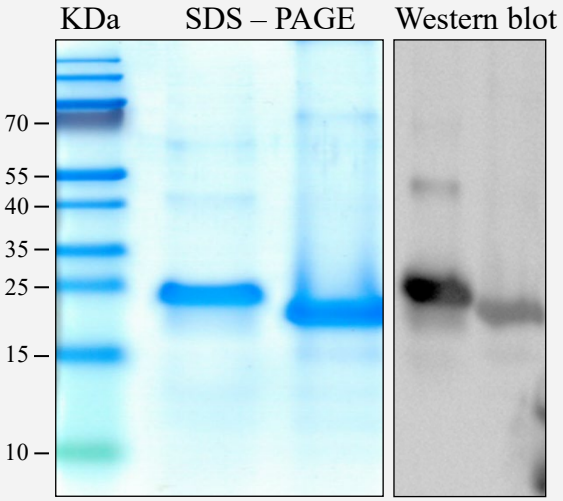
Fiebig U, Abicht JM, Mayr T, Längin M, Bähr A, Guethoff S, Falkenau A, Wolf E, Reichart B, Shibahara T, DennerJ Distribution of Porcine Cytomegalovirus in Infected Donor Pigs and in Baboon Recipients of Pig Heart. Transplantation. Viruses. 2018 Feb 6;10(2):66

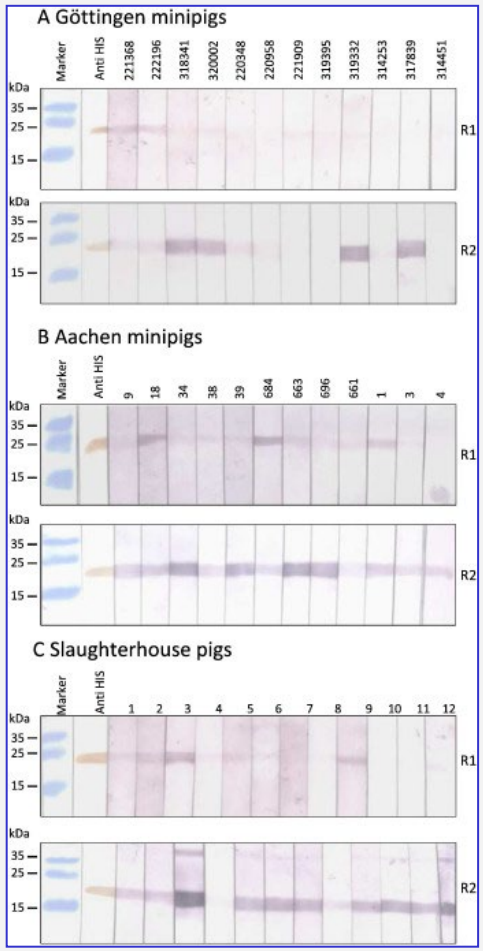
Detection of antibodies against PCMV/PRV: Adult pigs



R1

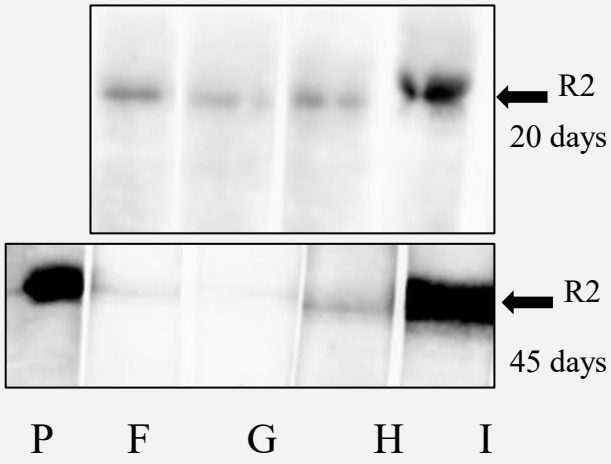
R2





Plotzki E, Keller M, Ivanusic D, Denner J. A new Western blot assay for the detection of porcine cytomegalovirus (PCMV). J Immunol Methods. 2016;437:37-42.

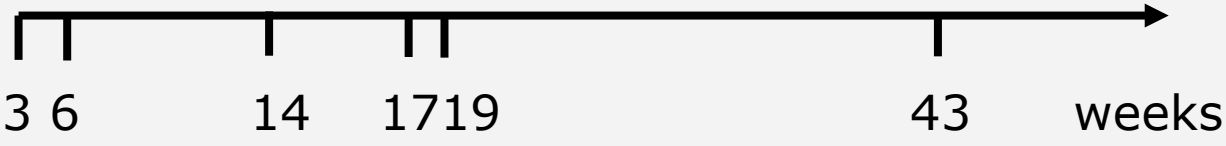
Detection of antibodies against PCMV/PRV: Young pigs



Halecker S, Hansen S, Krabben L, Ebner F, Kaufer, Denner J. How, where and when to screen for porcine cytomegalovirus (PCMV) in donor pigs for xenotransplantation. Scientific Reports, submitted

Detection of PCMV: General overview

PCR	+ +	+	+ -	-	infected
	- -	-	- -	-	uninfected
WB	+ -	+	+	+	infected
	+ -	-	-	-	uninfected/ mother infected
	- -	-	-	-	uninfected / mother uninfected



Halecker S, Hansen S, Krabben L, Ebner F, Kaufer, Denner J. How, where and when to screen for porcine cytomegalovirus (PCMV) in donor pigs for xenotransplantation. Scientific Reports, submitted

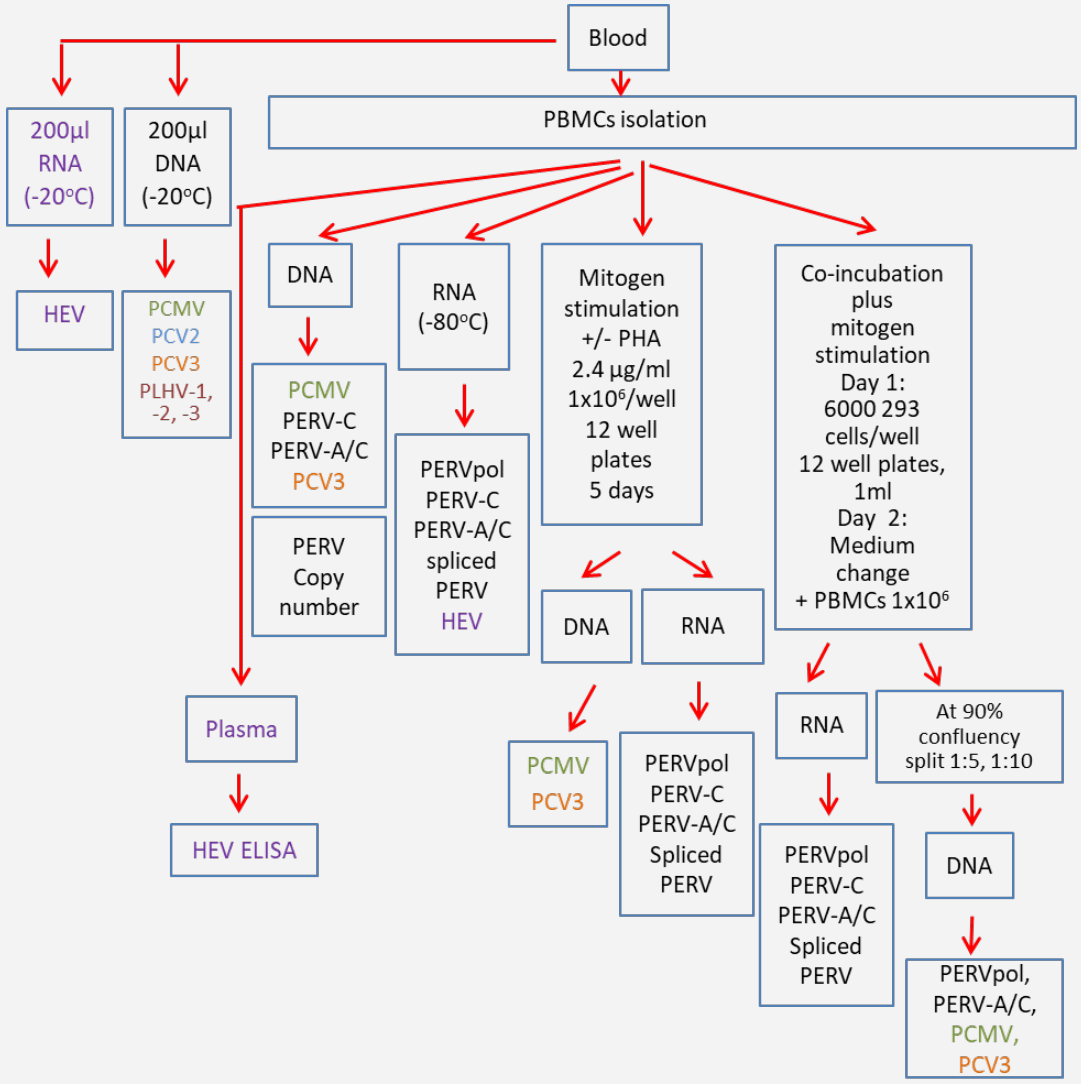
Can non-invasive detection methods be used: PCMV/PRV

Young Aachen minipigs

Samples/methods	PCR (positive/total)	Nested PCR (positive/total)	Uniplex real-time PCR (positive/total)	Duplex real-time PCR (positive/total)	Genome equivalents detected
Sera	1/10	1/10	6/10	0/10	10 – 150/ml
Anal swabs	0/10	3/10	7/10	0/10	5 – 100/mg
Oral swabs	10/10	10/10	10/10	9/10	$5x10^3$-$1x10^5$/mg
Ear biopsies	0/10	1/10	7/10	3/10	25 - $5x10^3$/mg
Total	11/40	15/40	30/40	12/40	

Morozov VA, Heinrichs G, Denner J. Effective Detection of Porcine Cytomegalovirus Using Non-Invasively Taken Samples from Piglets. Viruses. 2017 Jan 12;9(1).

Simultaneous detection of different viruses



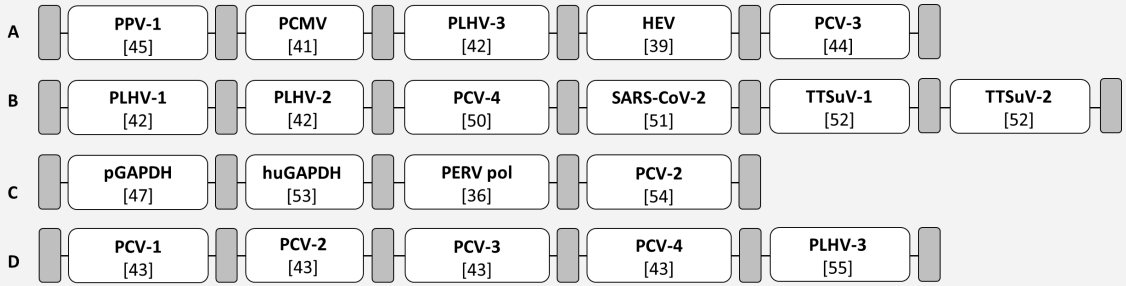
Krüger L, Kristiansen Y, Reuber E, Möller L, Laue M, Reimer C, Denner J. A Comprehensive Strategy for Screening for Xenotransplantation-Relevant Viruses in a Second Isolated Population of Göttingen Minipigs. Viruses. 2019;12(1):38.

Which additional viruses should be included

- Pseudorabies virus (PrV, or suid herpesvirus 1, SuHV-1)

  eliminated in Germany and other countries, but still in wild boars
- Porcine lymphotropic viruses -1, -2, -3 (PLHV-1,-2,-3) PCR, real-time PCR
- Porcine circoviruses 1,2,3,4 (PCV1,2,3,4) real-time PCR
- Porcine parvovirus 1 real-time PCR
- SARS-CoV-2 (does not infect pigs)
- Torque teno sus virus -1, -2 (TTSuV-1, -2) real-time PCR

Use of gene blocks as positive controls



Halecker S, Metzger J, Strube C, Krabben L, Kaufer B, Denner J. Virological and Parasitological Characterization of Mini-LEWE Minipigs Using Improved Screening Methods and an Overview of Data on Various Minipig Breeds. Microorganisms. 2021;9(12):2617

Conclusion

- Sensitive detection systems for numerous xenotransplantation-relevant porcine viruses (PCMV, PERV, HEV and others) are available.
- PCMV and HEV - known zoonotic (disease causing) viruses.
- PERV - still unclear whether it poses a risk for xenotransplantation, no additional experimental approaches are available to test this.
- Detection systems should be improved and extended.

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