

Sensitive detection systems for infectious agents in xenotransplantation*

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Abstract

Xenotransplantation of pig cells, tissues, or organs may be associated with transmission of porcine microorganisms, first of all of viruses, to the transplant recipient, potentially inducing a disease (zoonosis). I would like to define detection systems as the complex of sample generation, sample preparation, sample origin, time of sampling, and the necessary negative and positive controls along with the specific detection methods, either PCR-based, cell-based, or immunological methods. Some xenotransplantation-relevant viruses have already been defined; others are still unknown. The PCR-based methods include PCR and real-time PCR for DNA viruses, and RT-PCR and real-time RT-PCR for RNA viruses as well as for virus expression studies at the RNA level. Furthermore, droplet digital PCR (ddPCR) can be used for the determination of virus and provirus copies. To detect expression at the protein level, immunofluorescence, immunohistochemistry, and Western blot analyses can be used. To detect virus production and to detect infectious viruses, electron microscopy and infection assays can be used. Furthermore, immunological methods such as Western blot analysis or ELISA can be used to detect virus-specific antibodies. Detection of antiviral antibodies is a reliable and sensitive indirect detection method. For these immunological methods, purified viruses, recombinant viral proteins, or synthetic peptides are used as antigens and control sera and control antigens are needed. All these methods have been used in the past for the characterization of different pig breeds including genetically modified pigs generated for xenotransplantation and for the screening of recipients in preclinical and clinical xenotransplantations. Whereas in preclinical trials a few porcine viruses have been transmitted to the non-human primate recipients, in first clinical trials no such transmissions to humans were observed. Further improvement of the detection systems and their application in virus elimination programs will lead to clean donor animals and a safe xenotransplantation.

KEYWORDS

clinical trials, detection methods, elimination programs, PCR, preclinical trials, virus safety, Western blot analysis

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1 | INTRODUCTION

In the last years, xenotransplantation made a great progress as shown by long survival times of pig transplants in preclinical trials.¹⁻⁴ Most importantly, at the same time safety research also significantly improved. Numerous assays for the detection of porcine microorganisms, first of all of viruses, have been developed and applied. Systematic analysis of pig colonies bred for xenotransplantation and used in first preclinical and clinical trials as well as other pig breeds has been performed (Table 1,⁵⁻¹⁶). Successful elimination of viruses such as PCMV¹⁷ and PCV¹⁶ from breeding colonies has been achieved. On the other hand, successful preclinical trials have been performed without analyzing viruses in the donor pig and host animals, for example, reversal of diabetes for more than 100 days was achieved in cynomolgus macaques after intraportal transplantation of cultured islets from genetically unmodified pigs without Gal-specific antibody manipulation.¹⁸

Here, I will concentrate on the detection methods developed in our laboratory and their application for screening donor pigs and preclinical and clinical xenotransplantations. The pig virome contains numerous viruses.¹⁹ Since it is unclear, which porcine microorganisms can be zoonotic in the recipient, some viruses had been selected as xenotransplantation-relevant.²⁰ Detailed explanations of why HEV, PCMV, PERV, PCV, and some others were included were given in several reviews.²¹⁻²⁵ HEV (genotype 3, gt3) is a well-known zoonotic virus. It can be transmitted to humans by undercooked pork or contact with pigs,²³ and transmission from human to human by blood transfusion was reported.²⁶ PCMV at least is responsible for a shorter survival time of pig transplants in non-human primates,^{21,22,27,28,29} PERVs, although not yet transmitted in preclinical and clinical trials, are suspected (as all retroviruses) to induce immunodeficiency and tumors.²⁴ Such diseases had been reported for the nearest relatives of PERV, the feline leukemia virus, the murine leukemia virus, and the koala retrovirus. Safety analyses of porcine endogenous retroviruses (PERVs) will therefore be presented in greater detail due to their specific properties.

2 | DETECTION SYSTEMS FOR PORCINE ENDOGENOUS RETROVIRUSES (PERVS)

PERVs pose a special risk for xenotransplantation since they are integrated into the genome of all pigs and cannot be eliminated by the means other viruses can be eliminated such as selection of virus-negative animals, vaccination, treatment with antiviral drugs, early weaning, colostrum deprivation, cesarean delivery, and embryo transfer. PERVs can be detected as integrated DNA provirus in the genome of the pig or after transcription and translation of the genetic information at the level of mRNA and protein or as infectious enveloped RNA virus (Table 2). PERV-A and PERV-B are present in all pigs, whereas PERV-C is present in many, but not all pigs.²⁴ PERV-B is the phylogenetically oldest virus, PERV-C the youngest. PERV-A and PERV-B are polytropic viruses infecting cells of many

species including human cells, and PERV-C is an ecotropic virus infecting only pig cells. They are highly related with exception of their envelope protein, mainly the receptor-binding site, their long terminal repeats (LTR), and their receptor usage. The two receptors of PERV-A on human cells are known: the human porcine endogenous retrovirus-A receptor 1 (huPAR1, also called human riboflavin transporter 3, hRFT3, or solute carrier family 52A1, SLC52A1) and the human porcine endogenous retrovirus-A receptor 2 (huPAR2, also called human riboflavin transporter 1, hRFT1, or solute carrier family 52A2, SLC52A2). The receptors of PERV-B and PERV-C are still not known. Recombinants between PERV-A and PERV-C have been described, which acquired the receptor-binding domain of PERV-A, enabling the PERV-A/C recombinants to infect human cells. Since PERV-C is not present in all pigs, PERV-C-free animals should be selected as source for xenotransplantation, and this will prevent PERV-A/C recombination. In order to detect all PERVs, primers specific for the pol gene, which is highly conserved among PERV-A, PERV-B, and PERV-C, can be used. To identify the subtype, specific primers binding the sequences in the envelope protein of the corresponding virus type were used. When we developed methods to detect PERV-C in order to select PERV-C-free animals,³⁰ we detected new variants of PERV-C which had not been detected with primers usually used.³¹

To make sure that testing using methods shown in Table 2 is accurate, a validation of the methods is required. For example, in the case of real-time PCR assays, this means that different operators test at different time points and use different concentrations of the material. In all tests, reproducible results should be obtained (Figure 1).

Of great interest is the exact determination of the copy number of PERV proviruses in the genome of pigs. Different methods had been used in the past such as Southern blot analysis, PCR titration, real-time PCR, fluorescence in situ hybridization (FISH), and genome-wide sequencing, and different copy numbers were reported, in some cases over 100 copies (for review, see³²). The ddPCR seems the most reliable methods to measure copy numbers for the following reasons: Absolute quantification based on the principles of sample partitioning and Poisson statistics is provided, thus overcoming the normalization and calibrator issues; it has shown increased precision and sensitivity; and it is relatively insensitive to PCR inhibitors and directly provides the result of the analysis expressed as number of copies of target per microliter of reaction.³³ Analyzing the PERV copy number in different PK15 cells and pig breeds may be complicated by the fact that the number of the reference genes, used in these studies, for example, actin and GAPDH, is not two as expected for a diploid genome and therefore different copy numbers were determined using one or the other reference genes.³⁴ It was surprising that the copy number of an endogenous retrovirus, which is part of the germline and therefore should be identical in all cells of the body, was different in different organs and that the number increased with age.^{34,35} This is not the result of mistakes of the method used, but indicates that PERV is actively replicating in the living pig, and infecting and de novo integrating in certain somatic cells.³² However, there is no direct correlation between the copy number and the potential risk

TABLE 1 Comprehensive screening strategies applied to larger pig herds

Pig breed	Microorganisms including viruses screened for	Viruses detected in addition to PERV	References	Preclinical/clinical trials with these animals as donors	Output: Transmission of viruses including PERV	References
Auckland Island pigs	14 viruses ^a , 10 bacteria ^b , toxoplasma	None	Garkavenko et al ⁴ ; Wynyard et al ⁵	Preclinical trial: encapsulated islet cells into cynomolgus monkeys. Clinical trials ^c : islet cells in New Zealand and Argentina	No transmission	Garkavenko et al ⁶ ; Wynyard et al ⁷ ; Morozov et al ⁸
Large White Yorkshire x Landrace	28 viruses, 2 bacteria, 11 vaccinations	PRCV ^d , PRRSV, PPV, EMCV, EEEV, VEEV, WEEV	Gazda et al ⁹	Preclinical trial: encapsulated islet cells into diabetic cynomolgus monkeys	No transmission	Gazda et al ⁹
Landrace x Large White	31 viruses	PLHV-2, PLHV-3 in adults, none in piglets derived by cesarean section	Hartline et al ¹⁰	Not done		
CDCD ^e pigs	24 bacteria 3 fungi 6 parasites 21 viruses arthropods	PCV2 ^f	Noordergraaf et al ¹⁶	Not done		
Göttingen minipigs (Ellegaard)	88 different microorganisms including: 20 virus groups 20 bacteria groups 2 nematode, worm 3 protozoa 4 fungi	PCMV ^g , HEV, PLHV-1, PLHV-2, PCV2	Semaan et al ¹³ Morozov et al ¹¹ Morozov et al ¹² Heinze et al ¹⁴	Preclinical trial: macroencapsulated islet cells into cynomolgus monkeys	HEV? ^h (1/8)	Morozov et al ¹⁴

^aPCV1, porcine circovirus type 1; PCV2, porcine circovirus type 2; PLHV, porcine lymphotropic 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, Aujeszky's disease; PPV, porcine parvovirus; PRRSV, porcine reproductive and respiratory syndrome virus; EMCV, porcine encephalomyocarditis virus.

^bLeptospira tarassovi, Leptospira hardjo, Leptospira Pomona, Mycobacteria hyopneumoniae, Campylobacter, Isospora, Cryptosporidium, Yersinia, E coli K88, Salmonella.

^cSee Table 4.

^dPRCV, porcine respiratory coronavirus; PRRSV, porcine reproductive and respiratory syndrome virus; PPV, porcine parvovirus; EMCV, encephalomyocarditis virus; EEEV, eastern equine encephalomyelitis virus; VEEV, Venezuelan equine encephalomyelitis virus; WEEV, western encephalomyelitis virus.

^eCDCD, cesarean-derived, colostrum-deprived piglets.

^fTwo outbreaks happened, but PCV was eliminated each time.

^gFor details, see Table 3.

^hMaybe unspecific reaction.

TABLE 2 Detection methods: PERV

Detection method	Viral target, goal	Required tools	Equipment
Direct detection methods			
PCR using pol primers	Detection of all integrated proviruses	Primers	PCR machinery
PCR using primers specific for envA, envB, and envC	Detection of the specific class of PERV	Primers	PCR machinery
Real-time PCR and ddPCR	Quantification of proviruses	Primers and probes	PCR and ddPCR machinery
RT-PCR and real-time RT-PCR	Detection and quantification of viral RNA	Primers and probes	PCR machinery
Western blot of cell or virus lysates, immunohistochemistry, immunofluorescence, and immunoperoxidase assay	Detection of virus protein expression	Specific antiviral control sera	Electrophoresis and blotting apparatus, and specific microscopes
RT assay	Detection of reverse transcriptase activity	Specific kits, PERT assay ^a	ELISA washer and reader or PCR machinery
Electron microscopy	Detection of viral particles	Embedding material	Electron microscope
Infection assay	Detection of infectious virus	Target cells (human cells—human-tropic viruses; pig cells—ecotropic viruses)	Cell culture
Indirect detection methods			
Western blot, ELISA	Detection of antiviral antibodies	Viral proteins (purified virus, recombinant viral proteins) and positive control sera	Electrophoresis and blotting apparatus or ELISA washer and reader

^aProduct-enhanced reverse transcriptase assay

posed by these viruses, because many of the integrated proviruses are defective. It is of great value to identify the replication-competent sequences as described.³⁶

The expression of PERV can be characterized by measuring the mRNA and the protein expression. The amount of mRNA was found to differ in different pigs and different organs of a single pig.^{32,37} As just mentioned, not all of the proviruses are replication-competent. A prerequisite for replication competence is an intact provirus without deletions and mutations, a high expression of the full-length and spliced mRNA, protein production, and budding of the virus. PERV has two mRNA, the full-length mRNA, which is identical with the genomic RNA and which is used for the translation of the gag and pol genes, and the spliced mRNA, which allows translation of the env gene. The full-length mRNA is commonly expressed in normal tissues, whereas the detection of spliced mRNA

is an indicator of PERV expression at a level which allows virus production.^{20,38}

Viral proteins can be detected by immunofluorescence, immunohistochemistry, and immunoperoxidase assays. Using PERV-specific antibodies, the production of PERV has been shown in PERV-infected 293 cells, pig melanoma cells, or mink cells by immunofluorescence or immunoperoxidase assay.³⁹⁻⁴² Immunohistochemistry has been used to demonstrate expression of PERV proteins in different organs of a pig with high expression of PERV at the RNA level.⁴³ The same method has been used to demonstrate PERV protein-positive cells in different organs of the recipient baboons after orthotopic transplantation of pig hearts from PCMV-positive genetically modified pigs.⁴⁴

Another assay to detect virus particles, especially in the supernatant of PERV-infected cell cultures or mitogen-stimulated pig PBMCs, is the reverse transcriptase (RT) assay. This assay measures

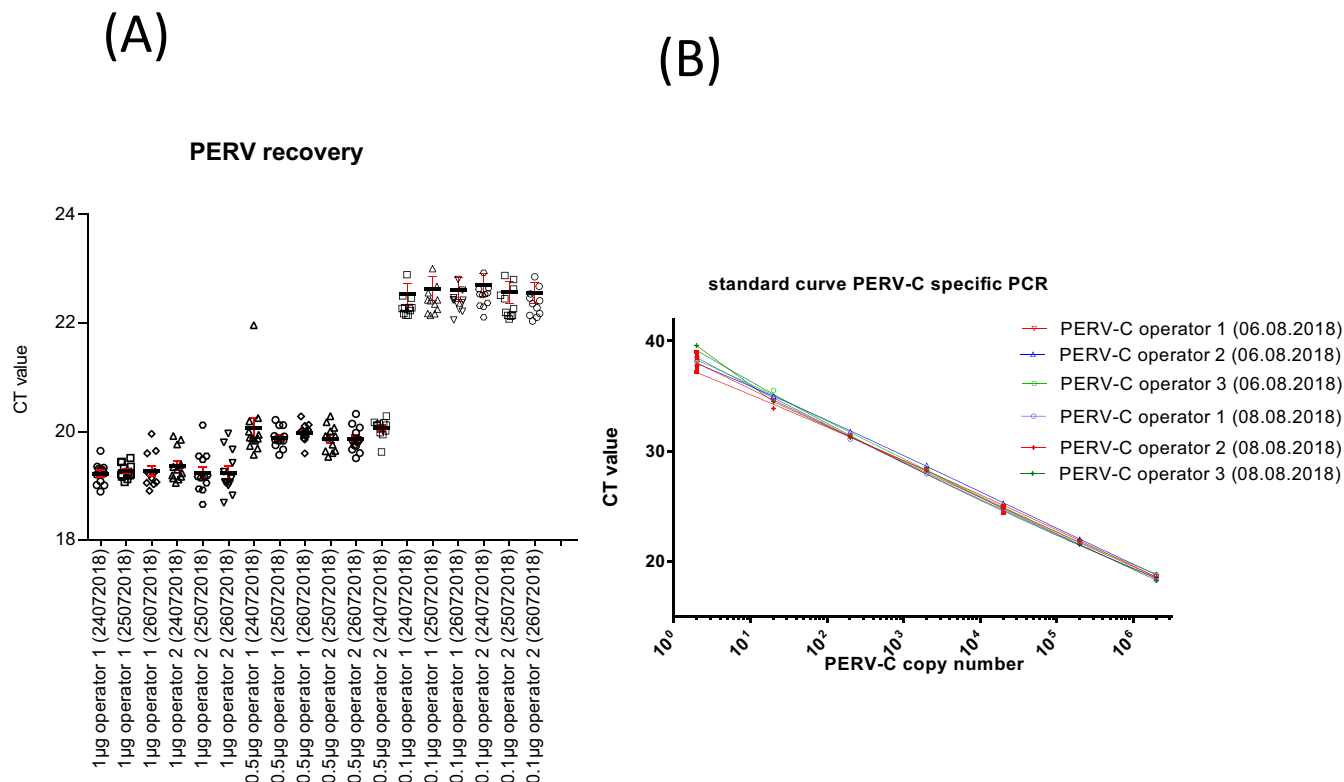
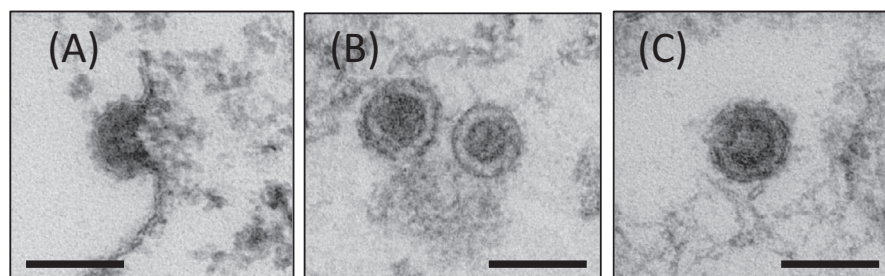


FIGURE 1 Validation of real-time PCR assays. A, Recovery of spiked PERV DNA from 100 µL human blood. Experiments were carried out from two operators on three different days ten times for each concentration of porcine DNA (0.1, 0.5, and 1 µg). B, Titration of a PERV-C plasmid from 2 to 2×10^6 copies per reaction using the generic PERVpol primer set. The experiments for in-house validation of this assay were carried out on three different days by three different members of the laboratory

FIGURE 2 Electron microscopy of PERV particles released from PBMCs of a Göttingen minipig²⁰ and produced by human 293 cells. A, budding virus; B, virus particles in the process of maturation; and C, mature virus. The bar corresponds to 200 nm (courtesy of L. Möller, M. Lau, Robert Koch Institute)



the RT activity in the virus particles allowing quantification of virus release.^{37,45} Mitogen treatment has been shown to enhance viral mRNA expression and virus release and is therefore a good tool to increase the sensitivity of these detection methods.^{20,37,46} A special form of the RT assay is the product-enhanced reverse transcriptase (PERT) assay,⁴⁷ which was also used to detect PERV.⁴⁸

Production of PERV particles was shown by electron microscopy (Figure 2),^{20,41,42,46} and using PERV-specific antibodies, the specificity has been demonstrated by immune gold electron microscopy.^{39,40} Infectious virus particles can be detected by infection assays using human cells for human-tropic PERVs and pig cells for pig-tropic (ecotropic) viruses. Since only the release of human-tropic viruses is relevant for xenotransplantation, human 293 cells were used as target cells. These cells lack intracellular restriction factors such as APOBEC and are therefore highly susceptible for PERV infection.

However, this co-incubation infection assay using mitogen-stimulated pig PBMCs with susceptible 293 cells is very insensitive and time-consuming. In a recent study, 50 pigs from different breeds were analyzed for release of human-tropic PERVs and only one animal, a Göttingen minipig with high expression of PERVpol and PERV-C in mitogen-stimulated PBMCs, was able to release particles and infect human cells.²⁰ The particles were found to be PERV-A/C recombinants and were characterized by electron microscopy (Figure 2). Release of human-tropic PERV-A and PERV-B from pig PBMCs was not yet detected by this assay. To improve the risk evaluation, it would be helpful to substitute the co-incubation assay with 293 cells with more sensitive molecular assays.⁴⁹

To detect antibodies against PERV, Western blot assays were developed, which were based either on recombinant proteins corresponding to the viral p27Gag, the surface envelope protein gp70,

TABLE 3 Testing of pig herds for xenotransplantation-relevant porcine viruses

Pig breed	Viruses in addition to PERV-A and PERV-B screened for by PCR methods (animals positive/tested)	Antibodies detected against (animals positive/tested)	Reference
Göttingen minipigs (Ellegaard)	PERV-C (28/28), HEV (9/40), PCMV (10/22), PLHV-1 (1/10), PLHV-3 (0/10), PCV 2 (3/21), PCV3 (0/10)	PLHV (1/10) HEV (1/22) PCMV (8/67)	Semaan et al ¹⁴ Morozov et al ¹² Morozov et al ¹³ Heinze et al ⁶⁷ Plotzki et al ⁶²
Göttingen minipigs (University of Göttingen)	PERV-C (11/11), PERV-A/C in PBMCs (4/11), PCV2 (2/11)		Krüger et al ²⁰
Aachen minipigs	PERV-C (10/10), PERV-A/C in spleen (2/10), PCMV (5/18), PLHV-2 (5/18), PLHV-3 (2/18), HEV (12/18), PCV2 (11/21)	PCMV (17/77)	Plotzki et al ⁶² Heinze et al ⁶⁷ Plotzki et al ⁶⁴
Genetically modified pigs used for heart transplantation	PERV-C (17/17), PCMV (5/17), HEV (4/17), PLHV-1, PLHV-2 (11/17), PCV3 (4/17)	HEV (4/17)	Abicht et al ⁶⁰ Morozov et al ⁶¹ Fiebig et al ³⁴ Denner et al ²⁹
Slaughterhouse pigs	PCV2 (0/22)	PCMV (39/85)	Plotzki et al ⁶⁶

TABLE 4 Transmission and absence of transmission of porcine viruses in preclinical and clinical trials

Trial	Viruses transmitted to the recipient	Viruses not transmitted despite the presence in the donor pigs	Reference
Preclinical			
Macroencapsulated islet cells from Göttingen minipigs into cynomolgus monkeys	HEV? ^a 1/8	PERV 0/8	Morozov et al ¹⁵
Orthotopic heart from CD46, GTKO, and hTM pigs into baboons	PCMV 4/17 PCV3 4/17	HEV 4/17 PERV 0/17 PLHV1 11/17	Abicht et al ⁶⁰ Morozov et al ⁶¹ Fiebig et al ³⁴ Denner et al ²⁹
Islet cells from LEA29Y pigs into marmosets	None 0/14	PCMV 4/14	Plotzki et al ⁷¹
Clinical			
Islet cells from Auckland island pigs into humans (New Zealand)	None 0/14	PERV 0/14	Wynyard et al ⁸
Islet cells from Auckland island pigs into humans (Argentina)	None 0/8	PERV 0/8	Morozov et al ⁹
Acute liver failure patients treated with a bioreactor based on porcine liver cells	None 0/7	n.t. ^b	Irgang et al ⁵⁴

^aUnclear infection.^bNot tested.

and the transmembrane envelope protein p15E or on lysates from highly purified virus particles.⁵⁰⁻⁵⁴ In addition, peptides derived from domains of the transmembrane envelope protein p15E and corresponding to peptides of the transmembrane envelope protein gp41 of HIV-1, known to be highly diagnostic for HIV-1, have been used.^{50,52} To validate the Western blot assays, goat anti-sera against the recombinant viral proteins and viral lysates were generated and applied.⁵⁰⁻⁵⁴

3 | DETECTION SYSTEMS FOR HEPATITIS E VIRUS (HEV)

HEV is an RNA virus and therefore not easy to detect in donor pigs, partially due to the fact that the genomic RNA is fragile. Therefore,

it may be useful to apply indirect detection methods, for example, to detect HEV-specific antibodies. HEV can be detected by RT-PCR, nested RT-PCR, and real-time RT-PCR.^{12,55,56} The amplicons can be used for sequencing in order to determine the genotype of HEV; in a few Göttingen minipigs, HEV gt3, which is common in pigs, was found. For the detection of antibodies against HEV, some commercial detection kits are on the market, which, however, are not always reliable. We used two recombinant viral proteins in ELISA or Western blot analysis.¹² The recombinant proteins correlated to sequences of the HEV capsid protein containing partially or as a whole the immunodominant epitope of this protein.¹² The generation of additional recombinant proteins corresponding to different viral proteins and their use in ELISA and Western blot analyses may be useful.

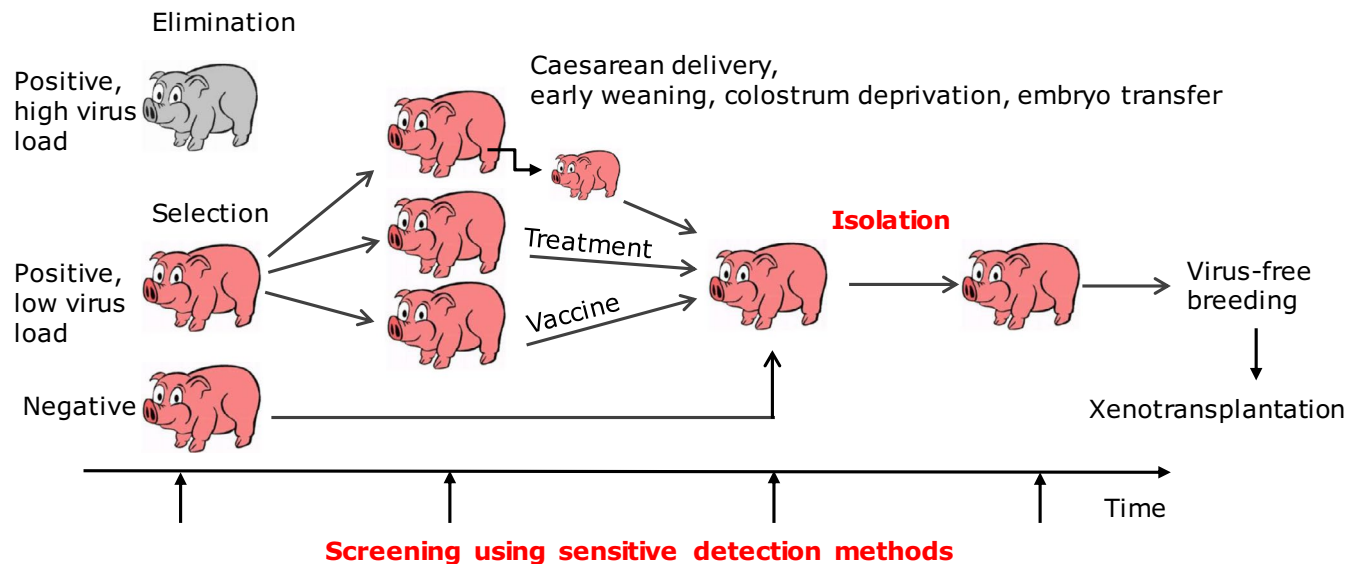


FIGURE 3 Schematic presentation of elimination programs in order to obtain virus-free donor pigs for xenotransplantation

4 | DETECTION SYSTEMS FOR HERPESVIRUSES

Herpesviruses, especially the porcine cytomegalovirus (PCMV), are potentially zoonotic viruses. PCMV is actually a porcine roseolovirus (PRV) closely related to the human herpesviruses 6A, 6b, and 7 (HHV-6A, HHV-6B, and HHV-7) and will be therefore named as PCMV/PRV (for review, see⁵⁶). PCMV/PRV has been shown to decrease significantly the survival time of kidney transplants in baboons and cynomolgus monkeys^{21,27,28} as well as the survival time of pig hearts in baboons,²² (Denner et al, submitted). There are excellent PCR and nested PCR methods available to detect PCMV.^{13,55,58,59} In addition, PCR methods resulting in longer amplicons which allow better sequencing and classification of the detected CMV/PRV are also available.⁵⁸ The case that PCMV/PRV was not detected in the blood of a donor pig by PCR, but nevertheless the recipient baboon got infected,^{60,61} indicates that blood is not a good source material to screen the donor pigs. Better results were obtained with different organs but, these are not available before transplantation. Isolation of PBMCs from the blood of donor pigs and cultivation of them in the presence of mitogens significantly increased the sensitivity of PCMV/PRV detection.⁴⁴ Alternatively, organs of siblings should be tested.⁴⁴ Good results were also obtained, when non-invasive samples from pigs (oral swabs, anal swabs, and ear biopsies) were tested.⁵⁹

Immunological methods measuring specific antibodies against PCMV/PRV were also developed. For this, sequences corresponding to the N-terminal and C-terminal part of the glycoprotein gB of PCMV/PRV were cloned and expressed, and the recombinant proteins were purified and used as antigens in a Western blot assays.⁶² A related sequence of the C-terminal part has been used by others in an ELISA.⁶³ Antibodies against these antigens were found in Göttingen and Aachen Minipigs and slaughterhouse pigs.⁶⁴

Other herpesviruses tested were the porcine lymphotropic herpesviruses 1, 2, and 3 (PLHV-1, PLHV-2, and PLHV-3). PCR methods and Western blot analyses using home-made recombinant proteins had been established.^{13,65} In analogy to the antibody testing against PCMV, the recombinant glycoprotein gB1 of PLHV was produced and used as antigen and a goat serum against this protein was used for validation of the methods.⁶⁵

5 | DETECTION SYSTEMS FOR PORCINE CIRCOVIRUSES AND OTHER SINGLE-STRANDED DNA VIRUSES

Single-stranded (ss) DNA viruses such as viruses of the *Anelloviridae*, *Parvoviridae*, and *Circoviridae* families infecting humans and pigs are widely prevalent. Some of these viruses can infect human cells, but there is no evidence the pig ssDNA viruses infect humans and are zoonotic. Since some of them such as the Torque teno sus virus (TTSuV), the porcine parvovirus 1 (PPV1), the porcine circovirus 2 (PCV2), and PCV3 are directly or in combination with other factors, for example, other viruses pathogenic for pigs, they should be eliminated from the donor pigs (for review, see^{25,66}). For all these viruses, efficient PCR and real-time PCR methods with specific primers and probes were described.⁶⁶⁻⁶⁸ For PCV2, a vaccine exists and even slaughterhouse pigs get vaccinated. However, this vaccine does not always prevent infection, but in most cases, it prevents disease development. PCV2 was not found in Göttingen minipigs which will be used as donor pigs for clinical pig islet cell transplantation into humans in Germany, but PCV2 was found in some Aachen minipigs.⁶⁷ Interestingly, transmission of PCV3 was observed in a preclinical trial of orthotopic pig heart transplantation into baboon without evidence of a pathogenic effect.⁶⁹

6 | SCREENING OF DONOR PIGS

Using the above-described detection systems, pigs used as organ donors in preclinical trials, pigs to be used in future clinical trials, and slaughterhouse pigs have been tested (Table 3). Göttingen minipigs produced by the company Ellegaard in Denmark are well characterized.^{12-14,67} Since these pigs will be used as donors for islet cell transplantation in Germany, they were analyzed using detection methods for PERV, PCMV, PLHV, PCV, and others. Altogether, 88 microorganisms were included in testing and PCMV, HEV, PLHV-1, and PLHV-2 were detected in only a very few animals.^{12-14,67} However, when islet cells from these animals were transplanted into cynomolgus monkeys, no porcine viruses were transmitted¹⁵ (Table 1). Interestingly, in Göttingen minipigs produced isolated at the Göttingen University, neither PCMV, nor PLHV-1, nor PCV2 were detected.²⁰

7 | PRECLINICAL AND CLINICAL TRIALS

Using the above-described detection systems, transmission of porcine viruses in several preclinical and clinical trials was analyzed (Table 4). Most importantly, PERVs were not transmitted in all of these trials, and the reasons have been discussed elsewhere.⁷⁰ Testing of the recipients for PERV was performed with both PCR-based and immunological methods; using Western blot analyses or ELISA, no antibodies against PERVs were detected. Interestingly, also no antibodies were found in pigs since these are tolerant against PERV.⁷¹ Virus proteins are obviously expressed early during pig ontogenesis, and therefore, they are treated as "self." PCMV/PRV and PCV3 were transmitted in orthotopic heart transplantation in baboons, obviously only the PCMV had a negative effect, significantly reducing the survival time of the transplant (Denner et al, submitted). Porcine viruses were not transmitted after transplantation of islet cells from transgenic pigs into marmosets.⁷²

8 | VIRUS ELIMINATION PROGRAMS

As mentioned above, with exception of PERVs all potentially zoonotic viruses can be eliminated by selection of virus-negative animals, vaccination, treatment with antiviral drugs, early weaning, colostrum deprivation, cesarean delivery, and embryo transfer. For elimination programs based on these strategies, the above-described detection systems are necessary. All elimination steps should be accompanied by a sensitive screening of the animals before and after treatment (Figure 3). For example, when PCMV was eliminated by early weaning associated with the introduction of these pigs into a new breeding facility, more than 1300 tests were performed, screening the partially PCMV-positive mother sows, and the piglets of the first and second generation, showing that the animals in this facility are free of PCMV.¹⁷

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