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BRIEF COMMUNICATION

Limited availability of methods for the detection of xenotransplantation-relevant viruses in veterinary laboratories

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Abstract

Background: The German Xenotransplantation Consortium is in the process to prepare a clinical trial application (CTA) on xenotransplantation of genetically modified pig hearts. In the CTA documents to the central and national regulatory authorities, that is, the European Medicines Agency (EMA) and the Paul Ehrlich Institute (PEI), respectively, it is required to list the potential zoonotic or xenozoonotic porcine microorganisms including porcine viruses as well as to describe methods of detection in order to prevent their transmission. The donor animals should be tested using highly sensitive detection systems. I would like to define a detection system as the complex including the actual detection methods, either PCR-based, cell-based, or immunological methods and their sensitivity, as well as sample generation, sample preparation, sample origin, time of sampling, and the necessary negative and positive controls. Lessons learned from the identification of porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV) in the xenotransplanted heart in the recipient in the Baltimore study underline how important such systems are. The question is whether veterinary laboratories can supply such assays.

Methods: A total of 35 veterinary laboratories in Germany were surveyed for their ability to test for selected xenotransplantation-relevant viruses, including PCMV/PRV, hepatitis E virus, and porcine endogenous retrovirus-C (PERV-C). As comparison, data from Swiss laboratories and a laboratory in the USA were analyzed. Furthermore, we assessed which viruses were screened for in clinical and preclinical trials performed until now and during screening of pig populations.

Results: Of the nine laboratories that provided viral diagnostics, none of these included all potential viruses of concern, indeed, the most important assays confirmed in recent human trials, antibody detection of PCMV/PRV and screening for PERV-C were not available at all. The situation was similar in Swiss and US laboratories. Different viruses have been tested for in first clinical and preclinical trials performed in various countries.

Conclusion: Based on these results it is necessary to establish special virological laboratories able to test for all xenotransplantation-relevant viruses using validated assays, optimally in the xenotransplantation centers.

KEYWORDS
immunological methods, PCR, porcine cytomegalovirus, porcine roseolovirus, virus safety

1 | INTRODUCTION

The German Transregional Collaborative Centre 127, Biology of xenogeneic cell, tissue and organ transplantation—from the bench to the bedside [German Transregional Collaborative Centre, http://www.klinikum.uni-muenchen.de/SFB-TRR-127/de/index.html] is preparing a clinical trial application (CTA) on pig heart transplantation. This interdisciplinary consortium of basic and translational scientists, such as immunologists, genetic engineers, cell physiologists, virologists, and transplant surgeons, aims to develop pig-to-primate xenotransplantation from the experimental studies to clinical application. It is generating genetically modified pigs, and investigates the immunology and microbiological safety of xenotransplantation. Breakthroughs in macroencapsulated porcine islet transplantation into diabetic macaques and life-supporting orthotopic porcine heart transplantation into baboons have been published.[1–3] When writing the virological part of the CTA documents to the central and national regulatory authorities, that is, the European Medicines Agency (EMA) and the Paul Ehrlich Institute (PEI), we assessed whether veterinary laboratories in Germany have the tests to screen for the thirteen pig viruses selected by Fishman as not permitted in pigs with designated pathogen-free status[4] as well as for porcine endogenous retrovirus-C (PERV-C). PERV-A and PERV-B are integrated into the genome of all pigs and are able to infect human cells, whereas PERV-C is present in many, but not all pigs and it infects only pig cells. It is still unknown whether PERVs pose a risk for xenotransplantation. As a matter of fact, PERV was not transmitted until now in all preclinical and clinical trials.[5] Since recombinant viruses between PERV-A and PERV-C also can infect human cells and are characterized by a higher virus replication, there is agreement to select PERV-C negative animals in order to prevent recombination.

Different other porcine viruses may be transmitted during xenotransplantation and interfere with the outcome of transplant survival. For example, when pig hearts or kidneys were transplanted in preclinical trials into non-human primates and the porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV) was transmitted, the survival time of the xenotransplants was significantly reduced.[6–10] The virus load was high in the blood of the transplanted baboons and cells expressing viral protein were detected in all analyzed organs of the baboon.[11]

In order to prevent virus transmissions, the donor pigs should be carefully tested for potential zoonotic and xenozoonotic viruses. A virus is zoonotic if it causes a disease in humans, hepatitis E virus genotype 3 (HEV3) is a well-known example. A virus should be defined as xenozoonotic if it does not infect and harm humans under natural conditions, but causes a disease in the context of a xenotransplantation such as PCMV/PRV.[12] The experiences of the past show that not only the detection methods (PCR-based or immunological methods) and the sensitivity of the methods, but also other factors such as the time of testing, and the nature of the samples to be tested are essential for the optimal test outcome.[13,14] PCMV/PRV can be easily detected by PCR in the running nose using nasal swabs,[15] or using oral or anal swabs[16] in newly infected animals, mainly in piglets infected by the mother. In older infected animals, it is difficult to detect the virus by PCR because PCMV/PRV establishes latency like many other herpesviruses. Although detection of antibody cannot differentiate between active and latent infection, a positive antibody test will indicate that an individual may have been infected in the past or currently. However, virus-specific antibodies can also be found in young piglets, but these antibodies are usually derived from the colostrum of the virus-positive mothers.[13,17] If the antibody titer decreases over time, the piglet is not infected, if it increases, it is infected.

In the last years, excellent detection systems have been established in numerous laboratories dealing with xenotransplantation (for an overview see[14]). However, until now it is still unclear which viruses pose a risk for xenotransplantation. Lists of microorganisms potentially posing a risk for healthy and immunosuppressed individuals have been published,[18–20] and xenotransplantation-relevant viruses have been suggested.[21] Recently Fishman published a short list of thirteen viruses which are not permitted in swine with designated pathogen-free status.[4] On the other hand, at present only two viruses are known to pose a risk for xenotransplantation, PCMV/PRV[6–10] and HEV3.[22,23] PCMV/PRV is a xenozoonotic virus, it does not infect or harm humans, but induces disease in the context of xenotransplantation, whereas HEV3 represents a typical zoonotic virus.[12] After evaluating whether veterinary laboratories meet the criteria to screen for viruses posing a risk for xenotransplantation, clinical and some preclinical trials as well as screenings of pig populations were analyzed to identify and to compare the viruses tested in these trials or screenings.

2 | METHODS

2.1 | Data acquisition Germany

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) a systematic analysis of German veterinary laboratories was performed starting with a list of lab-

oratories in Vet-Magazin (https://vet-magazin.de/deutschland-magazin.html?SID=ZbkDqSkFCbpkJwSBjvhTQgAA). Altogether websites of 35 veterinary laboratories in Germany were analyzed whether they are testing for porcine viruses, especially whether they test for thirteen viruses selected by Fishman as not permitted in pigs with designated pathogen-free status[4] as well as for porcine endogenous retrovirus-C (PERV-C). The selected viruses were pig adenovirus, hepatitis E virus genotype 3 (HEV3), pig influenza virus, encephalomyocarditis virus (EMCV), porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV), porcine circoviruses 1, 2, 3 (PCV1, 2, 3), porcine lymphotropic herpesvirus (PLHV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), pseudorabies virus (PrV), also called suid herpesvirus 1 (SuHV-1) or Aujeszky-Virus, und rabies virus (Table 1). Data was confirmed by email and telephone.

2.2 | Data acquisition abroad

For comparative purposes, information on Swiss veterinary laboratories was obtained by email. Furthermore, the website of one selected laboratory in the United States of America, ZOOLOGIX, 725 Lakefield Rd, Westlake Village, CA, 91361, USA (https://www.zoologix.com), was screened for diagnostic test methods.

2.3 | Literature research

Literature reporting clinical and preclinical xenotransplantation trials as well as describing strategies of pig virus testing in different pig populations were analyzed.

3 | RESULTS

3.1 | PCR-based methods

When 35 commercial veterinary laboratories in Germany were analyzed concerning their capacity to screen for 13 porcine viruses selected by Fishman as not permitted in pigs with designated pathogen-free status,[4] and PERV-C, altogether 14 viruses, only nine laboratories were able to test for porcine viruses. Although there are known three PLHV variants, PLHV-1, -2, and -3, they were counted only as one. Among the laboratories testing for pig viruses, one laboratory tested for nine viruses, one for seven viruse, two laboratories tested for six viruses, three tested for four, and one for three viruses (Table 1). Some viruses such as porcine adenoviruses, EMCV, and PLHV were not tested for at all.

Most importantly, PCMV/PRV and HEV3 were tested only in one laboratory each by PCR, and HEV3 in one laboratory by antibody testing. As mentioned above, these viruses are the only known xenozoonotic or zoonotic viruses posing a risk for xenotransplantation until today. However, their importance for pig breeding and meat production is relatively low. As mentioned, for the detection of PCMV/PRV immunological methods are required in order to detect virus infections in the case the PCR tests were negative. Assays to detect PLHV were also not available. PrV was only tested in four laboratories. The viruses tested best in all laboratories are swine influenza, PCV2, PRRSV-1, PRRSV-2, and PPV. These viruses are of importance for the pig industry.

In Switzerland, the following viruses are tested routinely at the Institute of Virology of the Vetsuisse faculty in Zurich: adenovirus (pan-adeno PCR), HEV, influenza, PCMV (pan-herpes nested PCR), PLHV (pan-herpes nested PCR), PPV, and PrV. The Institute of Virology and Immunology in Bern is testing for ECMV, PRRSV, and rabies virus.

A well-known American laboratory, Zoologix, tests for a broader number of viruses, but mainly using PCR-based methods (Table 2). The laboratory did not detect PCMV/PRV in our positive samples as published.[24]

3.2 | Immunological methods

In addition to PCR-based tests, also indirect detection assays detecting antibodies against SIV, PCV2, PRRSV, and PPV were available in a few of the laboratories (Table 1). One laboratory tested each for antibodies against rabies virus and HEV, and two against PrV. The most needed assay detecting antibodies against PCMV/PRV was available in none of the laboratories.

3.3 | Viruses tested in first trials and when screening pig populations

3.3.1 | Clinical trials

Testing of the Auckland Island pigs used as donors for the first transplantations of pig islet cells into diabetic patients in New Zealand and Argentina was performed in the laboratories of Living Cell Technologies (LCT), Auckland, New Zealand. Fourteen viruses were shown to be absent in these animals, including PCMV/PRV, and therefore were not transmitted to the patients.[25,26] In these trials PERV was also not transmitted[26,27] (Table 3, clinical trials, CT).

The donor pig of the heart transplanted into the Baltimore patient that transmitted PCMV/PRV was tested by the University of Minnesota Veterinary Diagnostic Laboratory.[28] PCMV/PRV was not detected in the donor pig because the laboratory tested nasal swabs by PCR. However, nasal swabs are only applicable at the stage of rhinitis, which is typical for an acute PCMV/PRV infection. Rhinitis can only be observed a short time after infection, which happens usually in young piglets upon transmission of the virus from the mother.[15,29] In infected adult animals the virus is usually not detectable by PCR, as the virus establishes a quiescent, latent stage. In this case, the presence of the virus can be detected by antibody tests.[13]

TABLE 1 Availability of test methods for different xenotransplantation-relevant viruses in German commercial veterinary laboratories. Empty boxes mean that no assays are available.

Laboratory/virus	PERV-C	Adeno-virus (pig)	EMCV	Hepatitis E virus	Influenza virus	PCMV/PRV	PCV 1, 2,3	PLHV	PRRSV	PPV	PrV	Rabies virus
aCare Lab GmbH & Co.KG, Leipzig					SIV PCR, Ab		PCV2 PCR, Ab		PRRSV-1 PCR, Ab	PCR, Ab		
AniCon Labor GmbH, Höltinghausen					SIV PCR, Ab		PCV2[a] PCR, Ab PCV3 PCR		Ab, PRRSV-1[a], -2[a] PCR	PCR, Ab	Ab	
BioCheck Labor für Veterinärdiagnostik, Leipzig					SIV PCR, Ab		PCV2 PCR, Ab		PCR, Ab	Ab		
Biocontrol, Ingelheim am Rhein							PCV2 PCR		PCR	PCR		
IVD Gesellschaft für Innovative Veterinärdiagnostik mbH, Seelze-Letter					PCR		PCV2, PCV3 PCR		PCR			
Laboklin GmbH & Co. KG, Kissingen					SIV PCR, Ab		PCV2 PCR, Ab[b]		PCR, Ab[b]	PCR, Ab[b]	TCA	Ab[b]
LUFA Nord-West, Oldenburg					SIV PCR, Ab		PCV2 PCR, Ab PCV3 PCR		PCR, Ab	PCR	Ab	
Synlab Vet GmbH und Co.KG, Augburg					SIV PCR, Ab		PCV2 PCR, Ab		PRRSV[a]-1, -2 PCR, Ab			
Vaxxinova Diagnostics GmbH, Leipzig				PCR, Ab	SIV[a] PCR, Ab	PCR	PCV2[a] PCR, Ab PCV3 PCR		PRRSV-1, -2 PCR, Ab	PCR	Ab	

Abbreviations: EMCV, encephalomyocarditis virus; PCMV/PRV, porcine cytomegalovirus/porcine roseolovirus; PCV, porcine circovirus; PERV-C, porcine endogenous retrovirus - C; PLHV, porcine lymphotropic herpesvirus; PPV, porcine parvovirus, PRRSV, porcine reproductive and respiratory syndrome virus; PrV, pseudorabies virus, also called suid herpesvirus 1 (SuHV-1) or Aujeszky-Virus, TCA, tissue culture assay.

[a]Genotyping, sequencing available.

[b]To be tested in a partner laboratory.

TABLE 2 Virus tests available at Zoologix, an experienced US American laboratory.

Virus	Test method
African swine fever (ASF)	Qualitative real time PCR
Pseudorabies virus (PRV)/suid herpesvirus-1 (SHV-1)/Aujeszky's disease virus	Qualitative real time PCR
Classical swine fever virus (CSFV)	Qualitative reverse transcription real time PCR
Foot and Mouth Disease virus (FMD)	Qualitative reverse transcription real time PCR
Hepatitis E virus (HEV)	Qualitative reverse transcription real time PCR
Nipah virus (NiV)	Qualitative reverse transcription real time PCR
Porcine adenovirus (PAdV)	Qualitative real time PCR
Porcine circovirus type 1 (PCV1)	Qualitative real time PCR
Porcine circovirus type 2 (PCV2)	Qualitative real time PCR
Porcine cytomegalovirus (PCMV)	Qualitative real time PCR
Porcine endogenous retroviruses-A, -C (PERV-A, -C)	Qualitative reverse transcription real time PCR
Porcine endogenous retroviruses-C (PERV-C)	Qualitative real time PCR
Porcine enteroviruses (PEV 8, 9 and 10)	Qualitative reverse transcription real time PCR
Porcine epidemic diarrhea virus (PEDV)	Qualitative reverse transcription real time PCR
Porcine hemagglutinating encephalomyelitis virus (PHEV)	Qualitative reverse transcription real time PCR
Porcine lymphotropic herpesviruses-1, -2 (PLHV-1, PLHV-2)	Qualitative real time PCR
Porcine lymphotropic herpesviruses-3 (PLHV-3)	Qualitative real time PCR
Porcine parvovirus (PPV)	Qualitative real time PCR
Porcine reproductive and respiratory syndrome virus (PRRSV),	Qualitative reverse transcription real time PCR
Porcine respiratory coronavirus (PRCV)	Qualitative reverse transcription real time PCR
Porcine transmissible gastroenteritis virus (TGEV)	Qualitative reverse transcription real time PCR
Rabies virus (RV)	Qualitative reverse transcription real time PCR
Reoviruses	Qualitative reverse transcription real time PCR

3.3.2 | Preclinical trials

Gazda et al.[30] screened Large White—Yorkshire x Landrace pigs for 28 viruses and found PRCV, PRRSV, PPV, EMCV, Eastern equine encephalomyelitis virus (EEEV), Venezuelan equine encephalomyelitis virus (VEEV), and Western encephalomyelitis virus (WEEV) in the screened animals (Table 3, preclinical trial 1, PCT1). However, these viruses and PERV were not transmitted when encapsulated islet cells were transplanted into cynomolgus monkeys.

Genetically modified pigs used as donors for orthotopic heart transplantation were screened for 17 viruses. In a few cases, PCMV/PRV[9,11] and PCV3[31] were transmitted to the recipients. The virus testing was performed by the virological laboratories of the German Transregional Collaborative Centre (Table 3, preclinical trial 2, PCT2).

3.3.3 | Screening pig populations

Noordergraaf et al.[32] screened caesarean derived, colostrum deprived (CDCD) pigs for 21 viruses and found only two outbreaks of PCV in their source animals barrier facility (Table 3, screening pig population 2, SPP2). The most extensive screening was performed by Hartline et al.[33] They looked for 31 viruses in 9 Large White—Yorkshire x Landrace pigs and their piglets derived by caesarean section (Table 3, screening pig population 1, SPP1). Gammaherpesviruses (PLHV-2, PLHV-3) were the most frequent detected in the adult animals, which were also transmitted partially to the piglets. In a study analyzing Mini-LEWE minipigs, 13 viruses were tested using duplex-real time PCR or real-time reverse transcription PCR. For the first time, synthetic gene blocks containing partial sequences of several viruses were used as a positive control.[34] In an extended study analyzing Göttingen minipigs diseased with the dippity pig syndrome as model, 15 viruses were tested including PERV-A/C[35] (Table 3, screening pig population 3, SSP3). The methods worked and detected PCMV/PRV, PCV1, PCV3, PLHV-3, and PERV-A/C in one or more animals. Otabi et al.[36] developed PCR detection methods to screen for 41 viruses, some of them are not common in Europe such as the Nipha virus, Getah virus, and the Menangle virus (Table 3, screening pig population 5, SPP5). Using these PCR methods, a small number of uterectomy-born piglets was tested, focusing on the transplacental transmission of viruses. In contrast to this, in the screening of the Göttingen minipigs with dippity pig syndrome and in a newer study screening indigenous Greek black pigs[37] (Table 3, SPP4), the established methods were evaluated under natural conditions testing animals infected with a broad range of viruses. In the case of indigenous Greek black pigs using the same methods to detect 15 viruses as in the case of the animals with the dippity pig syndrome, PCMV/PRV, PLHV-1, PLHV-2, PLHV-3, PVC3, and PERV-C were detected in most of the animals.[37]

TABLE 3 Viruses tested in clinical trials (CT), preclinical trials (PCT) and screening of pig populations (SPP) (marked boxes means these viruses were tested). Viruses marked in light grey in the full names column were defined by Fishman not permitted in swine with designated pathogen-free status.[4]

Nr.	Full name	Abbreviation	CT	PCT1	PCT2	SPP1	SPP2	SPP3 SSP4	SPP5
1	Astrovirus								
2	Bovine viral diarrhea virus	BVDV							
3	Chikungunya virus	CHIKV							
4	Encephalomyocarditis virus	EMCV							
5	Hepatitis E virus	HEV							
6	Infectious bovine rhinotracheitis virus	IBRV							
7	Influenza A virus	IAV							
8	Influenza B virus	IBV							
9	Lymphocytic choriomeningitis virus	LCMV							
10	Norovirus genogroup II	NoV GII							
11	Porcine adenovirus	PAdV							
12	Porcine cytomegalovirus/porcine roseolovirus	PCMV/PRV							
13	Porcine circovirus 1	PCV1							
14	Porcine circovirus 2	PCV2							
15	Porcine circovirus 3	PCV3							
16	Porcine circovirus 4	PCV4							
17	Porcine encephalomyocarditis virus	PEMCV							
18	Porcine epidemic diarrhea virus	PEDV							
19	Porcine endogenous retrovirus-A, -B	PERV-A, -B							
20	Porcine endogenous retrovirus-C	PERV-C							
21	Porcine endogenous retrovirus-A/C	PERV-A/C							
22	Porcine enterovirus type 1	PEV-1							
23	Porcine hemagglutinating encephalomyelitis virus	PHEV							
24	Porcine hokovirus	PHoV							
25	Porcine lymphotrophic herpesvirus 1	PLHV-1							
26	Porcine lymphotrophic herpesvirus 2	PLHV-2							
27	Porcine lymphotrophic herpesvirus 3	PLHV-3							
28	Porcine parvovirus	PPV							
29	Porcine reproductive and respiratory syndrome virus	PRRSV							
30	Porcine respiratory coronvirus	PRCV							
31	Pseudorabies virus/suid herpesvirus 1 (SuHV-1)/Aujeszky-Virus	PrV							
32	Rabies virus	RV							
33	Reovirus 1								
34	Reovirus 2								
35	Reovirus 3								
36	Rotavirus	RV							
37	Sapovirus								
38	Seneca valley A virus	SVA							

(Continues)

TABLE 3 (Continued)

Nr.	Full name	Abbreviation	CT	PCT1	PCT2	SPP1	SPP2	SPP3 SSP4	SPP5
39	Transmissible gastroenteritis virus	TGEV							
40	Torque Teno sus viruses	TTSuV1, TTSuV2							
41	Vesicular stomatitis Indiana virus	VSV-IN							
42	Vesicular stomatitis New Jersey virus	VSB-NJ							
43	Eastern equine encephalomyelitis virus	EEEV							
44	Venezualan equine encephalomyelitis virus	VEEV							
45	Western equine encephalomyelitis virus	WEEV							
46	West Nile virus	WNV							
47	Apoi virus								
48	Borna disease virus	BoDV							
49	Food and mouth disease virus	FMDV							
50	Getah virus								
51	Japanese encephalitis virus	JEV							
52	African swine fever virus	ASFV							
53	Classical swine fever virus	CSFV							
54	Lyssa virus genotype 1-7								
55	Menangle virus	MenPV							
56	Nipha virus	NIV							
57	Swine vesicular virus	SVV							
58	Swine pox virus	SWPV							

CT, clinical trial, pig islet cells into human diabetes patients in New Zealand and Argentina, green.[26,27]
PCT1, preclinical trial 1, pig islet cells into cynomolgus monkeys, light blue.[31]
PCT 2, preclinical trial 2, orthotopic pig heart transplantation into baboons, dark pink.[9]
SPP1, screening pig population 1, screening Caesarian derived colostrum deprived (CDCD) piglets, light brown.[32]
SPP2, screening pig population 2, screening Large White- Yorkshire x Landrace F1 hybrid animals, orange.[33]
SPP3, screening pig population 3, screening Göttingen minipigs with dippity pig syndrome, dark grey.[35]
SPP4, screening population 4, screening indigenous Greek black pigs.[37]
SPP5, screening pig population 5, uterectomy-born piglets, light pink.[36]
Viruses marked in light grey in the first column were defined by Fishman not permitted in swine with designated pathogen-free status.[4]

4 | DISCUSSION

It is not surprising that most of the xenotransplantation-relevant viruses cannot be tested in commercial veterinary laboratories in Germany because their actual task is to detect viruses that are relevant for pig breeding and pig production. Methods detecting the known zoonotic or xenozoonotic viruses like HEV3 and PCMV/PRV were not available in these veterinary laboratories.

The situation in other countries is similar. For example, a well-known American laboratory, Zoologix; is able to test for a larger number of viruses, but not for antibodies against PCMV/PRV needed to detect the infection when the virus is in latency. However, also their PCR-based testing was not sensitive enough: When we tested 11 adult animals using DNA from sera for PCMV/PRV in 2016, using a new home-made nested PCR and a new home-made real-time PCR with a sensitivity of about 5 and 2–5 copies PCMV/reaction,[24] three animals were found positive using the nested PCR and four animals were found positive using the real-time PCR. In parallel, we did send the same samples to Zoologix. They tested using a PCR and none of the samples were found positive.[24]

To note, xenotransplantation has taken a promising development in recent years, to name new genetically modified donor pigs, new immunosuppressiva, first clinical trials with pig islet cells, the compassionate use of genetically modified pig heart and the development of sensitive detection methods for potentially zoonotic or xenozoonotic viruses in different laboratories of the world (for reviews see[14,28,38–40]).

The absence of crucial tests for xenotransplantation-relevant viruses, especially for the zoonotic virus HEV3 and the xenozoonotic virus PCMV/PRV, indicates that the veterinary laboratories will not

be able to perform testing for future xenotransplantations. Testing in the first trials were mainly performed by the institutions performing the xenotransplanations or private companies. Testing of the Auckland Island pigs used for the first transplantations of pig islet cells into diabetic patients was performed in the laboratories of LCT. Fourteen viruses were shown to be absent in these animals, including PCMV/PRV and therefore not transmitted to the treated patients.[25,26] Testing of the animals used for the transplantation of islet cells to cynomolgus monkey was mainly performed by SGS Vitrology.[30] In this non-immunosuppressed streptozotocin-induced diabetic cynomolgus monkey surveillance, no evidence of viral infection with PCV2, PLHV-1, 2 and 3, PRRSV, PCMV, or PERV in samples collected longitudinally post-transplant was described.[30]

It is an open question whether commercial veterinary laboratories will include the necessary tests for xenotransplantation into their repertoire because the expense of establishing and validating methods is high and the number of expected tests, at least in the first years, is low. Testing for antibodies against PCMV/PRV, for example, requires cloning of the glycoprotein B sequences, expression and purification of the recombinant protein, and selection of positive and negative control sera.[13]

It is important to note that the assays which will be used for the screening of the donor pigs should be carefully validated, this means all samples, for example, blood, serum, tissues, or swabs require a separate validation process. Validation also requires multiple laboratory input using anonymized samples and a standard operating procedure. Of importance are also interlaboratory comparison tests. Since our laboratory at the Institute of Virology of the Free University in Berlin, the laboratory of Dr. Tönjes at the PEI in Langen, and the laboratory of Dr. Scobie at the Glasgow Caledonian University in Glasgow are planning interlaboratory comparison tests, we may include also veterinary laboratories at least with the assays, which are available in these laboratories.

When we analyzed which viruses were tested in different laboratories in the context of clinical or preclinical trials or in the context of screening pig populations in order to test the efficacy of the established methods, enormous differences in the selection of viruses were found. Therefore, it is recommended that an international group of virologists, pig breeders, and regulatory bodies should prepare a short list of the xenotransplantation-relevant viruses.

5 | CONCLUSION

Veterinary laboratories in Germany and abroad do not have the methods needed for screening for xenotransplantation-relevant viruses. Tests are available detecting viruses which influence the health of pigs. Therefore, it is necessary to establish special virological laboratories be able to test for all xenotransplantation-relevant porcine viruses using validated assays, optimally at the xenotransplantation centers. In order to establish an internationally standardized list of viruses to be tested, experts in the field of virology and production of genetically modified pigs should collaborate with regulatory authorities.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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