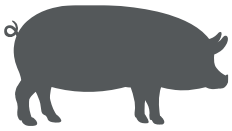
September 2022 | EN

SVANOVIR® PRV gE-Ab

SVANOVIR® PRV gB-Ab

Validation Report

For detection of antibodies to *Pseudorabies/Aujeszky's disease*



INDICAL BIOSCIENCE

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1. Introduction

1.1. Characteristics

SVANOVIR® PRV gE-Ab is an Enzyme Linked Immunosorbent Assay (ELISA) for the detection of PRV gE antibodies in porcine serum and plasma samples. The test will discriminate between animals infected and animals vaccinated with a gE-deleted vaccine.

The SVANOVIR® PRV gB-Ab is an ELISA for the detection of specific antibodies to PRV in porcine serum and plasma samples. These products are available in 2-plate (single strips) and 10-plate (whole plates) package sizes (Art. No. SV-104902/SV-104905 and SV-104904/SV-104906, respectively).

To discriminate between infected and vaccinated with a gE-deleted vaccine animals, it is recommended to use the SVANOVIR® PRV gE-Ab test.

1.2. General information

Pseudorabies Virus (PRV) or *Suid Herpesvirus type 1*, also known as Aujeszky's disease (AD) is a critical pathogen in swine production. PRV, first isolated by Aujeszky (1902) from ox with signs of rabies, has the pig as a primary host, while cattle and some other animal species are secondary hosts with infected pigs as the initial source of infection.

In countries with a high prevalence of endemic pseudorabies this disease causes severe losses annually [Gustafson, 1986]. In piglets, but also in older pigs, neurological disorders are intermingled with symptoms from the respiratory tract. High mortality can be seen, particularly in piglets. The disease is less pronounced in older pigs, and after recovery, a latent lifelong infection can persist. Virus transmission occurs mainly through nasal secretions by contact or even by the aerogenic route [Gustafson, 1986]. The infection of pregnant sows may reach the fetuses via the placenta and cause abortion, mummification, or stillbirth.

All infected herds in endemic regions should be monitored for the presence of infection and uninfected herds should be protected by control measures. Some countries practice vaccination, while others try to control the spread by culling seropositive pigs or by combination of both measures.

Virus isolation, PCR and serological tests are test methods available for diagnosis of the disease. Virus isolation is most likely successful from samples from the trigeminal ganglion however latent infections can be difficult to diagnose. ELISAs can differentiate between antibodies of infected and vaccinated pigs [OIE 2018].

Differentiation of vaccinated from infected pigs

If both control measures, i.e., vaccination and eradication by culling seropositive pigs are (or are to be) introduced, a differentiation of vaccinated pigs from those infected must be introduced, as both infected and vaccinated pigs are seropositive. Most vaccinal strains used are well-known attenuated strains with a deletion in their genome (DNA) of the gene sequence coding for the

virulence factor, the glycoprotein E, (gE/gI) [Rziha et al., 1982]. Consequently, vaccinated pigs lack antibodies against the gE antigen. The differentiation is thus based on the presence of antibodies to gE in infected pigs but the absence of these antibodies in vaccinated pigs [Baskerville et al., 1973]. Since both the vaccinal and field strains of PRV possess another glycoprotein, gB (gII), antibodies to gB are present in sera of both infected and vaccinated pigs but absent in sera of non-infected and non-vaccinated pigs [Soerensen et al., 1986].

Serological Tests

There are several serological test methods for the detection of antibodies to *Pseudorabies/ Aujeszky Virus*. According to the OIE manual both the virus neutralization test (VNT) and the enzyme-linked immunosorbent assay (ELISA) are prescribed tests. However ELISA is more commonly used due to its suitability for high volume testing and superior sensitivity figures. Criteria for suitability and performance of the ELISAs are described in EU directive 2001/618/EC, and SVANOVIR® PRV gE-Ab and SVANOVIR® PRV gB-Ab both conform to the directive. The SVANOVIR® assays are based on these premises:

SVANOVIR® PRV gE-Ab	The assay can discriminate between serologic response to gE-deleted vaccinal strains and field virus, and enables the detection of Aujeszky's disease in vaccinated pig populations.
SVANOVIR® PRV gB-Ab	The assay detects both vaccinated and naturally infected pigs and is a useful tool in the eradication programmes in non-vaccinated pig populations.

Complementary products

Complementary products from Indical Sweden AB for multi-objective surveillance of highly infectious diseases.

SVANOVIR® TGEV/ PRCV-Ab. Keep Transmissible Gastroenteritis Virus (TGEV) out of your herd.

SVANOVIR® Brucella-Ab C-ELISA. The best way to detect brucellosis in livestock herds.

1.3. Description of the test principle

1.3.1. SVANOVIR® PRV gE-Ab

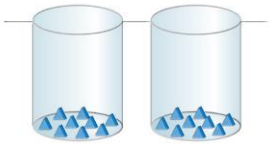

- Antigen


- Antigen non-specific antibody

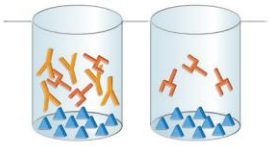

- Antigen specific antibody

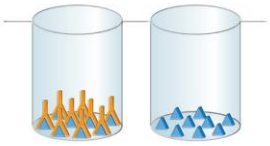

- Antigen specific HRP-conjugated monoclonal antibody

The kit procedure is based on the blocking Enzyme Linked Immunosorbent Assay (ELISA).

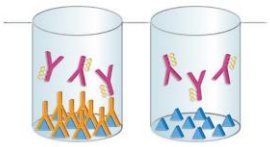


The assay is a blocking ELISA based on full antigen and anti-gE (gI) monoclonal antibodies. In this procedure, samples are exposed to PRV antigen, known to possess both the glycoprotein gE and gB, coated onto wells of microtiter plates.

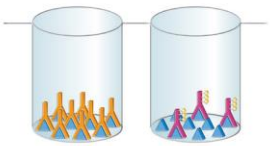




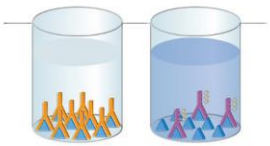
PRV gE antibodies will bind to the antigen in the wells.



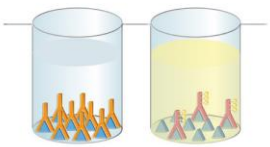
The indicator used for the detection of presence or absence of antibody against gE respectively, is a monoclonal antibody (mAb) specifically directed against gE. The mAbs are conjugated with the horse-radish peroxidase (HRP). If antibodies are absent in the test serum, i.e., from non-infected/non-vaccinated or vaccinated pigs, the gE epitopes will remain free and the conjugated gE mAb will subsequently bind.



The enzyme present (HPR) will catalyze the added substrate, which results in a colour reaction. If antibodies against gE are present in the serum when tested in the gE assay, i.e., from pigs infected with field virus, these antibodies will bind to the gE antigen in the well.



The gE antibodies will block the possibility of the conjugated mAb subsequently added to react with the gE antigen, respectively. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate.



The reaction is stopped by addition of stop solution; the colour changes to yellow. The optical density (OD) is measured with a spectrophotometer at 450 nm.

1.3.2. SVANOVIR® PRV gB-Ab

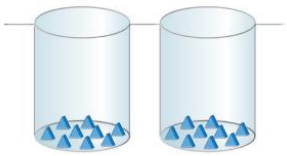
- Antigen

- Antigen non-specific antibody

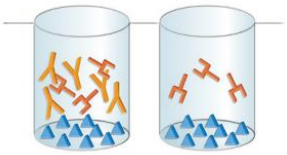
- Antigen specific antibody

- Antigen specific HRP-conjugated monoclonal antibody

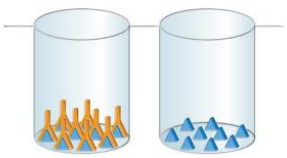
The kit procedure is based on the blocking Enzyme Linked Immunosorbent Assay (ELISA).



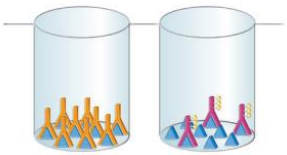
The assay is a blocking ELISA based on full antigen and anti-gB (gII) monoclonal antibodies. In this procedure, samples are exposed to PRV antigen, known to possess both the glycoprotein gE and gB, coated wells on microtiter plates.



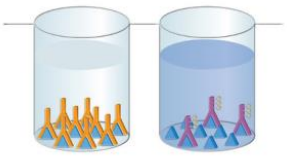
PRV gB antibodies will bind to the antigen in the wells.



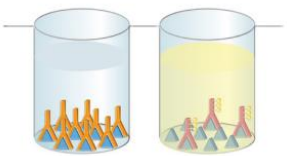
The indicator used for the detection of presence or absence of antibody against gB, is a monoclonal antibody (mAb) specifically directed against gB. The mAb are conjugated with the horse-radish peroxidase (HRP). If antibodies are absent in the test serum, i.e., from non-infected or non-vaccinated pigs, the gB antigen will remain free and the conjugated gB mAb subsequently added will bind.



The enzyme present (HPR) will catalyse the added substrate, which results in a colour reaction. If antibodies against gB are present in the serum when tested in the gB assay, i.e., from infected and/or vaccinated pigs, these antibodies will bind to the gB antigen in the well.



The gB antibodies will block the possibility of the conjugated mAb subsequently added to react with the gB antigen. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate.



The reaction is stopped by addition of stop solution; the colour changes to yellow. The optical density (OD) is measured with a spectrophotometer at 450 nm.

1.4. Kit contents

	SVANOVIR® PRV gE-Ab		**SVANOVIR® PRV gB-Ab**	
Cat. no.	SV-104902	SV-104904	SV-104905	SV-104906
Number of plates	2	10	2	10
Test Plate	2 Strip plates	10 Plates	2 Strip plates	10 Plates
Positive Control	1 x 2.5 ml	1 x 4.5 ml	1 x 2.5 ml	1 x 4.5 ml
Negative Control	1 x 2.5 ml	1 x 4.5 ml	1 x 2.5 ml	1 x 4.5 ml
Sample Dilution Buffer*	1 x 8 ml	1 x 40 ml	1 x 8 ml	1 x 40 ml
Wash Buffer, 20x concentrate	1 x 125 ml	3 x 125 ml	1 x 125 ml	3 x 125 ml
Conjugate, ready to use	1 x 24 ml	1 x 120 ml	1 x 24 ml	1 x 120 ml
Substrate (STORE IN THE DARK)	1 x 20 ml	1 x 100 ml	1 x 20 ml	1 x 100 ml
Stop Solution	1 x 10 ml	2 x 25 ml	1 x 10 ml	2 x 25 ml
Handbook	1	1	1	1

There is always a surplus volume for the liquid reagent. The volume mentioned on the label is the minimum attainable.

*Sample Dilution Buffer used for overnight (ON) incubation

1.5. Storage

The components of SVANOVIR® PRV gE-Ab / SVANOVIR® PRV gB-Ab should be stored at 2-8°C and are stable until the expiration date stated on the label. If test strips are provided with the kit, store the remaining test strips re-sealed with a desiccant bag at 2-8°C until next use. The test strips can be stored up to 4 weeks after opening the plate pouch.

1.6. Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Beakers
- Measuring cylinders
- Pipettes (adjustable)
- Multichannel pipettes (adjustable)
- Disposable pipette tips
- Distilled, deionized or any similar high-quality water
- Aluminum or adhesive foil for covering test plate
- Optional: device for delivery and aspiration of wash buffer
- Tubes or plates for diluting the samples
- Microtiter plate absorbance reader

1.7. Precautions

- Carefully read and follow all instructions.
- Handle all materials according to the Good Laboratory Practice.
- Do not mix the components or instruction manuals from different test kit batches.
- Care should be taken to prevent contamination of kit components.
- Do not use test kit beyond date of expiry.
- Include Positive and Negative Control serum on each plate or test strip series.
- When preparing buffers etc., measure the required volume.
- All unused biological material should be disposed according to the local, regional, and national regulations.

2. Procedure

2.1. Things to do before starting

All reagents should equilibrate to room temperature 18-25°C before use. Label each strip with a number. In case of precipitated salt crystals in the Wash Buffer (20x), dissolve by gentle swirling and warming.

Wash Buffer: Dilute Wash Buffer (20x) 1:20 in distilled water, e.g., for one Test Plate dilute 25 ml Wash Buffer (20x) in 475 ml distilled water and mix.

Serum samples: 100 µL of blood serum or plasma is required for each sample well. Fresh, refrigerated or previously frozen serum or plasma may be tested.

2.2. Protocol: Test procedure for short sample incubation

1. Add 100 µL of Positive Control (reagent A) and Negative Control (reagent B), into appropriate wells of the Test Plate.
2. Add 100 µl of undiluted serum sample to a selected well coated with PRV antigen. For confirmation purposes it is recommended to run the samples in duplicates. Record the positions of the controls and samples in a test protocol. The use of a multichannel pipette is recommended for the transfer of samples. Cover the Test Plate.
3. Incubate for 1 hour at 37°C.
4. Remove solution from the wells by aspiration or tapping.
5. Rinse each well 3 times with prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
6. Pipette 100 µl of the ready to use Conjugate to each well. Cover the Test Plate.
7. Incubate for 30 minutes at 37°C or 1 hour at room temperature (18-25°C).
8. Remove solution from the wells by aspiration or tapping.
9. Rinse each well 3 times with prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
10. Pipette 100 µl Substrate Solution to each well.
11. Incubate for 15 minutes at room temperature (18-25°C). Begin timing after first well is filled.
12. Stop the reaction by adding 50 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
13. Measure the optical density (OD) of the controls and samples in the plate reader at 450 nm within 15 minutes after stopping the reaction.

2.3. Protocol: Test procedure for overnight sample incubation

1. Add 25 µL of Sample Dilution Buffer to all wells
2. Add 100 µL of Positive Control (reagent A) and Negative Control (reagent B), into appropriate wells of the Test Plate.
3. Add 100 µl of undiluted serum sample to a selected well coated with PRV antigen. For confirmation purposes it is recommended to run the samples in duplicates. Record the positions of the controls and samples in a test protocol. The use of a multichannel pipette is recommended for the transfer of samples. Cover the Test Plate.
4. Incubate for overnight (12-18 hours) at room temperature (18-25°C).
5. Remove solution from the wells by aspiration or tapping.
6. Rinse each well 3 times with prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
7. Pipette 100 µl of the ready to use Conjugate to each well. Cover the Test Plate.
8. Incubate for 30 minutes at 37°C or 1 hour at room temperature (18-25°C).
9. Remove solution from the wells by aspiration or tapping.
10. Rinse each well 3 times with prepared (1x) Wash Buffer. Remove the buffer after each rinse by aspiration or tapping.
11. Pipette 100 µl Substrate Solution to each well.
12. Incubate for 15 minutes at room temperature (18-25°C). Begin timing after first well is filled.
13. Stop the reaction by adding 50 µl Stop Solution per well. Add the Stop Solution in the same order as the Substrate Solution was added.
14. Measure the optical density (OD) of the controls and samples in the plate reader at 450 nm within 15 minutes after stopping the reaction.

3. Data interpretation

Validation criteria

To ensure validity, the duplicate OD values of the negative control should not differ more than 25% from the mean value of the two duplicates. Additionally, the control values should fall within the following limits:

- ODp Positive Control < 30
- OD Negative Control 0.8 – 1.6

Should any of these criteria not be fulfilled, the test is invalid. For invalid tests, technique may be suspect, and the assay should be repeated.

Calculation

Calculate the mean OD values for each of the controls and samples.

Calculate the optical density percentage **(ODp)** for each sample and for the positive control sera using the following formula:

$$\mathrm{ODp} = \frac{\mathrm{OD}_{\text{Sample or Positive Control}}}{\mathrm{OD}_{\text{Negative Control}}} \times 100$$

Data interpretation

SVANOVIR® PRV gE-Ab	SVANOVIR® PRV gB-Ab
Short sample incubation ▪ **Samples with ODp values ≤ 50 are positive**. Serological diagnosis: Field virus infected. ▪ **Samples with ODp values > 60 are negative.** Serological diagnosis: Either a) non-infected with field virus and no vaccinated against Aujeszky's disease or b) non-infected with field virus but vaccinated with an Aujeszky's disease gE-deleted vaccine. ▪ **Samples with ODp values 51-60 are doubtful**. Retest the sample. If the test result is still doubtful, a new sample should be taken from the animal and both samples should be run in parallel.	**Short sample incubation** ▪ **Samples with ODp values ≤ 50 are positive**. Serological diagnosis: Field virus infected and/or vaccinated against Aujeszky's disease. ▪ **Samples with ODp values > 60 are negative.** Serological diagnosis: Neither infected with field virus nor vaccinated against Aujeszky's disease. ▪ **Samples with ODp values 51-60 are doubtful**. Retest the sample. If the test result is still doubtful, a new sample should be taken from the animal and both samples should be run in parallel.
Overnight sample incubation ▪ **Samples with ODp values ≤ 45 are positive**. Serological diagnosis: Field virus infected. ▪ **Samples with ODp values > 55 are negative.** Serological diagnosis: Either a) non-infected with field virus and no vaccinated against Aujeszky's disease or b) non-infected with field virus but vaccinated with an Aujeszky's disease gE-deleted vaccine. ▪ **Samples with ODp values 46-55 are doubtful**. Retest the sample. If the test result is still doubtful, a new sample should be taken from the animal and both samples should be run in parallel.	**Overnight sample incubation** ▪ **Samples with ODp values ≤ 45 are positive**. Serological diagnosis: Field virus infected and/or vaccinated against Aujeszky's disease. ▪ **Samples with ODp values > 55 are negative.** Serological diagnosis: Neither infected with field virus nor vaccinated against Aujeszky's disease. ▪ **Samples with ODp values 46-55 are doubtful**. Retest the sample. If the test result is still doubtful, a new sample should be taken from the animal and both samples should be run in parallel.
To evaluate whether the animal, for which a test serum score negative, is vaccinated, it is recommended to test the serum in the SVANOVIR® PRV gB-Ab test (Art. No. SV-104905/SV-104906).	To evaluate whether the animal, for which a test serum score negative, is vaccinated, it is recommended to test the serum in the SVANOVIR® PRV gB-Ab test (Art. No. SV-104902/SV-104904).

4. Characteristics of the test and validation studies

4.1. Inter assay precision – Repeatability

4.1.1. SVANOVIR® PRV gE-Ab

Objective

To investigate the homogeneity of SVANOVIR® PRV gE-Ab plates.

Material and Methods

Anti-PRV gE conjugate was added to all wells (96) of seven different plates from the same batch. The testing was performed according to the procedure for control of plate batches of SVANOVIR® PRV gE-Ab.

Results

Table 1. Intra plate variation of different plates of SVANOVIR® PRV gE-Ab

	SVANOVIR® PRV gE-Ab	
PLATES	**MEAN (OD_{450})**	**CV* (%)**
Plate 1	1.43	2.93
Plate 2	1.40	4.13
Plate 3	1.41	3.86
Plate 4	1.42	2.95
Plate 5	1.41	3.66
Plate 6	1.41	3.71
Plate 7	1.42	2.83

CV*= coefficient of variance is a normalized measure of dispersion of a probable distribution.

Table 2. Intra batch variation of SVANOVIR® PRV gE-Ab

	SVANOVIR® PRV gE-Ab
Average (OD_{450})	1.41
Standard deviation	0.009
Intra batch CV (%)	0.625

Conclusion

The results for the intra plate and intra batch variation studies show an excellent variation coefficient (CV %) of significantly less than 5 % in both cases.

4.1.2. SVANOVIR® PRV gB-Ab

Objective

To investigate the homogeneity of SVANOVIR® PRV gB-Ab plates.

Material and Methods

Homogeneity of plates of 5 different plate batches of SVANOVIR® PRV gB-Ab was tested by adding anti-gB conjugate to all wells of plates according to the procedure for control of plate batches of SVANOVIR® PRV gB-Ab.

Results

Table 3. Intra plate variation of different plates of SVANOVIR® PRV gB-Ab

	SVANOVIR® PRV gB-Ab	
PLATES	**MEAN (OD_{450})**	**CV (%)**
Plate 1	1.56	7.00
Plate 2	1.64	4.99
Plate 3	1.70	4.54
Plate 4	1.69	5.58
Plate 5	1.69	6.01

Table 4. Intra batch variation of SVANOVIR® PRV gB-Ab

	SVANOVIR® PRV gB-Ab
Average (OD_{450})	1.690
Standard deviation	0.060
Intra batch CV (%)	3.604

Conclusion

The results for the intra plate and intra batch variation studies show an excellent variation coefficient (CV %) of significantly less than 8 % in both cases.

4.2. Inter assay precision – Reproducibility

4.2.1. SVANOVIR® PRV gE-Ab

Objective

To investigate the inter assay variation on well-defined samples.

Method

Internal control serum (ICS) including negative, positive, and weak positive serum samples were tested on plates from 12 different batches of SVANOVIR® PRV gE-Ab in duplicates. The test results were normalized into ODp values. The mean value, standard deviation (STD) and CV were calculated.

Table 5. ODp values of internal control sera (ICS) from 12 batches of SVANOVIR® PRV gE-Ab

	SVANOVIR® PRV gE-Ab			
	ICS1	**ICS2**	**ICS3**	**ICS4**
Batch 1	17	34	59	78
Batch 2	17	37	55	7
Batch 3	16	33	55	70
Batch 4	18	33	56	69
Batch 5	19	39	57	72
Batch 6	17	31	51	67
Batch 7	19	39	60	76
Batch 8	17	33	49	66
Batch 9	16	33	55	70
Batch 10	16	33	54	74
Batch 11	17	31	54	67
Batch 12	18	35	54	73
Mean ODp	**17.3**	**34.3**	**54.9**	**71.0**
STD	**1.1**	**2.7**	**3.0**	**3.7**
CV%	**6**	**8**	**6**	**5**

Result

Low inter assay variation was observed in this study of 12 batches of SVANOVIR® PRV gE-Ab which was depicted by the low standard deviation and CV. This is a strong indication of high reproducibility among different batches of the product.

4.2.2. SVANOVIR® PRV gB-Ab

Objective

To investigate the inter assay variation using a panel of internal control samples.

Method

A panel of different internal control serum (ICS) samples including negative, positive, and weak positive samples were tested on 10 different batches of SVANOVIR® PRV gB-Ab in duplicates. The ODp value for the samples was calculated. The mean value, standard deviation and CV were calculated as well.

Table 6. ODp values of internal control sera (ICS) from 10 batches of SVANOVIR® PRV gB-Ab

	SVANOVIR® PRV gB-Ab			
	ICS1	**ICS2**	**ICS3**	**ICS4**
Batch 1	7	24	48	68
Batch 2	7	27	51	68
Batch 3	9	31	53	68
Batch 4	8	34	60	77
Batch 5	8	31	58	74
Batch 6	8	29	54	72
Batch 7	8	31	58	74
Batch 8	11	29	53	68
Batch 9	11	29	53	68
Batch 10	8	23	49	72
Mean ODp	**8**	**29**	**54**	**71**
STD	**1.3**	**3.4**	**3.6**	**3.0**
CV%	**14.91**	**11.74**	**6.74**	**4.26**

Result

The low standard deviation and CV demonstrate that SVANOVIR® PRV gB-Ab has a low inter assay variation.

5. Evaluation studies

5.1. SVANOVIR® PRV gE-Ab

5.1.1. Study 1

Sensitivity and Specificity

Objective

To evaluate the performance, specificity, and sensitivity of SVANOVIR® PRV gE-Ab in serum samples from domestic pigs.

Material and Method

- 1362 serum samples from naturally infected, vaccinated, non-vaccinated and negative pigs from Sweden, Yugoslavia and Germany have been tested and compared to VNT.
- Another 912 serum samples from Swedish pigs (officially free from Aujeszky's disease since 1996) were analyzed.
- Furthermore 999 sera from field infected, negative, and non-vaccinated pigs from Denmark's eradication programme were used and compared to the results of a Danish blocking test [Sörensen et al., 1986].

The samples were run according to the kit insert of SVANOVIR® PRV gE-Ab and compared with results of the aforementioned tests.

Results

The analysis of serum samples from field infected, negative, and non-vaccinated populations and farms revealed a high sensitivity and specificity of the SVANOVIR® PRV gE-Ab (Tables 7, 8 and 9). All 912 sera from the Swedish population with a history of freedom of infection revealed negative results (Table 9).

Table 7. Agreement between SVANOVIR® PRV gE-Ab and reference method VNT

	VNT		
SVANOVIR® PRV gE-Ab	**Pos.**	**Neg.**	**Total**
Pos.	675	3	687
Neg.	0	684	684
Total	675	687	1362

Table 8. Agreement between SVANOVIR® PRV gE-Ab and Danish blocking ELISA

	Danish blocking ELISA		
SVANOVIR® PRV gE-Ab	**Pos.**	**Neg.**	**Total**
Pos.	447	2	449
Neg.	5	545	550
Total	452	547	999

Table 9. Summary of results of study results analyzing the sensitivity and specificity of SVANOVIR® PRV gE- Ab

Serum	**Sensitivity**	**Specificity**	**Reference method**
Naturally infected, vaccinated, non-vaccinated herds n= 1362[a]	100 %	99.6 %	VNT
Naturally infected, vaccinated, non-vaccinated herds n= 999[b]	98.9 %	99.6 %	Danish blocking ELISA
Commercial pigs n= 912[c]	n.a.	100 %	Area free of disease

Samples originating from

[a] Sweden, Germany, Yugoslavia,

[b] Denmark,

[c] Sweden

SVANOVIR® PRV gE-Ab showed high agreement with the other tests and a high specificity in samples from a population with history of freedom of infection.

5.1.2. Study 2

Objective

To evaluate the specificity of SVANOVIR® PRV gE-Ab in serum samples from vaccinated pigs.

Material and Method

Sera from 155 pigs vaccinated with different gE-deleted vaccines were tested.

The samples were run on the SVANOVIR® PRV gE-Ab according to the kit insert.

Results

The specificity of SVANOVIR® PRV gE-Ab in vaccinated pigs was 100%, indicating high specificity of the assay with no-cross reactivity and low probability false positive results. This is essential to avoid unnecessary culling of animals and the implementation of costly sanitary measures.

Table 10. Specificity of SVANOVIR® PRV gE-Ab in vaccinated pig herd

Serum	Positive	Negative	Total	Specificity
Vaccinated pigs[a]	0	155	155	100 %

[a] serum samples from Germany

5.1.3. Study 3

Objective

The objective of the study was to validate the SVANOVIR® PRV gE-Ab and compare to an in-house ELISA assay from the National Reference Center for Aujeszky's Disease – IZSLER Brescia. The study was done by the National Reference Center for Aujeszky's Disease – IZSLER Brescia in Italy.

Material and Method

For the study 46 well-defined serum samples from the Aujeszky's disease ring trial 2009 were used. These samples represented several possible combinations of vaccination and/or infection.

The samples were tested in the SVANOVIR® PRV gE-Ab according to the kit insert as well as with the in-house Brescia PRV-gE ELISA (reference method).

Results

Table 11. Agreement between SVANOVIR® PRV gE-Ab and reference method

Reference method*	SVANOVIR® PRV gE-Ab			Total
	Neg.	Doubtful	Pos.	
Neg.	14	0	0	14
Doubtful	0	0	0	0
Pos.	0	1+	31	32
Total	14	1	31	46

*in-house ELISA Reference Lab Brescia, Italy
+The doubtful result was considered as a positive result in the calculation of agreement.

In the study a very high level of agreement was shown between the results of the reference method and SVANOVIR® PRV gE-Ab. 100 % agreement was obtained for all negative samples and 97 % agreement for the positive samples.

SVANOVIR® PRV gE-Ab was able to accurately discriminate between serum samples from infected and vaccinated animals.

5.1.4. Study 4

Ring test PRV gE

Objective

To evaluate the performance of SVANOVIR® PRV gE-Ab by participation in the ring test from the National Reference Laboratory for Aujeszky's disease in France.

Material and Method

Well defined serum samples from pigs (n=12) were included in the ring test: The sample pool consisted of:

- negative samples (n=2)
- serum samples from vaccinated animals (n=3)
- positive sample from a naturally infected animal (n=1)
- equivalents of EU reference sera in different dilutions (n=6)

The analysis was done according to the kit insert of SVANOVIR® PRV gE-Ab.

Result

SVANOVIR® PRV gE-Ab identified all 12 samples accurately.

5.1.5. Study 5

Benchmarking

Objective

To evaluate the performance of SVANOVIR® PRV gE-Ab to another widely used commercial assay.

Material and Method

24 serum samples (11 OIE reference samples and 13 negative samples originating from Sweden) were tested on SVANOVIR® PRV gE-Ab and with a widely used commercial gE-ELISA.

The samples were tested according to the respective kit inserts.

Result

Table 12. Test results of SVANOVIR® PRV gE-Ab and SVANOVIR® PRV gB-Ab and a competitor assay on PRV negative, infected, and vaccinated pig serum samples

Sample description	Official OIE Status	SVANOVIR® PRV gE-Ab	Widely used commercial gE assay
Multivaccinated pigs n=6	gE neg.	Neg.	Neg.
Infected pig n=1	gE pos.	Pos.	Pos.
Vaccinated gE deleted vaccine & infected n=1	gE pos.	Pos.	Doubtful
Vaccinated gE deleted vaccine & infected n=2	gE neg.	Neg.	Neg.
Vaccinated gE deleted vaccine & infected n=1	gE neg. or doubtful	Doubtful	Neg.
Swedish negative sera n=13	gE neg.	Neg.	Neg.

SVANOVIR® PRV gE-Ab showed equally good performance as another widely used commercial assay.

5.2. SVANOVIR® PRV gB-Ab

5.2.1. Study 1

Sensitivity and Specificity

Objective

To evaluate the performance of SVANOVIR® PRV gB-Ab in different pig herds.

Material and Method

- 1362 serum samples from naturally infected, vaccinated, non-vaccinated and negative pigs from Sweden, Yugoslavia and Germany have been tested and compared to VNT.
- Another 912 serum samples from Swedish pigs (officially free from Aujeszky's disease since 1996) were analyzed.
- Furthermore 1000 sera from field infected, negative, and non-vaccinated pigs from Denmark's eradication programme were used and compared to the results of a Danish blocking test [Sörensen et al., 1986].

The samples were run according to the kit insert of SVANOVIR® PRV gB-Ab.

Results

High agreement between the reference method and SVANOVIR® PRV gB-Ab was seen in all studies (Table 13, Table 14).

Table 13. Agreement between SVANOVIR® PRV gB-Ab and reference method VNT 1362 serum samples

	VNT		
SVANOVIR® PRV gB-Ab	**Pos.**	**Neg.**	**Total**
Pos.	675	3	687
Neg.	0	684	684
Total	675	687	1362

Table 14. Summary of results of study results analyzing the sensitivity and specificity of SVANOVIR® PRV gB-Ab

Serum	**Sensitivity**	**Specificity**	**Reference method**
Naturally infected, vaccinated, non-vaccinated herds n= 1362[a]	100 %	99.6 %	VNT
Naturally infected, vaccinated, non-vaccinated herds n= 1000[b]	99.6 %	99.3 %	Danish blocking ELISA
Commercial pigs n= 912[c]	n.a.	100 %	Area free of disease

Samples originating from

[a] Sweden, Germany, Yugoslavia,

[b] Denmark,

[c] Sweden

SVANOVIR® PRV gB-Ab showed very good correlation to the reference methods, with a sensitivity higher than 99.6% and specificity higher than 99.3% in all studies.

5.2.2. Study 2

Objective

The objective of the study was to validate the SVANOVIR® PRV gB-Ab to an in-house ELISA of the National Reference Center for Aujeszky's Disease - IZSLER Brescia. The study was done by the National Reference Center for Aujeszky's Disease – IZSLER Brescia in Italy.

Material and Method

For the study 46 well-defined serum samples from the Aujeszky's Disease ring trial 2009 were used. These samples represented several possible combinations of vaccination and/or infection.

The samples were tested in the SVANOVIR® PRV gB-Ab according to the kit insert as well as with the in-house Brescia PRV-gB ELISA (reference method).

Results

Table 15. Agreement between SVANOVIR® PRV gB-Ab and reference method

Reference method*	SVANOVIR® PRV gB-Ab			Total
	Neg.	Doubtful	Pos.	
Neg.	8	0	0	8
Doubtful	0	0	0	0
Pos.	0	0	38	38
Total	8	0	38	46

*in-house ELISA Reference Lab Brescia, Italy

In the study a high level of agreement of the results of the reference method (in-house ELISA) and SVANOVIR® PRV gB-Ab was shown. 100 % agreement was obtained for all negative samples and 97 % for the positive samples.

5.2.3. Study 3

PRV-Prevalence study in wild boar/part 1

[Vengust et al., 2006]

Objective

The objective of the study was to determine the seroprevalence against selected infectious pathogens, among them PRV, in wild boars from Slovenia.

Material and Method

For the study sera of 178 shot wild boars (non-vaccinated) were collected during hunting season 2003/2004.

The blood was taken by the hunters and sent to the lab. To avoid inaccurate results, only samples were included in the study when major hemolysis and protein denaturation were absent. The PRV tests were performed according to SVANOVIR® PRV gB-Ab kit insert.

Results

Table 16. Prevalence of PRV in Slovenian wild boars, part 1, 2006

	Positive	**Negative**	**Prevalence in Slovenia**
SVANOVIR® PRV gB-Ab (n=178)	55	123	31 %

SVANOVIR® PRV gB-Ab was suitable to test wild boar serum samples. Analysis of 178 sera of wild boars showed that antibodies against Aujeszky's disease were present in 55 sera indicating a prevalence of around 31 % in the Slovenian wild boar population.

5.2.4. Study 4

PRV-Prevalence study in wild boar/part 2

[Stukelj, M. et al., 2014]

Objective

The objective of the study was to report prevalence of a multitude of infectious diseases in the wild boar population in Slovenia. Slovenia's domestic pig population is officially free of PRV since 2010.

Material and Method

For the study sera of 184 shot wild boars (non-vaccinated) were collected during hunting season 2010/2011.

Results

Table 17. Prevalence of PRV in Slovenian wild boars, part 2, 2014

	Positive	Negative	Prevalence in Slovenia
SVANOVIR® PRV gB-Ab (n=184)	83	101	41.5 %

SVANOVIR® PRV gB-Ab was successfully used to test wild boar serum samples. Analysis of 184 sera of wild boars showed that antibodies against Aujeszky's disease were present in 83 sera indicating a prevalence of 41.5 % in the Slovenian wild boar population in 2011.

5.2.5. Study 5

Ring test PRV gB

Objective

To evaluate the performance of SVANOVIR® PRV gB-Ab by participation in a ring test from the National Reference Laboratory for Aujeszky's disease in France.

Material and Method

Well defined serum samples from pigs (n=12) were included in the ring test. The sample pool consisted of negative (n=3) and positive samples (n=2), a sample of a multi-vaccinated animal (n=1) as well as of equivalents of EU reference sera in different dilutions (n=6).

The analysis was done according to the kit insert of SVANOVIR® PRV gB-Ab.

Result

SVANOVIR® PRV gB-Ab identified all 12 samples accurately.

5.2.6. Study 6

Benchmarking

Objective

To evaluate the performance of SVANOVIR® PRV gB-Ab compared to another widely used commercial assay.

Material and Method

24 serum samples (11 OIE reference samples and 13 negative samples originating from Sweden, a population historically free of PRV) were tested on SVANOVIR® PRV gB-Ab and results compared to the analysis with a widely used commercial gB-ELISA.

The samples were tested according to the respective kit inserts.

Result

Table 18. Performance of SVANOVIR® PRV gB-Ab and a widely used commercial assay

Sample description	Official OIE Status	SVANOVIR® PRV gE-Ab	Widely used commercial gE assay
Multi-vaccinated pigs n=6	gE pos.	Pos.	Pos.
Infected pig n=1	gE pos.	Pos.	Pos.
Vaccinated gE deleted vaccine & infected n=1	gE pos.	Pos.	Pos.
Vaccinated gE deleted vaccine & infected n=2	gE pos.	Pos.	Pos.
Vaccinated gE deleted vaccine & infected n=1	gE pos.	Pos.	Pos.
Swedish negative sera n=13	gE neg.	Neg.	Neg. (n=10) Doubtful (n=2) Pos. (n=1).

SVANOVIR® PRV gB-Ab showed equally good performance on well-defined samples from OIE as compared to a widely used commercial gB-assay. On the population with a history of freedom of infection, SVANOVIR® PRV gB-Ab showed higher specificity and revealed less-false positive or doubtful results than the widely used commercial gB-assay.

6. Conclusions

SVANOVIR® PRV gE-Ab

The SVANOVIR® PRV gE-Ab is a thoroughly validated blocking ELISA with excellent performance. In several studies with well-defined samples from Reference Laboratories, the assay has demonstrated repeatedly high specificity in samples from vaccinated pigs and high specificity and sensitivity in samples from field infected pigs. This is essential in eradication programmes targeting at stamping out field virus exposed vaccinated animals and in situations when it is important to differentiate between vaccinated and field infected animals. Test results from benchmarking studies, performed on well-defined samples from OIE has demonstrated that SVANOVIR® PRV-gE performs equal to another widely used commercial assay.

There is evidence from the field that the combination of SVANOVIR® PRV-gE and a gE-deleted vaccine is an effective approach in eradication programmes for Aujeszky's disease. An example is the experience from the German eradication programme with integrated application of Ingelvac®Aujeszky Modified Live Virus Vaccine, Boehringer Ingelheim, and SVANOVIR® PRVgE-Ab.

The SVANOVIR® PRV gE-Ab has been to date, an integrated part of control programmes in several European countries such as Scandinavian countries, Germany and Poland and is officially registered in Germany, Poland, Russia, Spain, and Czech Republic.

SVANOVIR® PRV gB-Ab

SVANOVIR® PRV gB-Ab is a well validated and extensively used tool for Aujeszky's disease eradication and control programmes. The assay, used in programmes in many countries, is usefully applied in non-vaccinated pig populations. Vaccinated as well as infected positive animals need to be distinguished from seronegative livestock. Mainly in later stages of eradication programmes during monitoring and surveillance stage, when eradication strategies already were successful, SVANOVIR® PRV gB-Ab is a useful and robust serological diagnostic tool.

In several studies including studies on well-defined samples from OIE Reference labs and populations with a history of freedom of PRV, SVANOVIR® PRV gB-Ab has repeatedly shown high specificity and sensitivity. Equal to better results were achieved in a benchmarking study on samples from a population historically free of PRV compared to a widely used commercial gB- assay.

SVANOVIR® PRV gB-Ab was an effective tool for revealing prevalence of PRV in wild boar samples. The assay can be used, on a representative samples size for surveillance of PRV in population with a history of freedom from infection, for import and export of pigs and during eradication programmes in domestic and feral pigs.

Studies on the precision of both assays show that the SVANOVIR® PRV gE-Ab and SVANOVIR® PRV gB-Ab are robust assays providing repeatedly accurate results. This is essential in systematic surveillance and eradication programmes and gives confidence in reporting to animal health professionals.

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