BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2024-6

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

Sciensano/PT-program PRRS/2024-6/E

Biological health risks
Quality of laboratories
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A draft version of this report was submitted to the experts on 18/11/2024.

The experts were invited to send their comments via e-mail.

Responsibilities:
The National Reference Laboratory (NRL) of Sciensano was consulted for advice about the content of the global report, the interpretation of the results and the evaluation criteria. The responsibility for the choice of the samples used was carried out by the NRL.

Authorization of the report: by Ynse Van de Maele, coordinator

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All the global reports are also available on our webpage:

- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
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- EN: https://www.sciensano.be/en/external-quality-assessment/animal-health-pt-vet

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1 INTRODUCTION

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

2 AIM

The aim of the PT was to evaluate the ability of the participating laboratories to detect the agent of Porcine Reproductive and Respiratory Syndrome (PRRS) (PRRS virus) in serum either by ELISA (Ab) and/or by Real Time PCR.

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Porcine Reproductive and Respiratory Syndrome (PRRS) on serum samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97515
- 97532
- 97621

3.1.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral reemerging enzootic and bee diseases' in the department of 'Infectious diseases in animals Directorate', prepared the liquid sera samples. Participants were instructed to store the samples at 4°C until the analysis was carried out.

Information about the origin and preparation of the samples:

- **PT2024PRRSSER_NS1, PT2024PRRSSER_NS2** and **PT2024PRRSSER_NS3** are serum samples from 3 domestic pigs from a PRRSV-free herd in Flanders.
- **PT2024PRRSSER_PS1** is a serum sample from a domestic pig from a PRRSV-free herd that was experimentally infected with the Flanders-13 strain (PRRSV-1). The sample was collected 23 days after infection.
- **PT2024PRRSSER_PS2** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Porcilis PRRS vaccine at 3 weeks of age. The sample was collected 17 days after vaccination.
- **PT2024PRRSSER_PS3** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Ingelvac PRRS MLV vaccine at 3 weeks of age. The sample was collected 17 days after vaccination.

- **PT2024PRRSSER_PS4** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Unistrain PRRS vaccine at 3 weeks of age. The sample was collected 21 days after vaccination.
- **PT2024PRRSSER_PS5** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated twice with the Ingelvac PRRSFLEX EU vaccine at 3 and 6 weeks of age. The animal was experimentally infected with the Flanders-13 strain (PRRSV-1) 3 weeks after the second vaccination. The sample was collected 23 days after infection.
- **PT2024PRRSSER_PS6** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Suvaxyn PRRS MLV vaccine at 3 weeks of age. The sample was collected 21 days after vaccination.
- **PT2024PRRSSER_PS7** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Unistrain PRRS vaccine at 3 weeks of age. The sample was collected 14 days after vaccination.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (100 µL each) of each sample, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2024PRRSSER_PS1	POS
PT2024PRRSSER_PS2	POS
PT2024PRRSSER_PS3	POS
PT2024PRRSSER_PS4	POS
PT2024PRRSSER_PS5	POS
PT2024PRRSSER_PS6	POS
PT2024PRRSSER_PS7	POS
PT2024PRRSSER_NS1	NEG
PT2024PRRSSER_NS2	NEG
PT2024PRRSSER_NS3	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The stability of the samples was confirmed by comparing the pre-PT results with those obtained by the NRL during and after the PT. The samples were deemed stable.

3.1.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2024 PRRSSER_	97505	97507	97508
PS1	PRRSSER-2	PRRSSER-5	PRRSSER-5
PS2	PRRSSER-1	PRRSSER-8	PRRSSER-6
PS3	PRRSSER-9	PRRSSER-9	PRRSSER-7
PS4	PRRSSER-10	PRRSSER-7	PRRSSER-3
PS5	PRRSSER-4	PRRSSER-2	PRRSSER-2
PS6	PRRSSER-6	PRRSSER-10	PRRSSER-4
PS7	PRRSSER-5	PRRSSER-6	PRRSSER-9
NS1	PRRSSER-8	PRRSSER-4	PRRSSER-1
NS2	PRRSSER-7	PRRSSER-1	PRRSSER-10
NS3	PRRSSER-3	PRRSSER-3	PRRSSER-8

Sample content: PT2024 PRRSSER_	97515	97532	97621
PS1	PRRSSER-7	PRRSSER-3	PRRSSER-2
PS2	PRRSSER-8	PRRSSER-8	PRRSSER-10
PS3	PRRSSER-3	PRRSSER-6	PRRSSER-3
PS4	PRRSSER-2	PRRSSER-2	PRRSSER-8
PS5	PRRSSER-5	PRRSSER-4	PRRSSER-7
PS6	PRRSSER-4	PRRSSER-9	PRRSSER-9
PS7	PRRSSER-9	PRRSSER-7	PRRSSER-4
NS1	PRRSSER-1	PRRSSER-10	PRRSSER-1
NS2	PRRSSER-10	PRRSSER-5	PRRSSER-6
NS3	PRRSSER-6	PRRSSER-1	PRRSSER-5

3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the ten reference samples is at least 90%.

3.2 Virology (serum)

3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Porcine Reproductive and Respiratory Syndrome (PRRS) on serum samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97508
- 97514
- 97515
- 97532
- 97621

3.2.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral reemerging enzootic and bee diseases' in the department of 'Infectious diseases in animals Directorate', prepared the liquid sera samples. Participants were instructed to store the samples at 4°C until the analysis was carried out.

Information about the origin and preparation of the samples:
All samples were diluted 1:3 (v/v) in PrimeStore Molecular Transport Medium to inactivate and stabilize the samples.

- **PT2024PRRSVIR_NS1** and **PT2024PRRSVIR_NS2** are serum samples from 2 domestic pigs from a PRRSV-free herd in Flanders.
- **PT2024PRRSVIR_PS1** and **PT2024PRRSVIR_PS2** are serum samples from 2 domestic pigs from a PRRSV-free herd that were experimentally infected with the Flanders-13 strain (PRRSV-1). Both samples were collected 17 days after infection.
- **PT2024PRRSVIR_PS3** is a 10^{-2} dilution of a virus culture of the Flanders-13 strain (PRRSV-1) and has an estimated final concentration of 50000 $TCID_{50}$/ml.
- **PT2024PRRSVIR_PS4** is a 10^{-3} dilution of a virus culture of the Flanders-13 strain (PRRSV-1) and has an estimated final concentration of 5000 $TCID_{50}$/ml.
- **PT2024PRRSVIR_PS5** is a 10^{-4} dilution of a virus culture of the Flanders-13 strain (PRRSV-1) and has an estimated final concentration of 500 $TCID_{50}$/ml.
- **PT2024PRRSVIR_PS6** is a 10^{-5} dilution of a virus culture of the Flanders-13 strain (PRRSV-1) and has an estimated final concentration of 50 $TCID_{50}$/ml.
- **PT2024PRRSVIR_PS7** is a serum sample from a non-responder piglet that was vaccinated once with the Ingelvac PRRSFLEX EU vaccine (PRRSV-1) at 3 weeks of age and experimentally infected with the Flanders-13 strain (PRRSV-1) 6 weeks after vaccination. The sample was collected 42 days after infection.
- **PT2024PRRSVIR_PS8** is a serum sample from a domestic pig from a PRRSV-free herd that was vaccinated once with the Ingelvac PRRS MLV vaccine (PRRSV-2) at 3 weeks of age. The sample was collected 21 days after vaccination.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (100 µL each) of each sample, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2024PRRSVIR_PS1	POS EU / NEG NA
PT2024PRRSVIR_PS2	POS EU / NEG NA
PT2024PRRSVIR_PS3	POS EU / NEG NA
PT2024PRRSVIR_PS4	POS EU / NEG NA
PT2024PRRSVIR_PS5	POS EU / NEG NA
PT2024PRRSVIR_PS6	POS EU / NEG NA
PT2024PRRSVIR_PS7	POS EU / NEG NA
PT2024PRRSVIR_PS8	NEG EU / POS NA
PT2024PRRSVIR_NS1	NEG EU / NEG NA
PT2024PRRSVIR_NS2	NEG EU / NEG NA

(POS = positive; NEG = negative)

3.2.5 STABILITY

The stability of the samples was confirmed by comparing the pre-PT results with those obtained by the NRL during and after the PT. The samples were deemed stable.

3.2.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content: PT2024 PRRSVIR_	97505	97508	97514
PS1	PRRSVIR-9	PRRSVIR-5	PRRSVIR-10
PS2	PRRSVIR-2	PRRSVIR-4	PRRSVIR-2
PS3	PRRSVIR-10	PRRSVIR-2	PRRSVIR-1
PS4	PRRSVIR-3	PRRSVIR-3	PRRSVIR-4
PS5	PRRSVIR-5	PRRSVIR-9	PRRSVIR-8
PS6	PRRSVIR-1	PRRSVIR-8	PRRSVIR-9
PS7	PRRSVIR-6	PRRSVIR-7	PRRSVIR-6

Sample content: PT2024 PRRSVIR_	97505	97508	97514
PS8	PRRSVIR-7	PRRSVIR-1	PRRSVIR-5
NS1	PRRSVIR-8	PRRSVIR-6	PRRSVIR-3
NS2	PRRSVIR-4	PRRSVIR-10	PRRSVIR-7

Sample content: PT2024 PRRSVIR_	97515	97532	97621
PS1	PRRSVIR-4	PRRSVIR-5	PRRSVIR-8
PS2	PRRSVIR-10	PRRSVIR-2	PRRSVIR-6
PS3	PRRSVIR-6	PRRSVIR-10	PRRSVIR-4
PS4	PRRSVIR-5	PRRSVIR-3	PRRSVIR-9
PS5	PRRSVIR-2	PRRSVIR-1	PRRSVIR-3
PS6	PRRSVIR-9	PRRSVIR-7	PRRSVIR-5
PS7	PRRSVIR-3	PRRSVIR-6	PRRSVIR-2
PS8	PRRSVIR-1	PRRSVIR-9	PRRSVIR-7
NS1	PRRSVIR-7	PRRSVIR-4	PRRSVIR-1
NS2	PRRSVIR-8	PRRSVIR-8	PRRSVIR-10

3.2.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the ten reference samples is at least 90%.

4 TIMELINE

Transfer of the samples from NRL to QL: 09/09/2024
Randomisation of the samples by QL: Sero (09/09/2024) & Viro (13/09/2024)
Sending of samples to participants: 16/09/2024
Deadline for submitting the results: 11/10/2024
Individual report to the participants: 06/11/2024

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of ten different samples. No repetitions were included in the panel. One lab had chosen to test two different methods on the same samples, implying that there were two datasets submitted. These additional results are included in the tables below.

Sample content	Expected results	Total results	Observed results
PS1	POS	7	7 POS
PS2	POS	7	7 POS
PS3	POS	7	7 POS
PS4	POS	7	6 POS 1 NEG
PS5	POS	7	7 POS
PS6	POS	7	7 POS
PS7	POS	7	7 POS
NS1	NEG	7	7 NEG
NS2	NEG	7	7 NEG
NS3	NEG	7	7 NEG

(POS = positive; NEG = negative)

5.1.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name protocol/kit	N	NR	NCR	%
ELISA Indirect	IDEXX	IDEXX PRRS X3 Ab	**4**	**40**	**40**	**100**
ELISA Indirect	Indical (Qiagen)	Pigtype PRRSV Ab	**2**	**20**	**20**	**100**
ELISA Indirect	IDVet	ID Screen PRRS Indirect	**1**	**10**	**9**	**90**
TOTAL			**7**	**70**	**69**	**99**

(N= number of datasets; NR = number of results; NCR = number of correct results).

5.1.3 CONCLUSION

In 2024, six laboratories participated in the proficiency test Porcine Reproductive and Respiratory Syndrome serology (serum) organised by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by the laboratory are in agreement with the status of the reference samples assigned by the NRL of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Five out of six laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples. The remaining laboratory misclassified one aliquot, resulting in a level of agreement of 90%, which is still a satisfactory result.

5.2 Virology (serum)

5.2.1 RESULTS PER SAMPLE

The panel consisted of ten different samples. No repetitions were included in the panel.

The datasets of the laboratories submitting multiple datasets were taken together to get a final result for this part.

Sample content	Expected results	Total results	Observed results
PS1	POS EU / NEG NA	6	6 POS EU / NEG NA
PS2	POS EU / NEG NA	6	6 POS EU / NEG NA
PS3	POS EU / NEG NA	6	6 POS EU / NEG NA
PS4	POS EU / NEG NA	6	6 POS EU / NEG NA
PS5	POS EU / NEG NA	6	6 POS EU / NEG NA
PS6	POS EU / NEG NA	6	6 POS EU / NEG NA
PS7	POS EU / NEG NA	6	6 POS EU / NEG NA
PS8	NEG EU / POS NA	6	6 NEG EU / POS NA
NS1	NEG EU / NEG NA	6	6 NEG EU / NEG NA
NS2	NEG EU / NEG NA	6	6 NEG EU / NEG NA

(POS = positive; NEG = negative; EU = European genotype; NA = North American genotype)

5.2.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

Below, the table displays the results for each used extraction protocol/kit method.

Name producer	Name protocol/kit	N	NR	NCR	%
Indical Bioscience GmbH	IndiMag Pathogen Kit	**4**	**40**	**40**	**100**
Biosellal	BioExtract® Column	**1**	**10**	**10**	**100**
Applied Biosystems	MagMAX CORE Nucleic acid purification kit	**1**	**10**	**10**	**100**
TOTAL		**6**	**60**	**60**	**100**

(N= number of datasets; NR = number of results; NCR = number of correct results).

5.2.3 RESULTS PER USED PCR PROTOCOL/KIT

Below, the table displays the results for each used PCR protocol/kit method.

Name producer	Name protocol/kit	N	NR	NCR	%
Thermofisher	VetMAX PRRSV EU & NA 2.0 Kit	**2**	**20**	**20**	**100**
Thermofisher	VetMAX PRRSV EU & NA 3.0 Kit	**1**	**10**	**10**	**100**
Biosellal	Bio-T kit® PRRSV (Cat. N° BIOTK040)	**1**	**10**	**10**	**100**
Indical Bioscience GmbH	Virotype PRRSV 2.0 RT-PCR Kit (100 reactions)	**1**	**10**	**10**	**100**
/	Combination of methods: • RT-qPCR (Wernike *et al.* 2012) • Conventional RT-PCR (Donadeu *et al.* 1999) • Sequencing Sanger	**1**	**10**	**10**	**100**
TOTAL		**6**	**60**	**60**	**100**

(N= number of datasets; NR = number of results; NCR = number of correct results).

5.2.4 CONCLUSION

In 2024, six laboratories participated in the proficiency test Porcine Reproductive and Respiratory Syndrome serology (serum) organised by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by the laboratory are in agreement with the status of the reference samples assigned by the NRL of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

6 ANNEXES (NOT UNDER ACCREDITATION)

This quantitative data is not under BELAC-accreditation and is solely for the information of the laboratories.

6.1 Annex : Quantitative results

Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel. As there was no repetition of positive samples in either panel, no boxplots could be produced.

6.2 Annex: Additional information

The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2024
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2024
- EN: https://www.sciensano.be/nl/biblio/eqa-calendar-2024

END