



### BIOLOGICAL HEALTH RISKS QUALITY OF LABORATORIES

PROFICIENCY TEST IN VETERINARY DIAGNOSIS

## DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2024-1

INFECTIOUS BOVINE RHINOTRACHEITIS (IBR)

Sciensano/IBR/2024-1/E

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A draft version of this report was submitted to the experts on 23/07/2024.

#### **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.

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Date of publication: 13/11/2024

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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 Infectious Bovine Rhinotracheitis (IBR) serology was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies against IBR viruses in both serum (gB/gE) and milk (gB/gE) of ruminants, using ELISA.

## **3 MATERIALS AND METHODS**

## 3.1 Serology (serum gB)

#### 3.1.1 THE PARTICIPANTS

Seven laboratories participated in the proficiency test of Infectious Bovine Rhinotracheitis serology on serum gB. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509
- 97510
- 97516
- 97540

#### 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 gB samples. Participants were instructed to store the samples at 4°C until the analysis was carried out.

The samples originated from field collections or experimentation. Serum was harvested from collected blood before aliquotation and freezing. This detailed procedure ensures consistent preparation and handling of the samples, maintaining their integrity for accurate analysis.

#### 3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (250  $\mu$ L 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
PT2024IBRSERgB_PS1	POS/NEG/NI*
PT2024IBRSERgB_PS2	POS
PT2024IBRSERgB_PS3	POS
PT2024IBRSERgB_PS4	POS
PT2024IBRSERgB_NS1	NEG
PT2024IBRSERgB_NS2	NEG

(POS = positive; NEG = negative; NI = not interpretable)

\* = The positive sample PS1 represents a positive sample diluted to the limit of detection, implying that the result can be doubtful. Therefore, for this sample, POS. NEG or NI are accepted as correct results.

#### 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 ID: PT2024 IBRSERgB_	97505	97507	97508	97509
PS1 (1)	IBRSERgB24-4	IBRSERgB24-4	IBRSERgB24-1	IBRSERgB24-4
PS1 (2)	IBRSERgB24-5	IBRSERgB24-8	IBRSERgB24-9	IBRSERgB24-5
PS2 (1)	IBRSERgB24-1	IBRSERgB24-1	IBRSERgB24-2	IBRSERgB24-1
PS2 (2)	IBRSERgB24-2	IBRSERgB24-2	IBRSERgB24-10	IBRSERgB24-2
PS3	IBRSERgB24-8	IBRSERgB24-3	IBRSERgB24-5	IBRSERgB24-8
PS4 (1)	IBRSERgB24-3	IBRSERgB24-5	IBRSERgB24-3	IBRSERgB24-3
PS4 (2)	IBRSERgB24-6	IBRSERgB24-7	IBRSERgB24-6	IBRSERgB24-6
NS1	IBRSERgB24-10	IBRSERgB24-10	IBRSERgB24-8	IBRSERgB24-10
NS2 (1)	IBRSERgB24-7	IBRSERgB24-6	IBRSERgB24-4	IBRSERgB24-7
NS2 (2)	IBRSERgB24-9	IBRSERgB24-9	IBRSERgB24-7	IBRSERgB24-9

Sample ID: PT2024 IBRgBSER	97510	97516	97540
PS1 (1)	IBRSERgB24-4	IBRSERgB24-7	IBRSERgB24-1
PS1 (2)	IBRSERgB24-5	IBRSERgB24-8	IBRSERgB24-2
PS2 (1)	IBRSERgB24-3	IBRSERgB24-1	IBRSERgB24-4
PS2 (2)	IBRSERgB24-7	IBRSERgB24-5	IBRSERgB24-5
PS3	IBRSERgB24-8	IBRSERgB24-10	IBRSERgB24-7
PS4 (1)	IBRSERgB24-1	IBRSERgB24-3	IBRSERgB24-3
PS4 (2)	IBRSERgB24-10	IBRSERgB24-9	IBRSERgB24-6
NS1	IBRSERgB24-9	IBRSERgB24-4	IBRSERgB24-9
NS2 (1)	IBRSERgB24-2	IBRSERgB24-2	IBRSERgB24-8
NS2 (2)	IBRSERgB24-6	IBRSERgB24-6	IBRSERgB24-10

# 3.2 Serology (serum gE)

### 3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Infectious Bovine Rhinotracheitis serology on serum gE. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97509
- 97516
- 97540

#### 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 gE samples. Participants were instructed to store the samples at 4°C until the analysis was carried out.

The samples originated from field collections or experimentation. Serum was harvested from collected blood before aliquotation and freezing. This detailed procedure ensures consistent preparation and handling of the samples, maintaining their integrity for accurate analysis.

#### 3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (250  $\mu$ L 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
PT2024IBRSERgE_PS1	POS
PT2024IBRSERgE_PS2	POS
PT2024IBRSERgE_PS3	POS
PT2024IBRSERgE_NS1	NEG
PT2024IBRSERgE_NS2	NEG
PT2024IBRSERgE_NS3	NEG

(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 ID: PT2024 IBRSERgE_	97505	97507	97508	97509
PS1 (1)	IBRSERgE24-3	IBRSERgE24-1	IBRSERgE24-5	IBRSERgE24-3
PS1 (2)	IBRSERgE24-6	IBRSERgE24-5	IBRSERgE24-6	IBRSERgE24-6
PS1 (3)	IBRSERgE24-10	IBRSERgE24-8	IBRSERgE24-9	IBRSERgE24-10
PS2	IBRSERgE24-9	IBRSERgE24-9	IBRSERgE24-7	IBRSERgE24-9
PS3 (1)	IBRSERgE24-1	IBRSERgE24-3	IBRSERgE24-1	IBRSERgE24-1
PS3 (2)	IBRSERgE24-2	IBRSERgE24-4	IBRSERgE24-8	IBRSERgE24-2
NS1 (1)	IBRSERgE24-5	IBRSERgE24-6	IBRSERgE24-2	IBRSERgE24-5
NS1 (2)	IBRSERgE24-7	IBRSERgE24-10	IBRSERgE24-4	IBRSERgE24-7
NS2	IBRSERgE24-8	IBRSERgE24-7	IBRSERgE24-3	IBRSERgE24-8
NS3	IBRSERgE24-4	IBRSERgE24-2	IBRSERgE24-10	IBRSERgE24-4

Sample ID: PT2024 IBRSERgE_	97516	97540
PS1 (1)	IBRSERgE24-2	IBRSERgE24-1
PS1 (2)	IBRSERgE24-6	IBRSERgE24-6
PS1 (3)	IBRSERgE24-10	IBRSERgE24-10
PS2	IBRSERgE24-1	IBRSERgE24-3
PS3 (1)	IBRSERgE24-7	IBRSERgE24-8
PS3 (2)	IBRSERgE24-9	IBRSERgE24-9
NS1 (1)	IBRSERgE24-3	IBRSERgE24-4
NS1 (2)	IBRSERgE24-4	IBRSERgE24-5
NS2	IBRSERgE24-5	IBRSERgE24-7
NS3	IBRSERgE24-8	IBRSERgE24-2

## 3.3 Serology (milk gB)

The Milk gB section was reorganised for all participating laboratories. This means that newly randomised samples were sent to all participants. This was done due to a mix-up of samples during their preparation or randomisation. Therefore, the initial results are not discussed in this global report.

#### 3.3.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Infectious Bovine Rhinotracheitis serology on milk gB. The laboratory numbers of the participating laboratories are:

- 97505
- 97509
- 97511
- 97512

#### 3.3.2 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 lyophilized milk gB samples. Participants were instructed to reconstitute the milk by adding 1 ml of demineralized water at approximately 30°C. They were then asked to incubate the sample at room temperature, followed by placing the vial on a stirrer for 3 to 4 hours to ensure complete rehydration. After this, participants were asked to homogenize the sample by vortexing or pipetting. The storage conditions for the samples were defined as  $20^{\circ}C \pm 5^{\circ}C$ . Reconstituted milk was to be stored at  $4^{\circ}C \pm 3^{\circ}C$  and used within seven days.

The milk samples were harvested from bulk milk tanks in Belgium. The fat was skimmed before aliquotation and lyophilisation. This detailed procedure ensures consistent preparation and handling of the samples maintaining their integrity for accurate analysis.

#### 3.3.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (1 ml each) of each sample, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

#### 3.3.4 TARGET VALUES

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

Sample content	Expected result
PT2024IBRMILgB_PM1	POS
PT2024IBRMILgB_PM2	POS
PT2024IBRMILgB_PM3	POS
PT2024IBRMILgB_PM4	POS
PT2024IBRMILgB_PM5	POS
PT2024IBRMILgB_NM1	NEG
PT2024IBRMILgB_NM2	NEG
PT2024IBRMILgB_NM3	NEG
PT2024IBRMILgB_NM4	NEG
PT2024IBRMILgB_NM5	NEG

(POS = positive; NEG = negative)

#### 3.3.5 STABILITY

The stability was determined by comparison of the pre-proficiency test results with the results obtained by the NRL during and after the proficiency test. The samples were considered stable.

#### 3.3.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: PT2024 IBRMILgB_	97505	97509	97511	97512
PM1	IBRMILgB-3	IBRMILgB-10	IBRMILgB-6	IBRMILgB-2
PM2	IBRMILgB-8	IBRMILgB-6	IBRMILgB-3	IBRMILgB-9
PM3	IBRMILgB-4	IBRMILgB-5	IBRMILgB-1	IBRMILgB-4
PM4	IBRMILgB-7	IBRMILgB-3	IBRMILgB-2	IBRMILgB-7
PM5	IBRMILgB-1	IBRMILgB-2	IBRMILgB-10	IBRMILgB-5
NM1	IBRMILgB-10	IBRMILgB-1	IBRMILgB-7	IBRMILgB-1
NM2	IBRMILgB-6	IBRMILgB-7	IBRMILgB-8	IBRMILgB-3
NM3	IBRMILgB-2	IBRMILgB-9	IBRMILgB-5	IBRMILgB-6
NM4	IBRMILgB-5	IBRMILgB-8	IBRMILgB-4	IBRMILgB-10
NM5	IBRMILgB-9	IBRMILgB-4	IBRMILgB-9	IBRMILgB-8

# 3.4 Serology (milk gE)

### 3.4.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Infectious Bovine Rhinotracheitis serology on milk gE. The laboratory numbers of the participating laboratories are:

- 97505
- 97509
- 97511
- 97512

#### 3.4.2 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 lyophilized milk gE samples. Participants were instructed to reconstitute the milk by adding 1 ml of demineralized water at approximately 30°C. They were then asked to incubate the sample at room temperature, followed by placing the vial on a stirrer for 3 to 4 hours to ensure complete rehydration. After this, participants were asked to homogenize the sample by vortexing or pipetting. The storage conditions for the samples were defined as  $20^{\circ}C \pm 5^{\circ}C$ . Reconstituted milk was to be stored at  $4^{\circ}C \pm 3^{\circ}C$  and used within seven days.

The milk samples were harvested from bulk milk tanks in Belgium. The fat was skimmed before aliquotation and lyophilisation. This detailed procedure ensures consistent preparation and handling of the samples maintaining their integrity for accurate analysis.

#### 3.4.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (1 ml each) of each sample, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

#### 3.4.4 TARGET VALUES

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

Sample content	Expected result
PT2024IBRMILgE_PM1	POS
PT2024IBRMILgE_PM2	POS
PT2024IBRMILgE_PM3	POS
PT2024IBRMILgE_PM4	POS
PT2024IBRMILgE_PM5	POS
PT2024IBRMILgE_NM1	NEG
PT2024IBRMILgE_NM2	NEG
PT2024IBRMILgE_NM3	NEG
PT2024IBRMILgE_NM4	NEG
PT2024IBRMILgE_NM5	NEG

(POS = positive; NEG = negative)

#### 3.4.5 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.

#### 3.4.6 RANDOMISATION AND PANEL COMPOSITION

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

Sample ID: PT2024 IBRMILgE_	97505	97509	97511	97512
PM1	IBRMILgE24-3	IBRMILgE24-3	IBRMILgE24-7	IBRMILgE24-7
PM2	IBRMILgE24-7	IBRMILgE24-7	IBRMILgE24-2	IBRMILgE24-2
PM3	IBRMILgE24-6	IBRMILgE24-6	IBRMILgE24-10	IBRMILgE24-10
PM4	IBRMILgE24-2	IBRMILgE24-2	IBRMILgE24-3	IBRMILgE24-3
PM5	IBRMILgE24-10	IBRMILgE24-10	IBRMILgE24-6	IBRMILgE24-6
NM1	IBRMILgE24-1	IBRMILgE24-1	IBRMILgE24-9	IBRMILgE24-9
NM2	IBRMILgE24-4	IBRMILgE24-4	IBRMILgE24-1	IBRMILgE24-1
NM3	IBRMILgE24-8	IBRMILgE24-8	IBRMILgE24-4	IBRMILgE24-4
NM4	IBRMILgE24-9	IBRMILgE24-9	IBRMILgE24-5	IBRMILgE24-5
NM5	IBRMILgE24-5	IBRMILgE24-5	IBRMILgE24-8	IBRMILgE24-8

## **4 TIMELINE**

Transfer of the samples from NRL to QL: 28/03/2024 Randomisation of the samples by QL: 02/04/2024 Sending samples to participants: in the week of 08/04/2024 Deadline for submitting the results: 03/05/2024 Individual report to the participants (serum gB, serum gE, milk gE): 12/06/2024

#### Extra PT milk gB

Transfer of the samples from NRL to QL: 06/09/2024 Randomisation of the samples by QL: 06/09/2024 Sending samples to participants: in the week of 11/09/2024 Deadline for submitting the results: 04/10/2024 Individual report to the participants (milk gB): 23/10/2024

## 5.1 Serology (serum gB)

## 5.1.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, positive samples PS1, PS2 and PS4 were replicated twice. Additionally, negative sample NS2 was repeated twice. Therefore, the panel included ten samples in total.

Sample ID	Expected results	Total results	Observed results	
PS1	POS/NEG/NI	14	14 POS	
PS2	POS	14	14 POS	
PS3	POS	7	7 POS	
PS4	POS	14	14 POS	
NS1	NEG	7	7 NEG	
NS2	NEG	14	14 NEG	

(POS = positive; NEG = negative; NI = not interpretable)

### 5.1.2 RESULTS PER METHOD

Below, the table displays the results for each method. For this case, all participants utilised the IDEXX ELISA competition method, specifically the IBR gB X3 Ab Test.

Method	Name producer	Name kit	Ν	NR	NCR	%
ELISA competition	IDEXX	IBR gB X3 Ab	7	70	70	100
	7	70	70	100		

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

## 5.1.3 CONCLUSION

In 2024, seven laboratories participated in proficiency test of Infectious Bovine Rhinotracheitis (serology - serum gB) organized 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 this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories used the ELISA-kit IBR gB X3 Ab form IDEXX and succeeded in achieving the maximum score (100%) for this test.

# 5.2 Serology (serum gE)

### 5.2.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, positive sample PS1 was replicated three times and positive sample PS2 was duplicated. Additionally, negative sample NS1 was also duplicated. Consequently, the panel included a total of ten samples.

Three labs 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 ID	Expected results	Total results	Observed results	
PS1	POS	27	27 POS	
PS2	POS 9		9 POS	
PS3	POS	18	18 POS	
NS1	NEG	18	18 NEG	
NS2	NEG	9	9 NEG	
NS3	NEG 9		9 NEG	

(POS = positive; NEG = negative)

#### 5.2.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	Ν	NR	NCR	%
ELISA Competition	IDEXX	Bovine Rhinotracheitis Virus (IBR/BHV-1) gE Antibody Test Kit	4	40	40	100
ELISA Competition	ID.VET	ID SCREEN® IBR gE test kit	3	30	30	100
ELISA Indirect	IDEXX	Other*	2	20	20	100
TOTAL				90	90	100

(N= number of datasets; NR = number of results; NCR = number of correct results; \* = name of kit was not specified).

### 5.2.3 CONCLUSION

In 2024, six laboratories participated in proficiency test of Infectious Bovine Rhinotracheitis (serology - serum gE) organized 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 this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

## 5.3 Serology (milk gB)

## 5.3.1 RESULTS PER SAMPLE

The panel consisted of 10 different samples. No repetitions were included.

Sample ID	Expected results	Number of repetitions (total results)	Observed result
PM1	POS	1 (4)	4 POS
PM2	POS	1 (4)	3 POS 1 NEG
PM3	POS	1 (4)	3 POS 1 <mark>NEG</mark>
PM4	POS	1 (4)	3 POS 1 <mark>NEG</mark>
PM5	POS	1 (4)	4 POS
NM1	NEG	1 (4)	4 NEG
NM2	NEG	1 (4)	3 NEG 1 POS
NM3	NEG	1 (4)	4 NEG
NM4	NEG	1 (4)	3 NEG 1 POS
NM5	NEG	1 (4)	3 NEG 1 POS

(POS = positive; NEG = negative)

### 5.3.2 RESULTS PER METHOD

Below, the table displays the results for each method. For this case, all participants utilised the INDICAL ELISA Indirect method. specifically the Cattletype BHV1 gB AB.

Method	ethod Name producer Name kit		Ν	NR	NCR	%
ELISA Indirect	INDICAL	Cattletype BHV1 gB AB	4	40	34	85
	4	40	34	85		

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

### 5.3.3 CONCLUSION

In 2024, four laboratories participated in proficiency test of Infectious Bovine Rhinotracheitis (serology - milk gB) organized 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 this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories used the ELISA-kit Cattletype BHV1 gB AB form INDICAL. Three laboratories achieved the maximum score of 100% for this test. One laboratory achieved a score of 40%, which is below the minimum requirement of 90%. This unsatisfactory result is due to a data entry issue where the results were not submitted with the corresponding sample, leading to a reversal of results. To conclude, an overall score of 85% was achieved.

## 5.4 Serology (milk gE)

### 5.4.1 RESULTS PER SAMPLE

The panel consisted of 10 different samples. No repetitions were included.

Sample ID	Expected results	Total results	Observed results	
PM1	POS	4	4 POS	
PM2	POS	4	4 POS	
PM3	POS	4	3 POS 1 NI	
PM4	POS	4	3 POS 1 NEG	
PM5	POS	4	3 POS 1 NI	
NM1	NEG	4	4 NEG	
NM2	NEG	4	4 NEG	
NM3	NEG	4	4 NEG	
NM4	NEG	4	4 NEG	
NM5	NEG	4	4 NEG	

(POS = positive; NEG = negative; NI = not interpretable)

### 5.4.2 RESULTS PER METHOD

Below, the table displays the results for each method. For this case, all participants utilised the IN3 Diagnostic ELISA indirect method, specifically the Eradikit<sup>™</sup> BoHV1-gE Bulk Milk Surveillance kit.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Indirect	IN3 Diagnostic	Eradikit™ BoHV1-gE Bulk Milk Surveillance kit	4	40	37	93
	TOTAL			40	37	93

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

## 5.4.3 CONCLUSION

In 2024, four laboratories participated in proficiency test of Infectious Bovine Rhinotracheitis (serology - milk gE) organized 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 this laboratory is in agreement with the status of the reference serum samples assigned by the reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories used the ELISA-kit Eradikit<sup>™</sup> BoHV1-gE Bulk Milk Surveillance kit form IN3 Diagnostic. Three laboratories achieved the maximum score of 100% for this test. One laboratory achieved a score of 70%, which is below the minimum score of 90%, giving this laboratory an unsatisfactory result. An overall score of 93% was achieved.

## 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 1: Quantitative results

Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel.

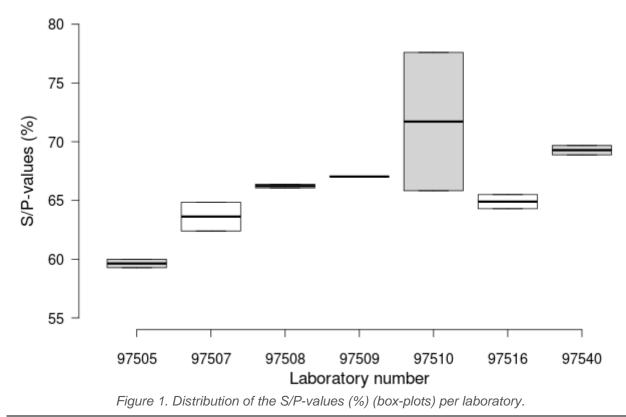
The boxplots, shown down below, were created by using the following software programme: <u>shiny.chemgrid.org/boxplotr/</u>.

#### 6.1.1 SEROLOGY (SERUM GB)

Sample PT2024IBRgBSER-PS1

Lab number	97505	97507	97508	97509	97510	97516	97540		
Method									
(ELISA			IDEX	X - IBR gB	X3 Ab				
protocol/kit)									
Cut-off	45	45	45	45	45	45	45		
	55	55	55	55	55	55	55		
S/P % (1)	59.99	62.40	66.07	67.02	77.60	64.30	68.88		
S/P % (2)	59.27	64.85	66.39	67.04	65.83	65.50	69.68		
Mean	59.63	63.63	66.23	67.03	71.72	64.90	69.28		
SD	0.51	1.73	0.23	0.01	8.32	0.85	0.09		
CV (%)	0.85	2.72	0.34	0.02	11.61	1.31	0.13		

Numbers were rounded to two significant decimal place. [S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation].



Lab number	97505	97507	97508	97509	97510	97516	97540
Method		•	•		•	•	
(ELISA			IDEX	X - IBR gB	X3 Ab		
protocol/kit)				5			
Cut-off	45	45	45	45	45	45	45
	55	55	55	55	55	55	55
S/P % (1)	91.81	92.56	97.31	93.49	96.32	96.10	103.00
S/P % (2)	92.33	93.58	97.31	93.57	96.21	93.40	102.87
Mean	92.07	93.07	97.31	93.53	96.27	94.75	102.94
SD	0.37	0.72	0.00	0.06	0.08	1.91	0.09
CV (%)	0.40	0.77	0.00	0.07	0.08	2.01	0.09

Numbers were rounded to two significant decimal place. [S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation].

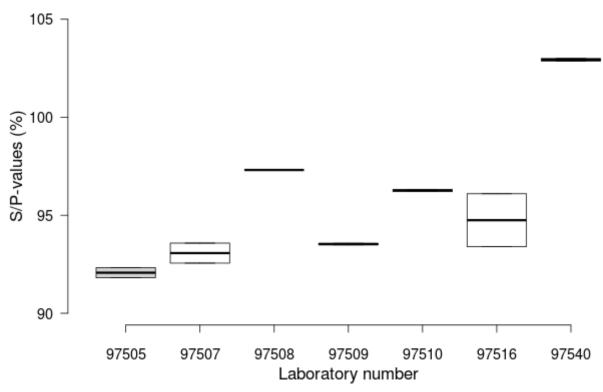


Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97505	97507	97508	97509	97510	97516	97540
Method							
(ELISA			IDEX	X - IBR gB	X3 Ab		
protocol/kit)				5			
Cut-off	45	45	45	45	45	45	45
	55	55	55	55	55	55	55
S/P % (1)	71.78	69.84	79.19	78.07	83.49	81.80	80.49
S/P % (2)	70.20	73.64	77.66	75.90	86.44	84.30	79.87
Mean	70.99	71.74	78.43	76.99	84.97	83.05	80.18
SD	1.12	2.69	1.09	1.54	2.09	1.77	0.44
CV (%)	1.57	3.75	1.38	2.00	2.46	2.13	0.55

Numbers were rounded to two significant decimal place. [S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation].

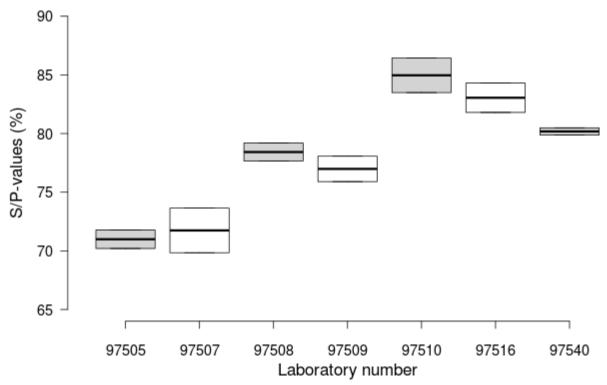


Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.

### 6.1.2 SEROLOGY (SERUM GE)

CV (%)

5.75

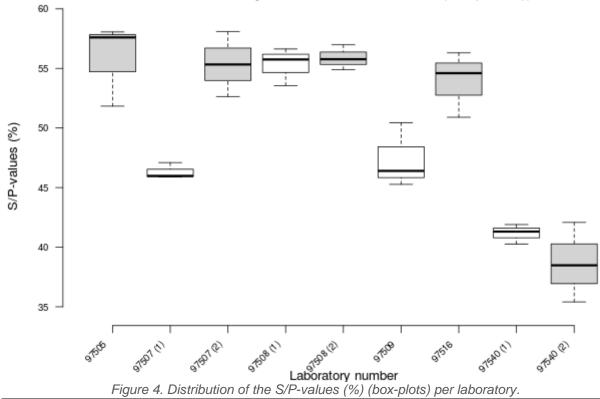
Lab number	97505	97507 (1)	97507 (2)	97508 (1)	97508 (2)
Method (ELISA protocol/kit)	$M_1$	M <sub>1</sub>	$M_2$	$M_1$	$M_2$
Cut-off	30 40	30 40	40 40	30 40	30 40
S/P % (1)	51.83	45.88	58.08	56.63	56.98
S/P % (2)	58.06	45.98	55.33	55.75	54.89
S/P % (3)	57.60	47.10	52.64	53.56	55.77
Mean	55.83	46.32	55.35	55.31	55.88
SD	3.47	0.68	2.72	1.58	1.05
CV (%)	6.22	1.46	4.91	2.86	1.88
Lab number	97509	97516	97540 (1)	97540 (2)	
Lab number Method (ELISA protocol/kit)	<b>97509</b> М <sub>3</sub>	97516 M <sub>3</sub>	97540 (1) M <sub>2</sub>	97540 (2) M <sub>1</sub>	
Method (ELISA	M <sub>3</sub> 30	M <sub>3</sub> 30	M <sub>2</sub> 60	M <sub>1</sub> 60	
Method (ELISA protocol/kit) Cut-off	M <sub>3</sub> 30 40	M <sub>3</sub> 30 40	M <sub>2</sub> 60 60	M <sub>1</sub> 60 70	
Method (ELISA protocol/kit) Cut-off S/P % (1)	M <sub>3</sub> 30	M <sub>3</sub> 30	M <sub>2</sub> 60	M <sub>1</sub> 60	
Method (ELISA protocol/kit) Cut-off	M <sub>3</sub> 30 40	M <sub>3</sub> 30 40	M <sub>2</sub> 60 60	M <sub>1</sub> 60 70	
Method (ELISA protocol/kit) Cut-off S/P % (1)	M <sub>3</sub> 30 40 46.40	M <sub>3</sub> 30 40 50.90	M <sub>2</sub> 60 60 40.27	M <sub>1</sub> 60 70 35.41	
Method (ELISA protocol/kit) Cut-off S/P % (1) S/P % (2)	M <sub>3</sub> 30 40 46.40 45.27	M <sub>3</sub> 30 40 50.90 56.30	M <sub>2</sub> 60 60 40.27 41.90	M <sub>1</sub> 60 70 35.41 42.08	
Method (ELISA protocol/kit) Cut-off S/P % (1) S/P % (2) S/P % (3)	M <sub>3</sub> 30 40 46.40 45.27 50.45	M <sub>3</sub> 30 40 50.90 56.30 54.60	M <sub>2</sub> 60 60 40.27 41.90 41.31	M <sub>1</sub> 60 70 35.41 42.08 38.48	

Numbers were rounded to two significant decimal place. [S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation;  $M_1 = IDEXX$  - Bovine Rhinotracheitis Virus (IBR/BHV-1) gE Antibody Test Kit;  $M_2 = ID.VET$  - ID SCREEN® IBR gE test kit;  $M_3 = IDEXX - Other$  (not specified)].

5.12

2.01

8.64



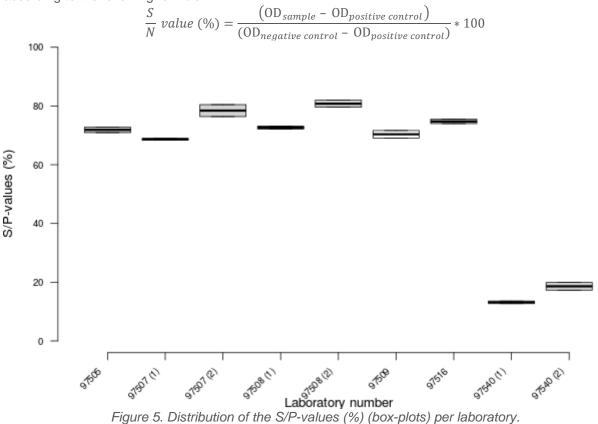
#### Sample PT2024IBRgESER-PS3

Lab number	97505	97507 (1)	97507 (2)	97508 (1)	97508 (2)
Method (ELISA	M <sub>1</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>1</sub>	M <sub>2</sub>
protocol/kit)	IVI1	IVI1	1112	IVI1	1012
Cut-off	30	30	40	30	30
	40	40	40	40	40
S/P % (1)	72.80	68.36	80.46	72.17	81.99
S/P % (2)	70.95	68.97	76.40	73.13	79.57
Mean	71.88	68.67	78.43	72.65	80.78
SD	1.31	0.43	2.87	0.68	1.71
CV (%)	1.82	0.63	3.66	0.94	2.11

Lab number	97509	97516	97540 (1) <b>*</b>	97540 (2)*
Method (ELISA protocol/kit)	$M_3$	$M_3$	$M_2$	$M_1$
Cut-off	30	30	60	60
	40	40	60	70
S/P % (1)	71.64	75.50	13.58	19.90
S/P % (2)	69.10	74.00	12.69	17.28
Mean	70.37	74.75	13.13	18.59
SD	1.80	1.06	0.63	1.86
CV (%)	2.56	1.42	4.77	9.98

Numbers were rounded to two significant decimal place. [S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation;  $M_1 = IDEXX - Bovine Rhinotracheitis Virus (IBR/BHV-1) gE Antibody Test Kit; <math>M_2 = ID.VET - ID SCREEN$  [BR gE test kit;  $M_3 = IDEXX - Other$  (not specified)].

\* = For this lab, no S/P % values were mentioned therefore normalization was automatically performed according to the following formula:



## 6.2 Annex 2: Additional information

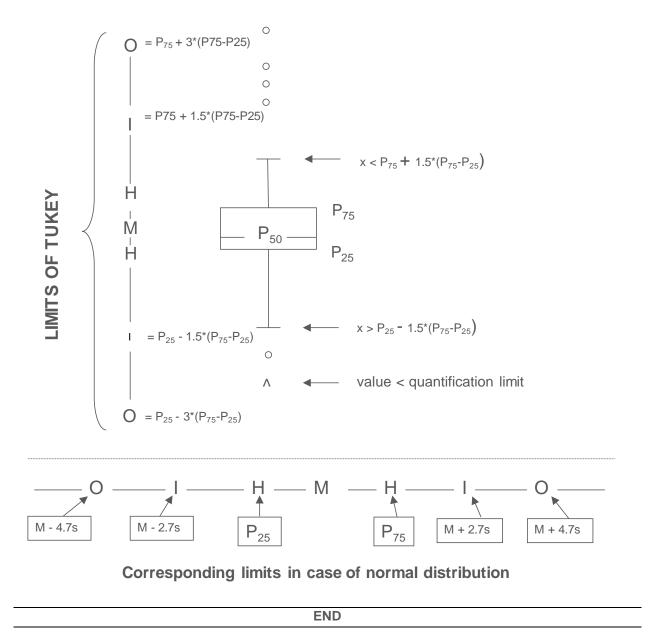
The **<u>calendar</u>** for Proficiency Testing in Veterinary diagnosis is available on our website:

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

#### Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 3 participants:

- a rectangle ranging from percentile 25 (P<sub>25</sub>) to percentile 75 (P<sub>75</sub>)
- a central line representing the median of the results (P<sub>50</sub>)
- a lower limit showing the smallest value  $x > P_{25} 1.5 * (P_{75} P_{25})$
- an upper limit representing the largest value x < P<sub>75</sub> + 1.5 \* (P<sub>75</sub> P<sub>25</sub>)
- all points outside this interval are represented by a dot.



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