

**BIOLOGICAL HEALTH RISKS  
QUALITY OF LABORATORIES**

**COMMITTEE OF EXPERTS**

**PROFICIENCY TEST  
IN VETERINARY DIAGNOSIS**

**DEFINITIVE GLOBAL REPORT  
VETERINARY MEDICINE  
BOVINE TUBERCULOSIS (BOV T)  
PROFICIENCY TEST 2023-12**

**Sciensano/PT VET BOV T/2023-12/E**  
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A draft version of this report was submitted to the expert(s) on 17/01/2024.

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**Date of publication: 01/02/2024**

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# TABLE OF CONTENTS

<b>1</b>	<b>INTRODUCTION</b>	<b>4</b>
<b>2</b>	<b>AIM</b>	<b>4</b>
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>4</b>
3.1	<b>Serology (serum)</b>	<b>4</b>
3.1.1	The participants	4
3.1.2	The samples	4
3.1.3	Homogeneity	4
3.1.4	Target values	5
3.1.5	Stability	5
3.1.6	Randomisation and panel composition	5
3.1.7	Threshold for qualification	6
3.2	<b>Gamma interferon (plasma)</b>	<b>6</b>
3.2.1	The participants	6
3.2.2	The samples	6
3.2.3	Homogeneity	6
3.2.4	Target values	7
3.2.5	Stability	7
3.2.6	Randomisation and panel composition	7
3.2.7	Threshold for qualification	8
<b>4</b>	<b>TIMELINE</b>	<b>8</b>
<b>5</b>	<b>RESULTS</b>	<b>9</b>
5.1	<b>Serology (serum)</b>	<b>9</b>
5.1.1	Results per sample	9
5.1.2	Used ELISA protocols/kits	9
5.1.3	Conclusion	9
5.2	<b>Gamma interferon (serum)</b>	<b>10</b>
5.2.1	Results per sample	10
5.2.2	Used ELISA protocols/kits	10
5.2.3	Conclusion	10
<b>6</b>	<b>ANNEXES (NOT UNDER ACCREDITATION)</b>	<b>11</b>
6.1	<b>Annex 1: Quantitative results</b>	<b>11</b>
6.1.1	Serology (serum)	11
6.1.2	Gamma interferon (serum)	15
6.2	<b>Annex 2: Additional information</b>	<b>18</b>

# 1 INTRODUCTION

Details relevant to the proficiency test 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

This proficiency test focused on identifying antibodies against the *Mycobacterium tuberculosis complex* in serum, as well as detecting gamma-interferon in bovine plasma using the ELISA.

## 3 MATERIALS AND METHODS

### 3.1 Serology (serum)

#### 3.1.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Bovine tuberculosis serology on serum samples. The names of the participating laboratories are:

- Sciensano, service of Veterinary Bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN

#### 3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology Service, Infectious diseases in animals Directorate, Sciensano.

Samples for the detection of specific antibodies to *Mycobacterium tuberculosis complex* in serum: Positive sera were collected from 3 animals and originated from 2 bovine tuberculosis outbreaks in Belgium. The 3 animals collected were either RT-PCR positive for the detection of Mycobacterium Tuberculosis complex and/or skin test positive. The four positive sera used for the proficiency test were used undiluted or prepared by dilution with negative sera.

#### 3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on three aliquots (three serum samples) of each sample using ELISA before and after the PT. The NRL obtained each time the same qualitative result. Therefore, the samples were considered as homogeneous.

### 3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of six different samples. However, repetitions were present in each of the samples. Therefore, the panel included twenty samples in total.

Sample content	Repetition	Expected result
PT2023BT_SP1	3	POS/NEG/NI*
PT2023BT_SP2	2	POS
PT2023BT_SP3	3	POS
PT2023BT_SP4	5	POS
PT2023BT_SN1	4	NEG
PT2023BT_SN2	3	NEG

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

\* = Sample SP1 has multiple interpretations (POS/NEG/NI) as revealed during homogeneity tests, where not all qualitative results were consistent. This variance is attributed to the serum's S/P value closely approaching the cut-off threshold.

### 3.1.5 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 as 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: PT2023BT_	97504	97507	97508	97509
SP1 (1)	BTSER23-5	BTSER23-5	BTSER23-7	BTSER23-2
SP1 (2)	BTSER23-19	BTSER23-10	BTSER23-10	BTSER23-15
SP1 (3)	BTSER23-20	BTSER23-13	BTSER23-16	BTSER23-18
SP2 (1)	BTSER23-1	BTSER23-2	BTSER23-12	BTSER23-10
SP2 (2)	BTSER23-4	BTSER23-19	BTSER23-13	BTSER23-13
SP3 (1)	BTSER23-14	BTSER23-4	BTSER23-4	BTSER23-1
SP3 (2)	BTSER23-16	BTSER23-17	BTSER23-5	BTSER23-3
SP3 (3)	BTSER23-17	BTSER23-18	BTSER23-6	BTSER23-4
SP4 (1)	BTSER23-2	BTSER23-6	BTSER23-4	BTSER23-6
SP4 (2)	BTSER23-10	BTSER23-12	BTSER23-5	BTSER23-8
SP4 (3)	BTSER23-12	BTSER23-14	BTSER23-6	BTSER23-9

Sample content: PT2023BT_	97504	97507	97508	97509
SP4 (4)	BTSER23-13	BTSER23-15	BTSER23-4	BTSER23-11
SP4 (5)	BTSER23-15	BTSER23-16	BTSER23-5	BTSER23-12
SN1 (1)	BTSER23-3	BTSER23-1	BTSER23-1	BTSER23-5
SN1 (2)	BTSER23-7	BTSER23-7	BTSER23-2	BTSER23-14
SN1 (3)	BTSER23-8	BTSER23-8	BTSER23-8	BTSER23-16
SN1 (4)	BTSER23-18	BTSER23-11	BTSER23-14	BTSER23-20
SN2 (1)	BTSER23-6	BTSER23-3	BTSER23-15	BTSER23-7
SN2 (2)	BTSER23-9	BTSER23-9	BTSER23-19	BTSER23-17
SN2 (3)	BTSER23-11	BTSER23-20	BTSER23-20	BTSER23-19

### 3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 reference samples is at least 90%.

## 3.2 Gamma interferon (plasma)

### 3.2.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of Bovine tuberculosis serology on serum samples. The names of the participating laboratories are:

- Sciensano, service of Veterinary Bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- IDVET – Grabels (France)
- AGES – Gesundheit für Mensch, Tier und Pflanze

### 3.2.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Veterinary Bacteriology Service, Infectious diseases in animals Directorate, Sciensano.

Samples for the detection of gamma-interferon in bovine plasma by ELISA: a positive plasma was first prepared by stimulation of bovine whole blood with pokeweed and then the different positive plasmas used for this proficiency test were prepared by dilution with negative serum.

### 3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on three aliquots (three serum samples) of each sample using ELISA before and after the PT. The NRL obtained each time the same qualitative result. Therefore, the samples were considered as homogeneous.

### 3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of five different samples. However, repetitions were present in each of the samples. Therefore, the panel included twenty samples in total.

Sample content	Repetition	Expected result
PT2023BT_IP1	4	POS
PT2023BT_IP2	6	POS
PT2023BT_IP3	4	POS
PT2023BT_IN1	3	NEG
PT2023BT_IN2	3	NEG

(POS = positive; NEG = negative)

### 3.2.5 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 as 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: PT2023 BT_	97504	97507	97508	97509	97522	97546
IP1 (1)	BTgIFN23-8	BTgIFN23-8	BTgIFN23-1	BTgIFN23-3	BTgIFN23-3	BTgIFN23-7
IP1 (2)	BTgIFN23-10	BTgIFN23-13	BTgIFN23-6	BTgIFN23-7	BTgIFN23-5	BTgIFN23-17
IP1 (3)	BTgIFN23-17	BTgIFN23-16	BTgIFN23-7	BTgIFN23-10	BTgIFN23-6	BTgIFN23-18
IP1 (4)	BTgIFN23-20	BTgIFN23-17	BTgIFN23-20	BTgIFN23-15	BTgIFN23-8	BTgIFN23-19
IP2 (1)	BTgIFN23-1	BTgIFN23-1	BTgIFN23-2	BTgIFN23-1	BTgIFN23-1	BTgIFN23-1
IP2 (2)	BTgIFN23-9	BTgIFN23-6	BTgIFN23-5	BTgIFN23-6	BTgIFN23-2	BTgIFN23-3
IP2 (3)	BTgIFN23-11	BTgIFN23-7	BTgIFN23-8	BTgIFN23-8	BTgIFN23-4	BTgIFN23-4
IP2 (4)	BTgIFN23-12	BTgIFN23-11	BTgIFN23-10	BTgIFN23-9	BTgIFN23-9	BTgIFN23-6
IP2 (5)	BTgIFN23-14	BTgIFN23-18	BTgIFN23-15	BTgIFN23-17	BTgIFN23-13	BTgIFN23-10
IP2 (6)	BTgIFN23-18	BTgIFN23-19	BTgIFN23-19	BTgIFN23-19	BTgIFN23-14	BTgIFN23-15
IP3 (1)	BTgIFN23-4	BTgIFN23-2	BTgIFN23-3	BTgIFN23-2	BTgIFN23-7	BTgIFN23-9
IP3 (2)	BTgIFN23-5	BTgIFN23-5	BTgIFN23-4	BTgIFN23-11	BTgIFN23-11	BTgIFN23-11
IP3 (3)	BTgIFN23-6	BTgIFN23-10	BTgIFN23-11	BTgIFN23-12	BTgIFN23-15	BTgIFN23-12
IP3 (4)	BTgIFN23-16	BTgIFN23-20	BTgIFN23-14	BTgIFN23-20	BTgIFN23-18	BTgIFN23-20

Sample content: PT2023 BT_	97504	97507	97508	97509	97522	97546
IN1 (1)	BTgIFN23-3	BTgIFN23-3	BTgIFN23-9	BTgIFN23-13	BTgIFN23-12	BTgIFN23-5
IN1 (2)	BTgIFN23-13	BTgIFN23-12	BTgIFN23-17	BTgIFN23-14	BTgIFN23-16	BTgIFN23-8
IN1 (3)	BTgIFN23-15	BTgIFN23-15	BTgIFN23-18	BTgIFN23-18	BTgIFN23-19	BTgIFN23-14
IN2 (1)	BTgIFN23-2	BTgIFN23-4	BTgIFN23-12	BTgIFN23-4	BTgIFN23-10	BTgIFN23-2
IN2 (2)	BTgIFN23-7	BTgIFN23-9	BTgIFN23-13	BTgIFN23-5	BTgIFN23-17	BTgIFN23-13
IN2 (3)	BTgIFN23-19	BTgIFN23-14	BTgIFN23-16	BTgIFN23-16	BTgIFN23-20	BTgIFN23-16

### 3.2.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 20 reference samples is at least 90%.

## 4 TIMELINE

Transfer of the samples from NRL to QL: 16/10/2023

Randomisation of the samples by QL: 26/10/2023

Sending samples to participants: 30/10/2023

- Storage of the samples : refrigerated (4°C)

Deadline for submitting the results: 17/11/2023

Individual report to the participants: 07/12/2023



## 5 RESULTS

### 5.1 Serology (serum)

#### 5.1.1 RESULTS PER SAMPLE

The panel consisted of six different samples. However, repetitions were present in each of the samples. Therefore, the panel included twenty samples in total.

Sample content	Status	Number of repetitions (total results)	Observed result
SP1	POS/NEG/NI	3 (12)	12 POS
SP2	POS	2 (8)	8 POS
SP3	POS	3 (12)	12 POS
SP4	POS	5 (20)	20 POS
SN1	NEG	4 (16)	16 NEG
SN2	NEG	3 (12)	12 NEG

#### 5.1.2 USED ELISA PROTOCOLS/KITS

In the table below, the ELISA protocols/kits used are listed along with the number of laboratories that have used this protocol/kit with their achieved score.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Indirect	IDEXX	M. bovis Ab Test	4	80	80	100
<b>TOTAL</b>			<b>4</b>	<b>80</b>	<b>80</b>	<b>100</b>

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

#### 5.1.3 CONCLUSION

In 2023, four laboratories participated in the proficiency test Bovine Tuberculosis serology (serum) 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 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.

## 5.2 Gamma interferon (serum)

### 5.2.1 RESULTS PER SAMPLE

The panel consisted of five different samples. However, repetitions were present in each of the samples. Therefore, the panel included twenty samples in total.

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	Status	Number of repetitions (total results)	Observed result
IP1	POS	4 (28)	28 POS
IP2	POS	6 (42)	42 POS
IP3	POS	4 (28)	28 POS
IN1	NEG	3 (21)	21 NEG
IN2	NEG	3 (21)	21 NEG

### 5.2.2 USED ELISA PROTOCOLS/KITS

In the table below, the ELISA protocols/kits used are listed along with the number of datasets and achieved score.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Indirect	ID.VET	ID Screen ruminant IFN-g	7	140	140	100
<b>TOTAL</b>			<b>7</b>	<b>140</b>	<b>140</b>	<b>100</b>

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

### 5.2.3 CONCLUSION

In 2023, six laboratories participated in the proficiency test Bovine Tuberculosis gamma interferon (serum) 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 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 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: [shiny.chemgrid.org/boxplotr/](https://shiny.chemgrid.org/boxplotr/).

#### 6.1.1 SEROLOGY (SERUM)

PT2023BT-SP1

Lab number	97504	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - M. bovis Ab Test			
S/P (REP1)	0,41	0,45	0,48	0,36
S/P (REP2)	0,47	0,51	0,45	0,37
S/P (REP3)	0,50	0,68	0,57	0,41
Mean	0,46	0,55	0,50	0,38
SD	0,044	0,12	0,062	0,023
CV (%)	9,63	21,41	12,37	6,07

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

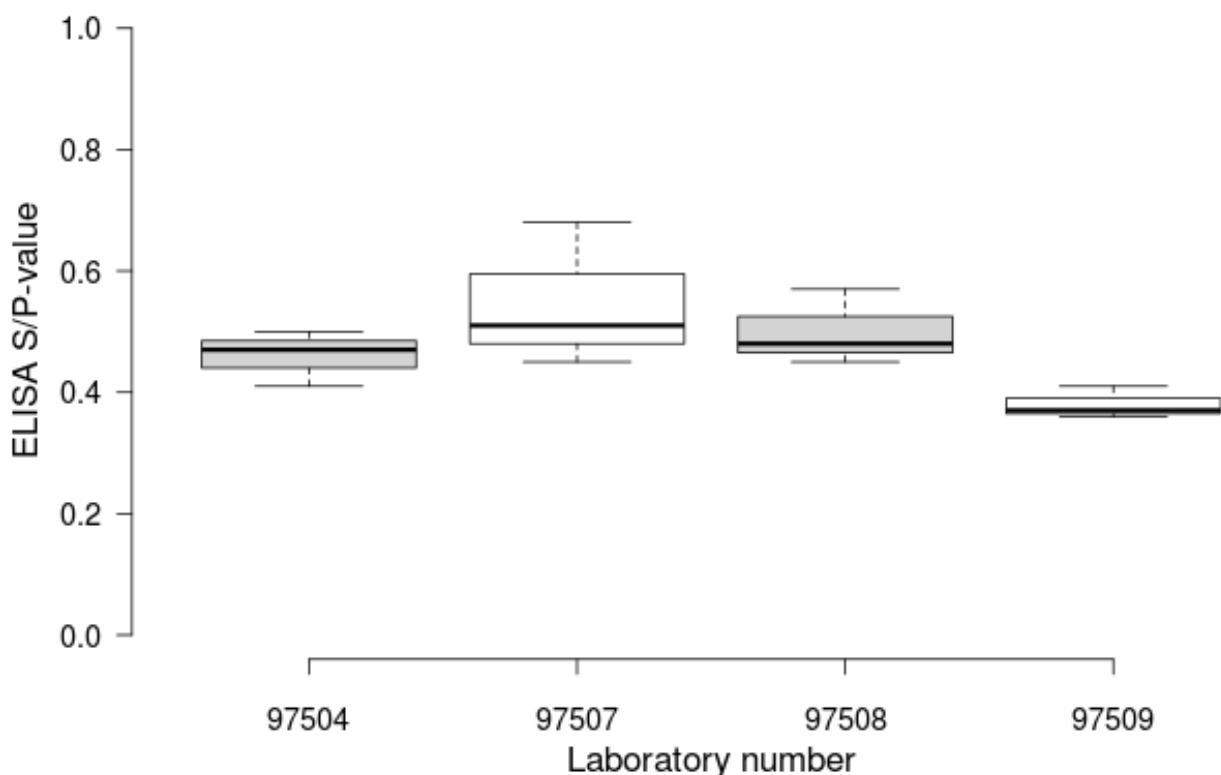


Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97504	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - M. bovis Ab Test			
S/P (REP1)	2,96	2,40	4,72	2,13
S/P (REP2)	3,04	2,86	4,70	1,82
Mean	3,00	2,63	4,71	1,98
SD	0,060	0,32	0,019	0,22
CV (%)	2,01	12,20	0,41	10,91

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

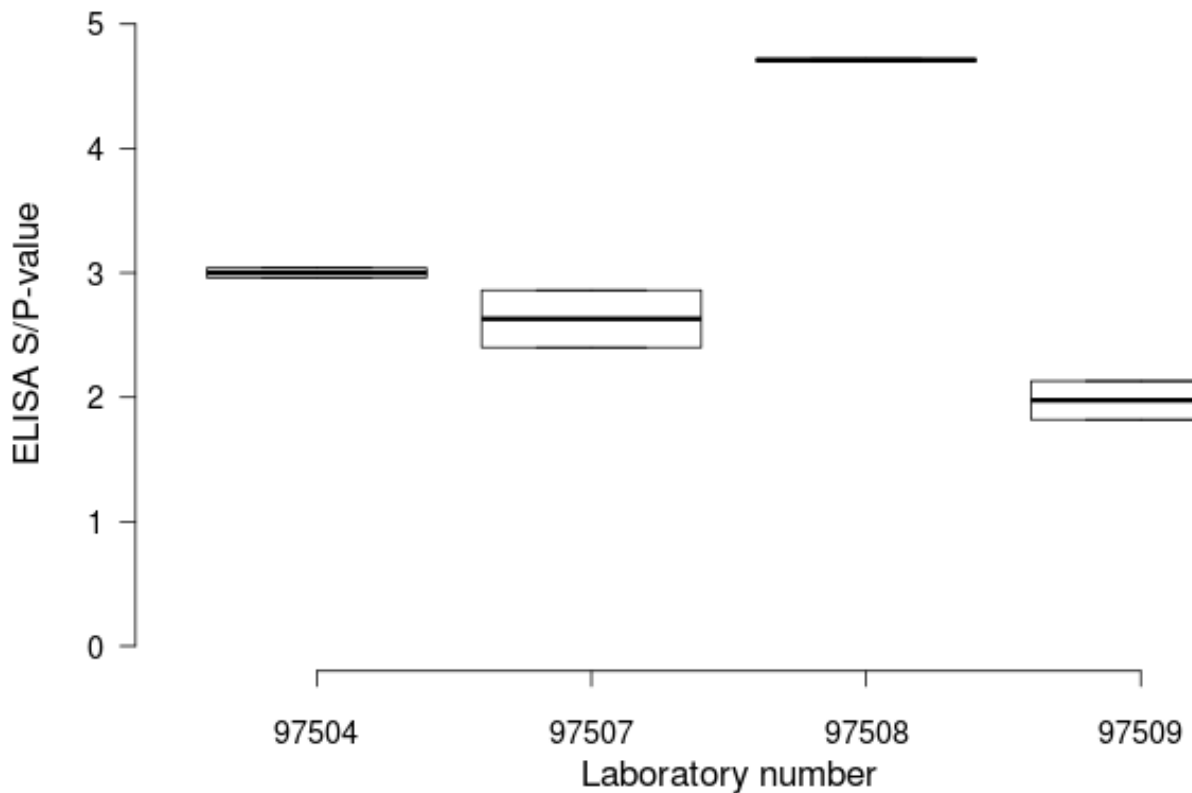


Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97504	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - M. bovis Ab Test			
S/P (REP1)	3,29	3,35	6,17	2,61
S/P (REP2)	3,67	4,15	6,59	2,96
S/P (REP3)	3,74	3,93	5,04	2,83
Mean	3,56	3,81	5,93	2,80
SD	0,24	0,41	0,80	0,18
CV (%)	6,80	10,82	13,50	6,36

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

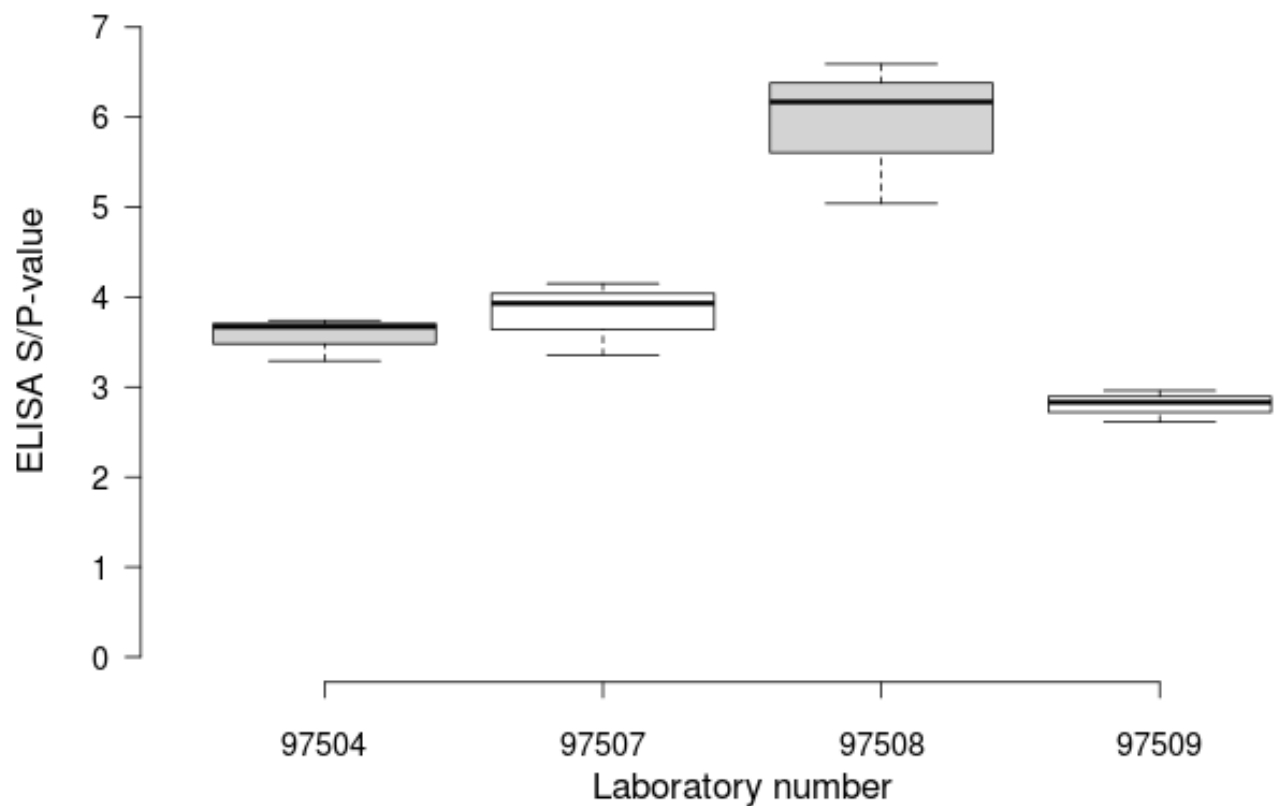


Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97504	97507	97508	97509
Method (ELISA protocol/kit)	IDEXX - M. bovis Ab Test			
S/P (REP1)	1,43	0,98	2,28	1,13
S/P (REP2)	1,32	1,15	1,78	1,01
S/P (REP3)	1,49	1,18	2,18	0,93
S/P (REP4)	1,02	0,91	2,37	1,39
S/P (REP5)	1,25	0,93	2,18	0,98
Mean	1,30	1,03	2,16	1,08
SD	0,18	0,13	0,23	0,18
CV (%)	14,06	12,17	10,43	16,83

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

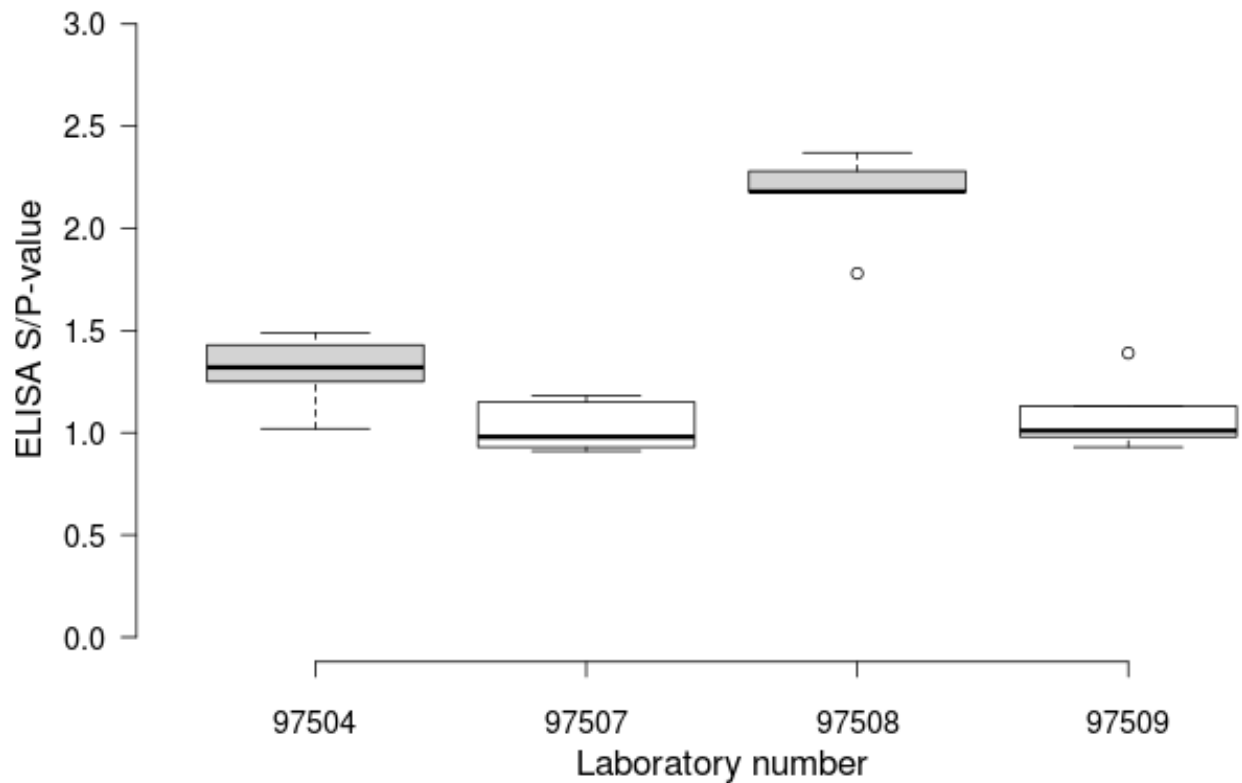


Figure 4. Distribution of the S/P-values (%) (box-plots) per laboratory.

## 6.1.2 GAMMA INTERFERON (SERUM)

PT2023BT-IP1

Lab number	97504	97507	97508	97509	97522	97546 (1)	97546 (2)
Method (ELISA protocol/kit)	ID.VET - ID Screen ruminant IFN-g						
S/P % (REP1)	111,00	116,57	132,67	131,30	102,00	105,26	37,58
S/P % (REP2)	116,00	138,36	120,10	130,57	112,00	91,92	43,38
S/P % (REP3)	115,00	133,43	119,77	129,44	112,00	97,31	41,54
S/P % (REP4)	114,00	129,85	124,24	131,85	108,00	93,97	39,28
Mean	114,00	129,55	124,19	130,79	108,50	97,12	40,45
SD	2,16	9,33	6,01	1,04	4,73	5,86	2,54
CV (%)	1,89	7,20	4,84	0,80	4,36	6,04	6,29

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

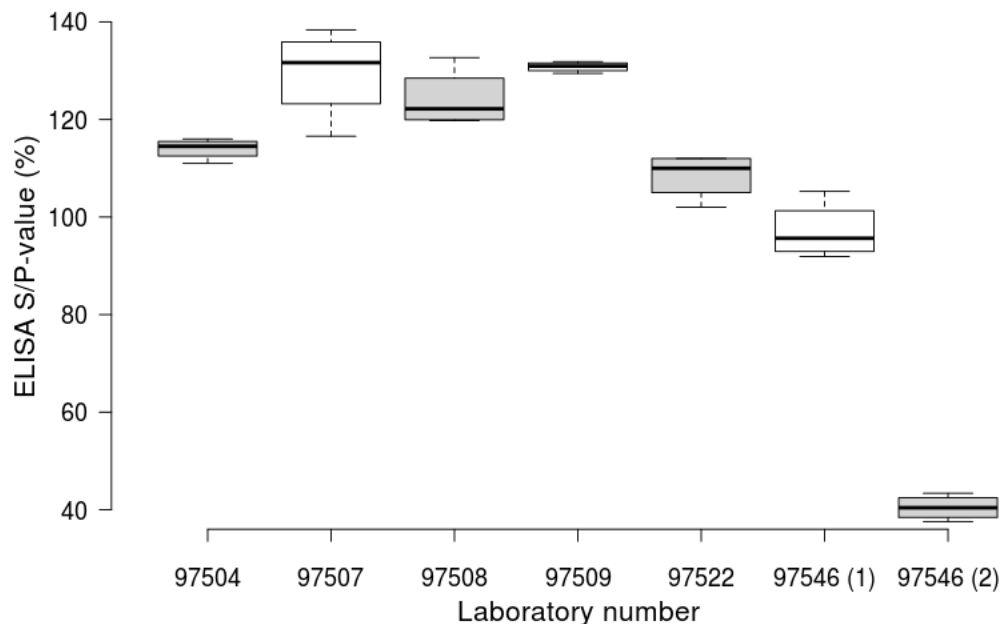


Figure 5. Distribution of the S/P-values (%) (box-plots) per laboratory.

Lab number	97504	97507	97508	97509	97522	97546 (1)	97546 (2)
Method (ELISA protocol/kit)	ID.VET - ID Screen ruminant IFN-g						
S/P % (REP1)	60,00	70,60	62,04	64,63	66,00	56,41	19,46
S/P % (REP2)	64,00	70,30	72,79	61,48	65,00	52,18	17,48
S/P % (REP3)	58,00	64,93	65,67	61,30	63,00	49,49	17,48
S/P % (REP4)	62,00	71,19	66,50	61,30	66,00	48,46	18,47
S/P % (REP5)	59,00	68,81	67,49	61,11	59,00	49,49	17,76
S/P % (REP6)	59,00	70,00	71,96	62,41	64,00	49,74	16,77
Mean	60,33	69,31	67,74	62,04	63,83	50,96	17,91
SD	2,25	2,28	4,04	1,35	2,64	2,94	0,94
CV (%)	3,73	3,30	5,97	2,18	4,13	5,77	5,24

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

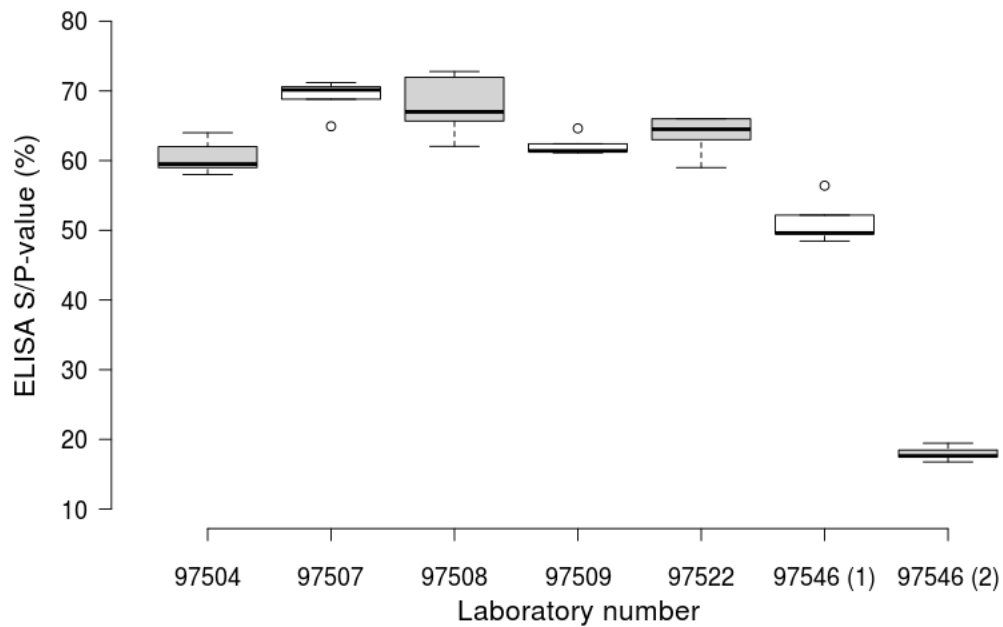


Figure 6. Distribution of the S/P-values (%) (box-plots) per laboratory.



Lab number	97504	97507	97508	97509	97522	97546 (1)	97546 (2)
Method (ELISA protocol/kit)	ID.VET - ID Screen ruminant IFN-g						
S/P % (REP1)	41,00	48,81	45,82	45,93	42,00	33,08	13,52
S/P % (REP2)	42,00	50,90	46,65	45,19	39,00	31,67	11,25
S/P % (REP3)	38,00	51,04	45,82	45,74	41,00	32,44	11,68
S/P % (REP4)	42,00	52,69	47,31	42,59	40,00	30,39	12,53
Mean	40,75	50,86	46,40	44,86	40,50	31,89	12,24
SD	1,89	1,59	0,72	1,54	1,29	1,16	1,00
CV (%)	4,65	3,13	1,55	3,44	3,19	3,63	8,17

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

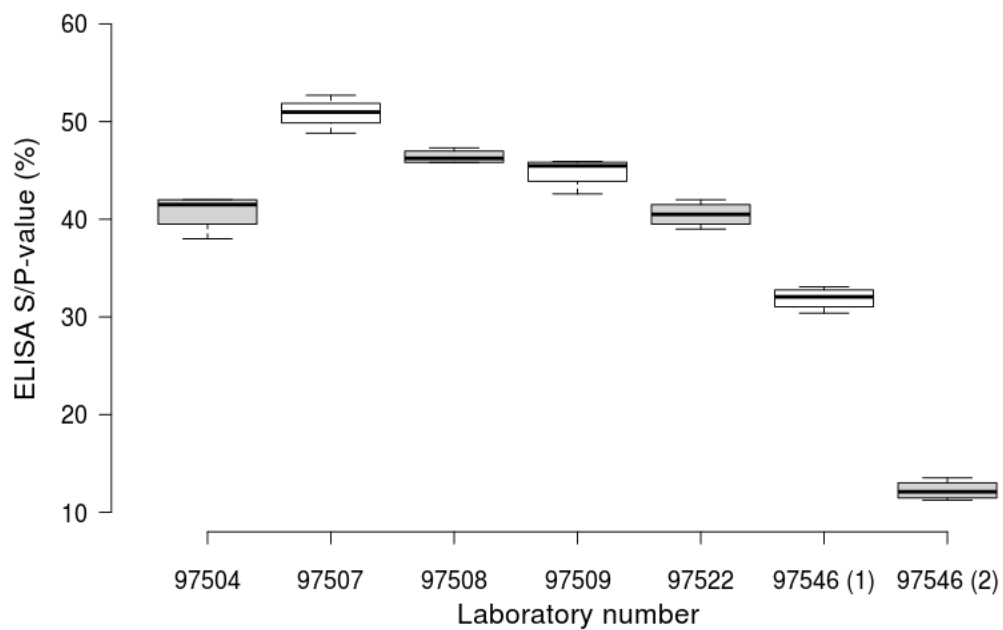


Figure 7. Distribution of the S/P-values (%) (box-plots) per laboratory.

## 6.2 Annex 2: Additional information

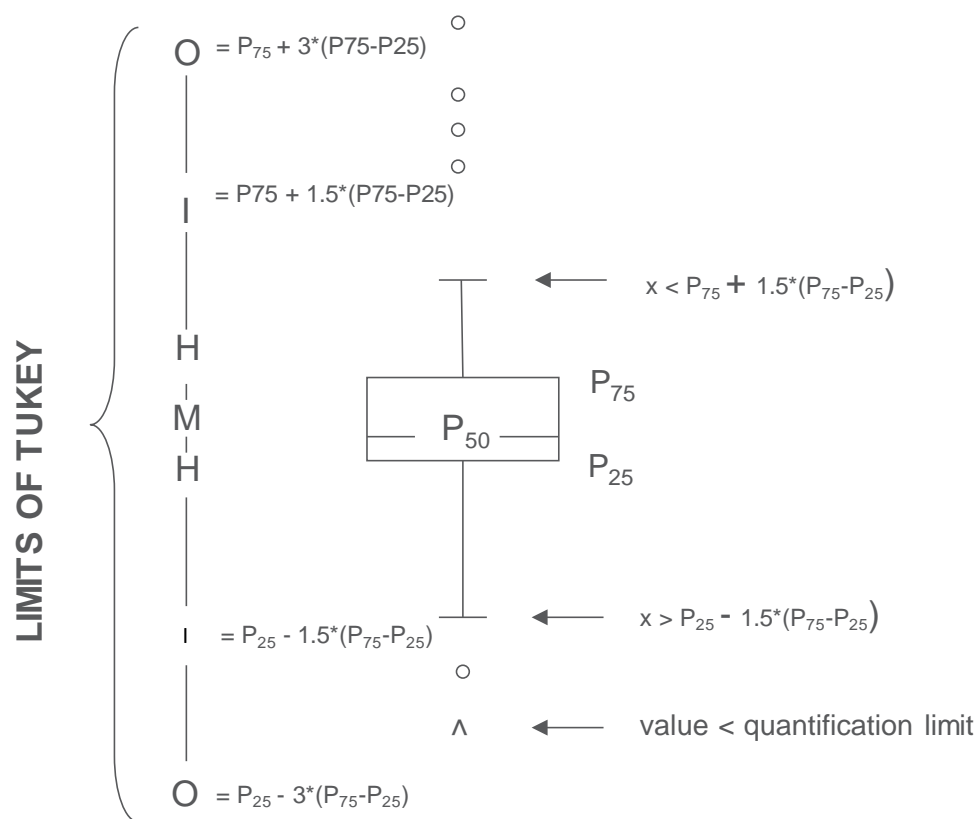
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

- NL: <https://www.sciensano.be/fr/biblio/eke-kalender-2023>
- FR: <https://www.sciensano.be/en/biblio/calendrier-eeq-2023>
- EN: <https://www.sciensano.be/en/biblio/eqa-calendar-2023>

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



**Corresponding limits in case of normal distribution**

END

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