BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

BRUCELLOSIS (BRU)

PROFICIENCY TEST 2023-6

Sciensano/PT VET BRU/2023-6/E
Biological health risks
Quality of laboratories
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- NL: https://www.sciensano.be/nl/kwaliteit-van-laboratoria
- FR: https://www.sciensano.be/fr/qualite-des-laboratoires
- EN: https://www.sciensano.be/en/quality-laboratories

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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 Brucellosis (serology) was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies against *Brucella spp.* in serum of cattle, using ELISA, Slow Agglutination (Wright's sero-agglutination) (SAW) or Rose Bengal Test (RBT) methods.

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

Five laboratories participated in the proficiency test of Brucellosis serology on serum. The names of the participating laboratories are:

- Sciensano, department of Veterinary Bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- Laboratoire de Médecine Vétérinaire de l'Etat du Grand-Duché de Luxembourg (LMVE)
- ANSES - Unité Zoonoses bactériennes (UZB) du laboratoire de santé animale

Laboratory number	ELISA (Ab)	SAW	RBT
97504	1	1	1
97507	1	1	1
97508	1	1	1
97516	0	0	1
97517	1	1	1

(0 = no participation; 1 = participation)

3.1.2 THE SAMPLES

The samples (lyophilized sera) were prepared by the National Reference Laboratory (NRL), department of Veterinary Bacteriology, Sciensano. Participants were instructed to reconstitute the serum with 1 mL of demineralized water and incubate the sample for 20 minutes at room temperature without shaking in order to allow full rehydratation. Thereafter, they were directed to homogenize the sample by vortexing and let rest for another 10 minutes before using it.

Information about the origin and preparation of the samples:

- PT2023BRUSERPS1 was a serum sample collected from a naturally infected bovine and diluted 1/16 in negative serum.
- PT2023BRUSERPS2 was a serum sample collected from a bovine, experimentally infected with Brucella abortus and used undiluted for this PT.
- PT2023BRUSERPS3: was a serum sample collected from a bovine, experimentally infected with Brucella abortus and used undiluted for this PT.
- PT2023BRUSERNS1 was a negative serum sample collected from field negative animal.
- PT2023BRUSERNS2 was a negative serum sample collected from field negative animal.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 3 aliquots (1 mL) of each sample using three different methods (ELISA, SAW, and RBT). The samples were considered as homogeneous.

For the laboratory, the criterion to consider that the homogeneity are correct is when the coefficient of variation (CV) between the 3 values is < 15%.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of different of samples: 6 positive and 4 negative samples.

Sample ID	Repetition	Status
PT2023BRUSERPS1	6	POS
PT2023BRUSERPS2	4	POS
PT2023BRUSERPS3	4	POS
PT2023BRUSERNS1	3	NEG
PT2023BRUSERNS2	3	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The criterion for stability is that the status of the sample in Post-PT remains the status assigned in pre-PT test. The stability check was conform.

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: PT2023 BRUSER	97505	97507	97508	97522	97532
PS1 (1)	BRUSER23-6	BRUSER23-3	BRUSER23-1	BRUSER23-1	BRUSER23-3
PS1 (2)	BRUSER23-11	BRUSER23-4	BRUSER23-2	BRUSER23-6	BRUSER23-6
PS1 (3)	BRUSER23-12	BRUSER23-6	BRUSER23-6	BRUSER23-7	BRUSER23-7
PS1 (4)	BRUSER23-15	BRUSER23-7	BRUSER23-11	BRUSER23-12	BRUSER23-10
PS1 (5)	BRUSER23-16	BRUSER23-13	BRUSER23-15	BRUSER23-13	BRUSER23-16
PS1 (6)	BRUSER23-20	BRUSER23-20	BRUSER23-18	BRUSER23-18	BRUSER23-19
PS2 (1)	BRUSER23-2	BRUSER23-8	BRUSER23-5	BRUSER23-5	BRUSER23-1
PS2 (2)	BRUSER23-3	BRUSER23-14	BRUSER23-9	BRUSER23-14	BRUSER23-9
PS2 (3)	BRUSER23-7	BRUSER23-16	BRUSER23-10	BRUSER23-15	BRUSER23-12
PS2 (4)	BRUSER23-9	BRUSER23-18	BRUSER23-20	BRUSER23-20	BRUSER23-18
PS3 (1)	BRUSER23-1	BRUSER23-10	BRUSER23-3	BRUSER23-2	BRUSER23-8
PS3 (2)	BRUSER23-13	BRUSER23-11	BRUSER23-7	BRUSER23-4	BRUSER23-15
PS3 (3)	BRUSER23-14	BRUSER23-12	BRUSER23-13	BRUSER23-9	BRUSER23-17
PS3 (4)	BRUSER23-19	BRUSER23-19	BRUSER23-19	BRUSER23-16	BRUSER23-20
NS1 (1)	BRUSER23-4	BRUSER23-9	BRUSER23-8	BRUSER23-3	BRUSER23-4
NS1 (2)	BRUSER23-8	BRUSER23-15	BRUSER23-16	BRUSER23-10	BRUSER23-11
NS1 (3)	BRUSER23-17	BRUSER23-17	BRUSER23-17	BRUSER23-11	BRUSER23-13
NS2 (1)	BRUSER23-5	BRUSER23-1	BRUSER23-4	BRUSER23-8	BRUSER23-2
NS2 (2)	BRUSER23-10	BRUSER23-2	BRUSER23-12	BRUSER23-17	BRUSER23-5
NS2 (3)	BRUSER23-18	BRUSER23-5	BRUSER23-14	BRUSER23-19	BRUSER23-14

4 TIMELINE

Transfer of the samples from NRL to QL: 19/06/2023
Randomization of the samples by QL: 22/06/2023
Sending samples to participants: in the week of the 26th of June 2023

- Samples serology: refrigerated at 4 °C

Deadline for submitting the results: 07/07/2023
Individual report to the participants: 07/08/2023

5 RESULTS

5.1 Serology (serum)

The panel consisted of 5 different samples. However, repetitions were included in both positive and negative samples (see tables down below). Therefore, in total, the panel consisted of 20 samples (14 positive and 6 negative samples).

5.1.1 ENZYME-LINKED IMMUNOASSAY (ELISA)

5.1.1.1 Results per sample

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	6 (24)	24 POS
PS2	POS	4 (16)	16 POS
PS3	POS	4 (16)	16 POS
NS1	NEG	3 (12)	12 NEG
NS2	NEG	3 (12)	12 NEG

(POS = positive; NEG = negative)

5.1.1.2 Used method

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Indirect	IDEXX	Brucellosis Antibody test kit	**3**	**60**	**60**	**100**
Home made	/	/	**1**	**20**	**20**	**100**
TOTAL			**4**	**80**	**80**	**100**

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

5.1.1.3 Conclusion

In 2023, four laboratories participated in proficiency test of Brucellosis serology (serum - ELISA, SAW & RB testing) 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.

5.1.2 SLOW AGGLUTINATION OF WRIGHT (SAW)

5.1.2.1 Results per sample

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	6 (24)	24 POS
PS2	POS	4 (16)	16 POS
PS3	POS	4 (16)	16 POS
NS1	NEG	3 (12)	12 NEG
NS2	NEG	3 (12)	12 NEG

(POS = positive; NEG = negative)

5.1.2.2 Used method

Name producer	Name reagent	N	NR	NCR	%
Synbiotics/Zoetis	SAW Antigen	**4**	**80**	**80**	**100**
TOTAL		**4**	**80**	**80**	**100**

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

5.1.2.3 Conclusion

In 2023, four laboratories participated in proficiency test of Brucellosis serology (serum - SAW) 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.

5.1.3 ROSE BENGAL TEST (RBT)

5.1.3.1 Results per sample

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	6 (30)	30 POS
PS2	POS	4 (20)	20 POS
PS3	POS	4 (20)	20 POS
NS1	NEG	3 (15)	15 NEG
NS2	NEG	3 (15)	15 NEG

(POS = positive; NEG = negative)

5.1.3.2 Used method

Name producer	Name reagent	N	NR	NCR	%
Synbiotics/Zoetis	Rose Bengale Ag	**1**	**20**	**20**	**100**
IDEXX	Rose Bengale Ag	**4**	**80**	**80**	**100**
TOTAL		**5**	**100**	**100**	**100**

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

5.1.3.3 Conclusion

In 2023, five laboratories participated in proficiency test of Brucellosis serology (serum - RBT) 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.

6 ANNEXES (NOT UNDER ACCREDITATION)

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

6.1 Annex 1: Quantitative results

6.1.1 ENZYME-LINKED IMMUNOASSAY (ELISA)

6.1.1.1 S/P values

PT2023BRUSERPS1

* = Laboratory 97504 did not report the S/P-values (%) since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
S/P (REP1)	/	238	213,86	239,55
S/P (REP2)	/	238	204,12	253,11
S/P (REP3)	/	240	212,21	253,70
S/P (REP4)	/	240	208,84	247,20
S/P (REP5)	/	253	209,42	247,17
S/P (REP6)	/	242	205,84	248,70
Mean	/	241,83	209,05	248,24
SD	/	5,67	3,69	5,12
CV (%)	/	2,35	1,76	2,06

Numbers were rounded to 2 significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

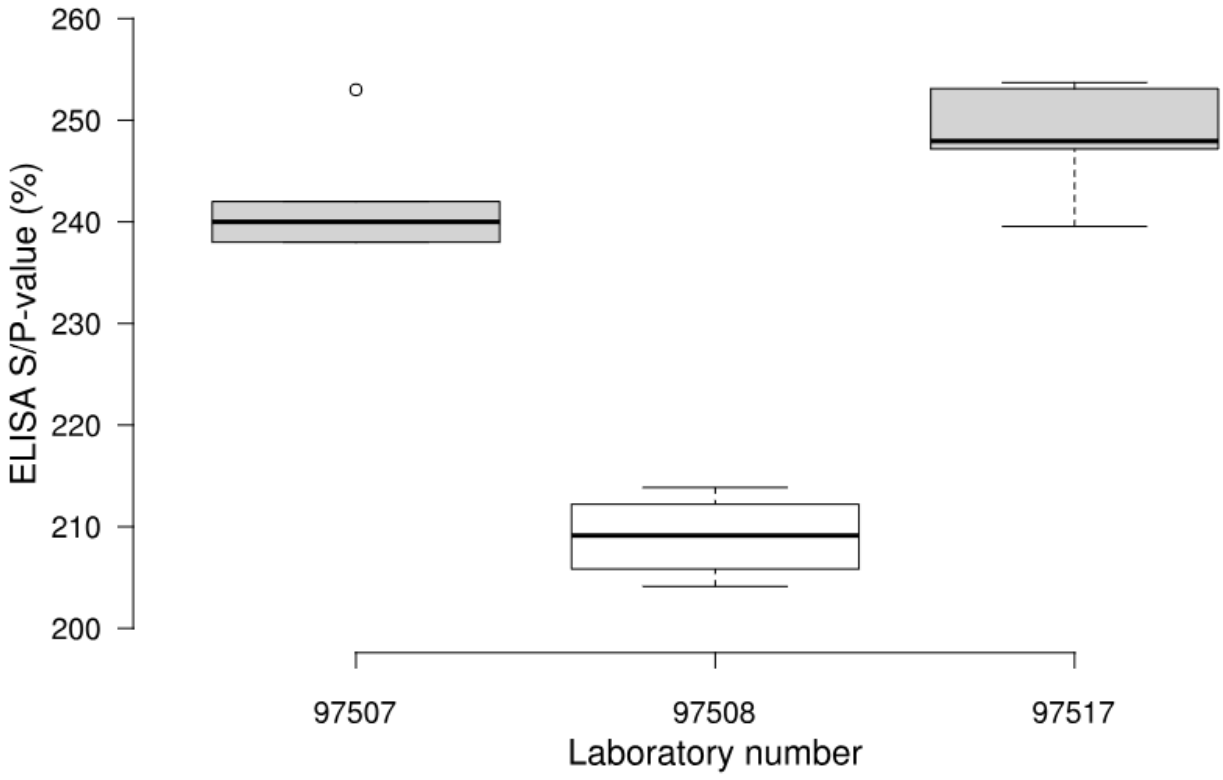


Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.

* = Laboratory 97504 did not report the S/P-values (%) since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
S/P (REP1)	/	198	172,54	203,21
S/P (REP2)	/	209	181,63	205,21
S/P (REP3)	/	209	150,77	199,04
S/P (REP4)	/	206	176,55	203,21
Mean	/	205,50	170,37	202,67
SD	/	5,20	13,59	2,60
CV (%)	/	2,53	7,98	1,28

Numbers were rounded to 2 significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

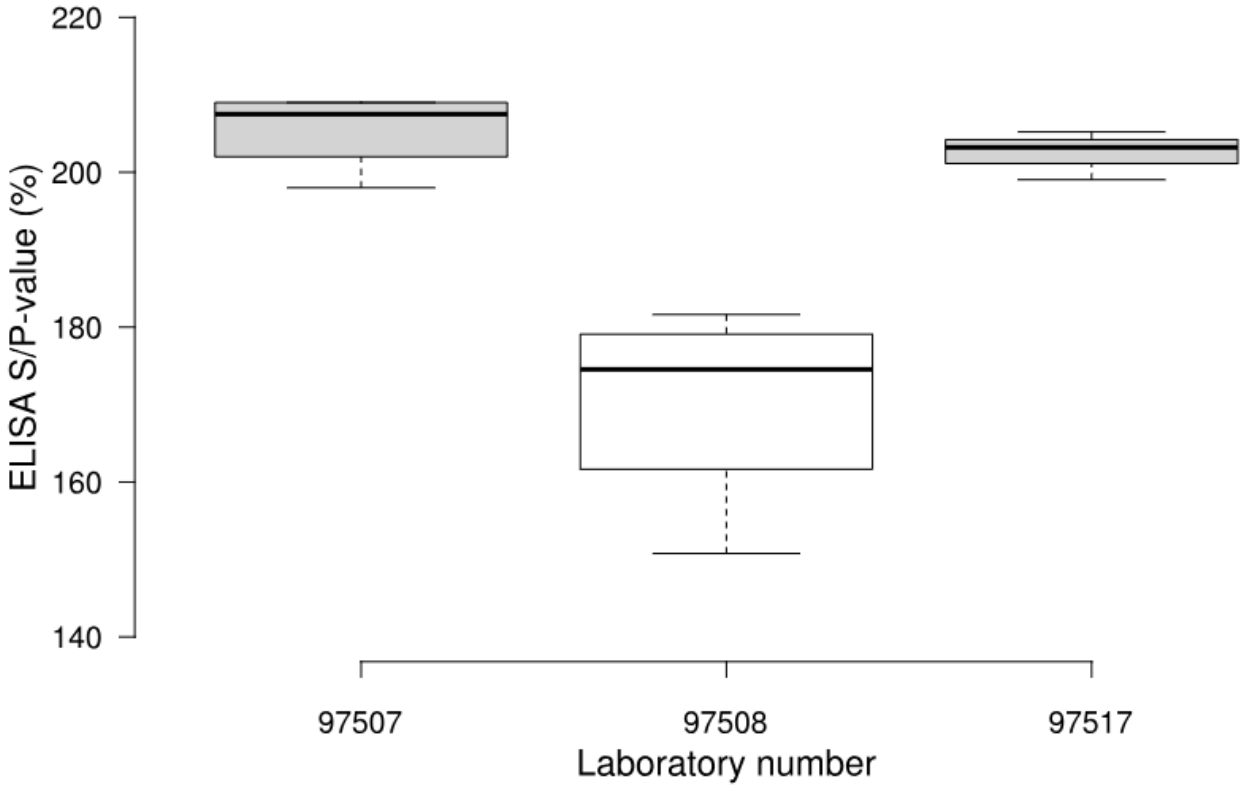


Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.

* = Laboratory 97504 did not report the S/P-values (%) since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
S/P (REP1)	/	234	200,39	227,71
S/P (REP2)	/	241	201,32	238,91
S/P (REP3)	/	232	200,97	228,52
S/P (REP4)	/	236	209,34	237,55
Mean	/	235,75	203,01	233,17
SD	/	3,86	4,241	5,88
CV (%)	/	1,64	2,09	2,52

Numbers were rounded to 2 significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

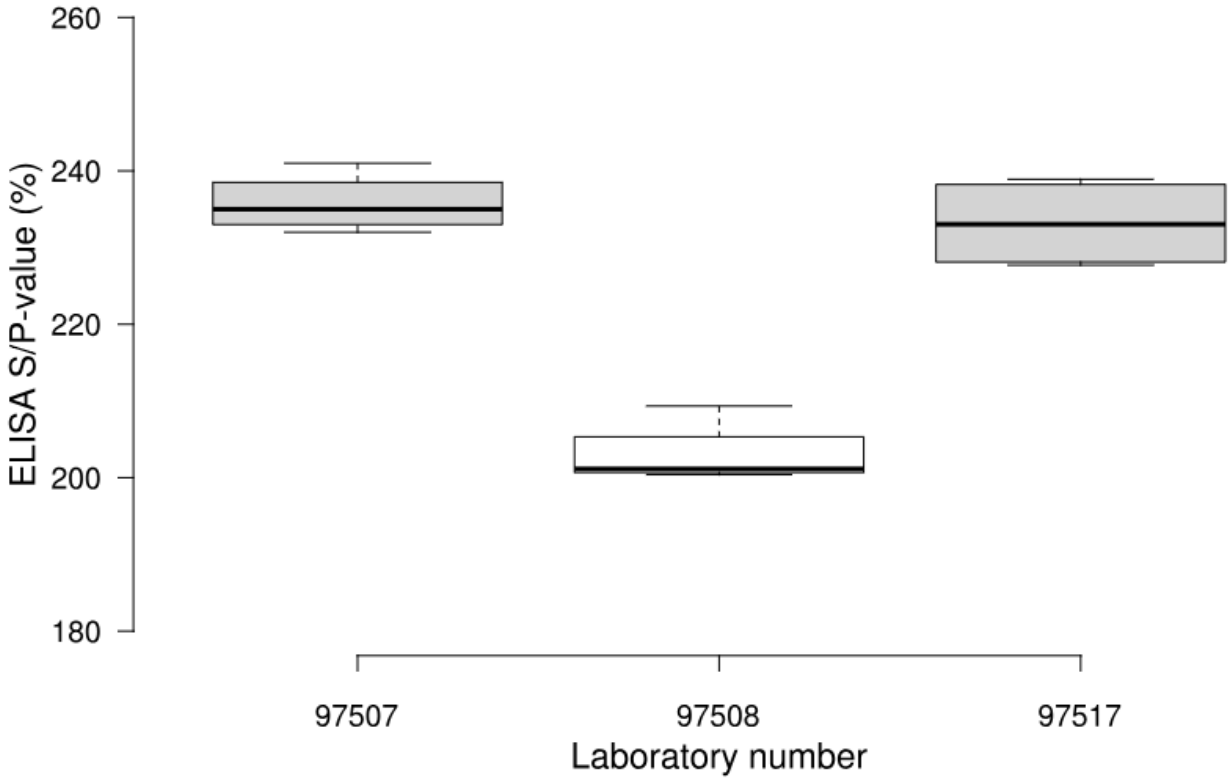


Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.

6.1.1.2 Optical density (OD)

PT2023BRUSERPS1

* = Laboratory 97504 did not report the optical densities since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
OD (REP1)	/	2,47	3,04	2,64
OD (REP2)	/	2,47	2,90	2,79
OD (REP3)	/	2,49	3,01	2,79
OD (REP4)	/	2,49	2,97	2,72
OD (REP5)	/	2,62	2,98	2,72
OD (REP6)	/	2,51	2,93	2,74
Mean	/	2,51	2,97	2,73
SD	/	0,057	0,051	0,055
CV (%)	/	2,26	1,73	2,03

Numbers were rounded to 2 significant decimal places. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

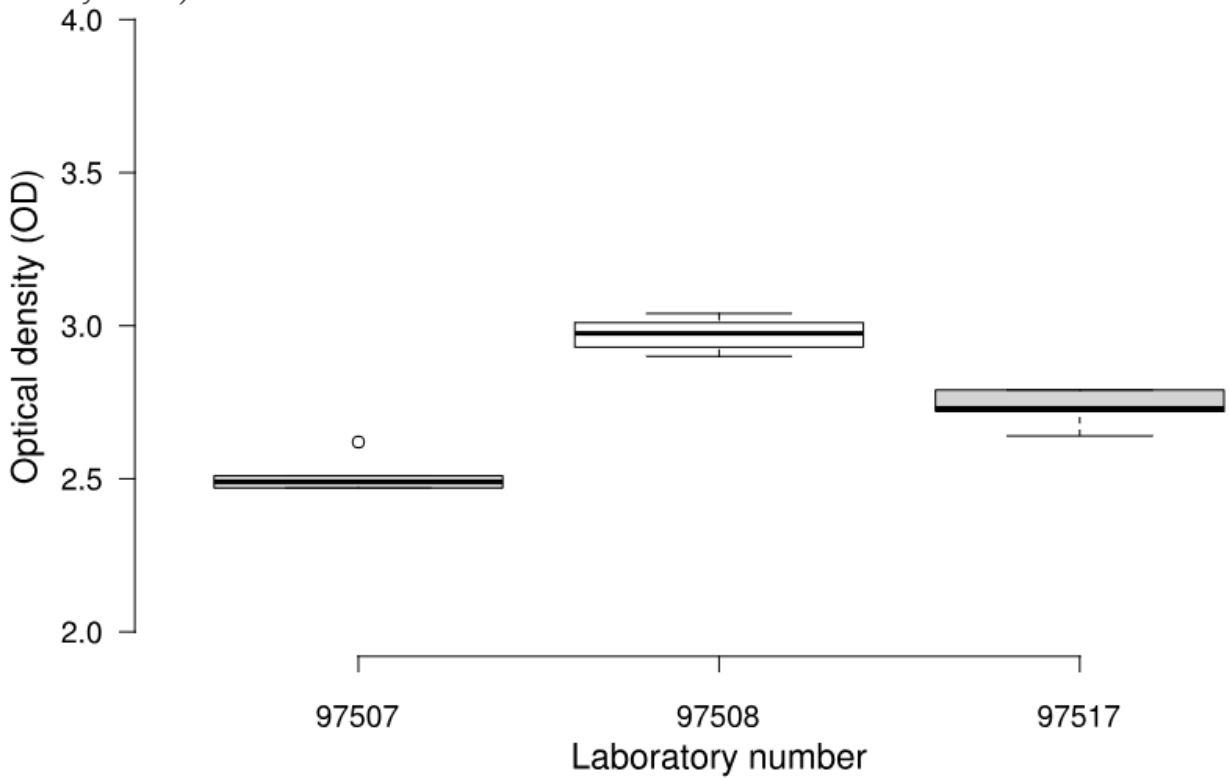


Figure 1. Distribution of the optical densities (box-plots) per laboratory.

* = Laboratory 97504 did not report the optical densities since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
OD (REP1)	/	2,07	2,46	2,25
OD (REP2)	/	2,18	2,59	2,27
OD (REP3)	/	2,18	2,16	2,20
OD (REP4)	/	2,15	2,52	2,25
Mean	/	2,15	2,43	2,24
SD	/	0,052	0,19	0,028
CV (%)	/	2,42	7,81	1,26

Numbers were rounded to 2 significant decimal places. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

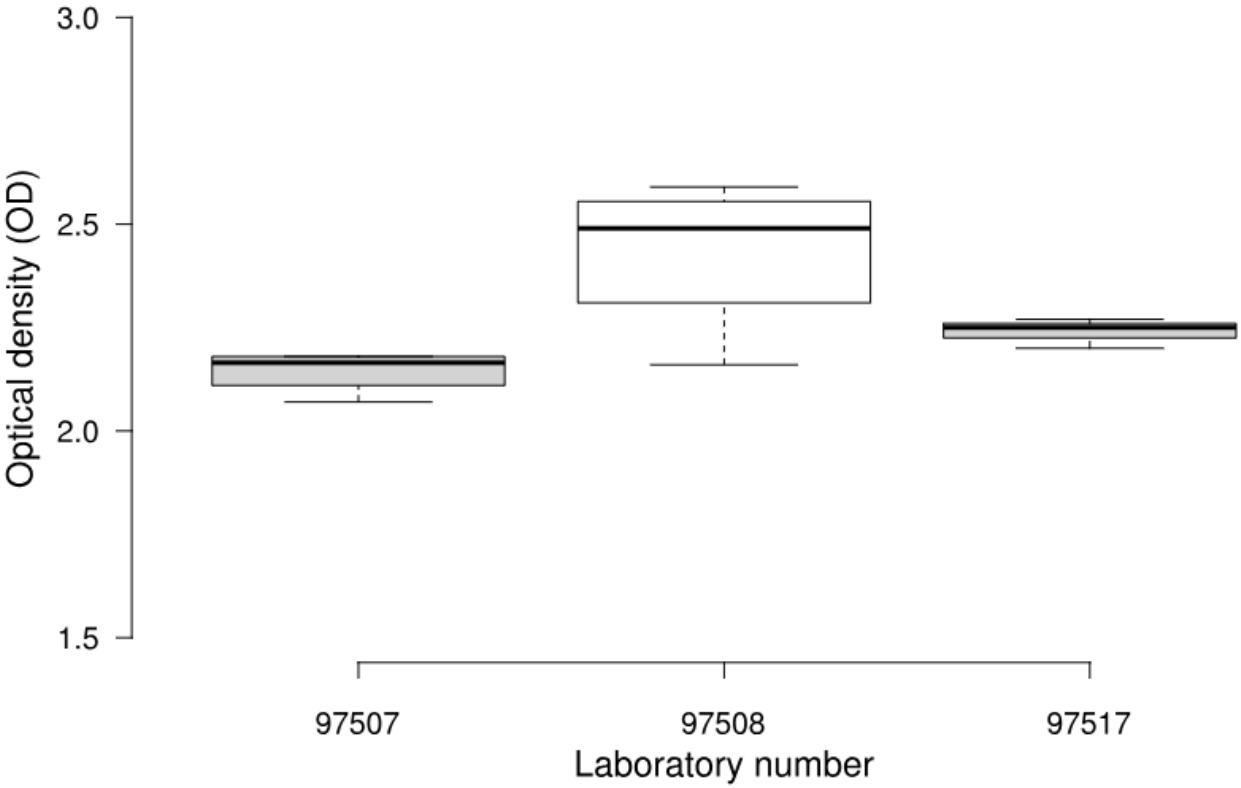


Figure 2. Distribution of the optical densities (box-plots) per laboratory.

* = Laboratory 97504 did not report the optical densities since they used a 'home-made' ELISA that uses a standard curve to make calculations in units/mL. Therefore, no boxplot is added for this laboratory.

Lab number	97504*	97507	97508	97517
Method	M_1	M_2	M_2	M_2
OD (REP1)	/	2,43	2,85	2,51
OD (REP2)	/	2,50	2,86	2,63
OD (REP3)	/	2,41	2,86	2,52
OD (REP4)	/	2,45	2,97	2,62
Mean	/	2,45	2,89	2,57
SD	/	0,039	0,059	0,064
CV (%)	/	1,58	2,05	2,48

Numbers were rounded to 2 significant decimal places. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation; M_1 = Home Made ELISA; M_2 = IDEXX - Brucellosis Antibody test kit).

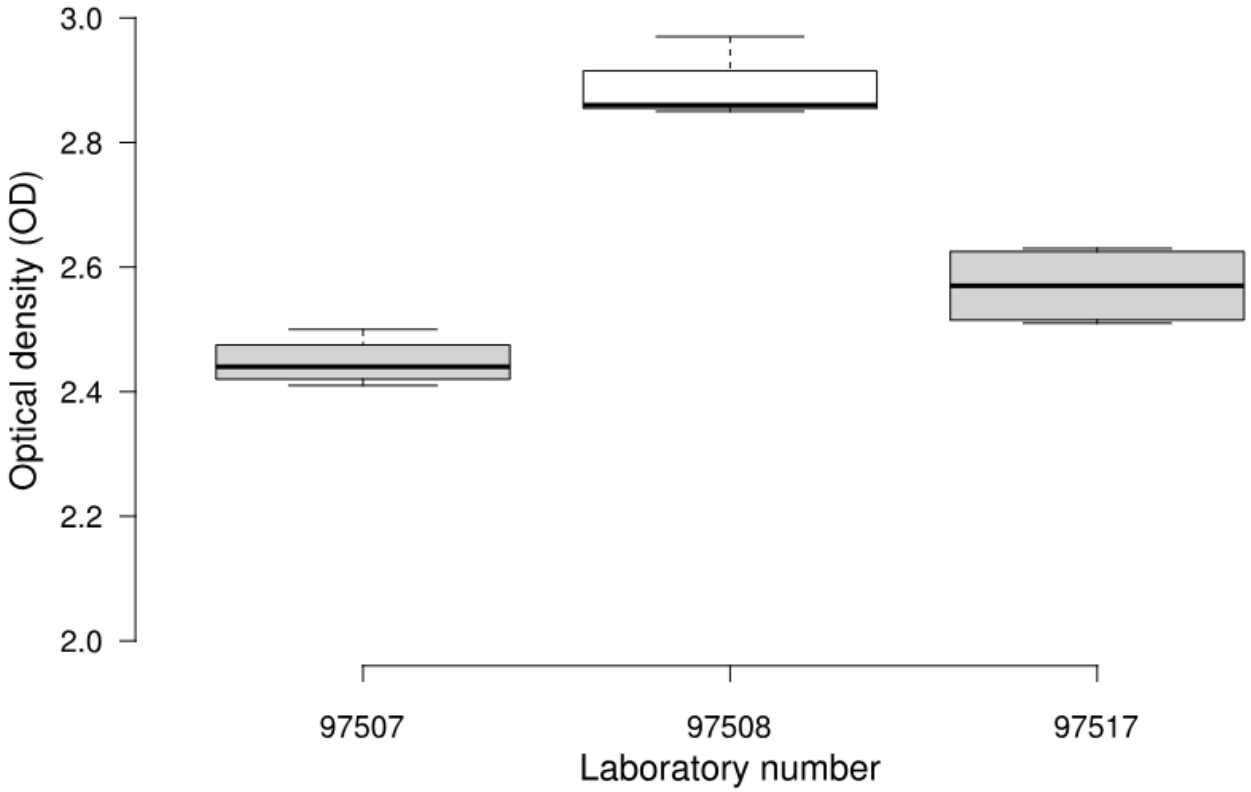


Figure 3. Distribution of the optical densities (box-plots) per laboratory.

6.1.2 SLOW AGGLUTINATION OF WRIGHT (SAW)

All four laboratories chose the SAW Antigen method form Synbiotics/Zoetis. No differences in results were observed.

Sample ID	Method used by all participants	Status	IU/mL	Observed result
PS1	Synbiotics/Zoetis - SAW Antigen	POS	50	Every laboratory responded the same value for each repetition
PS2		POS	>100	Every laboratory responded the same value for each repetition
PS3		POS	>100	Every laboratory responded the same value for each repetition

6.2 Annex 2: Additional information

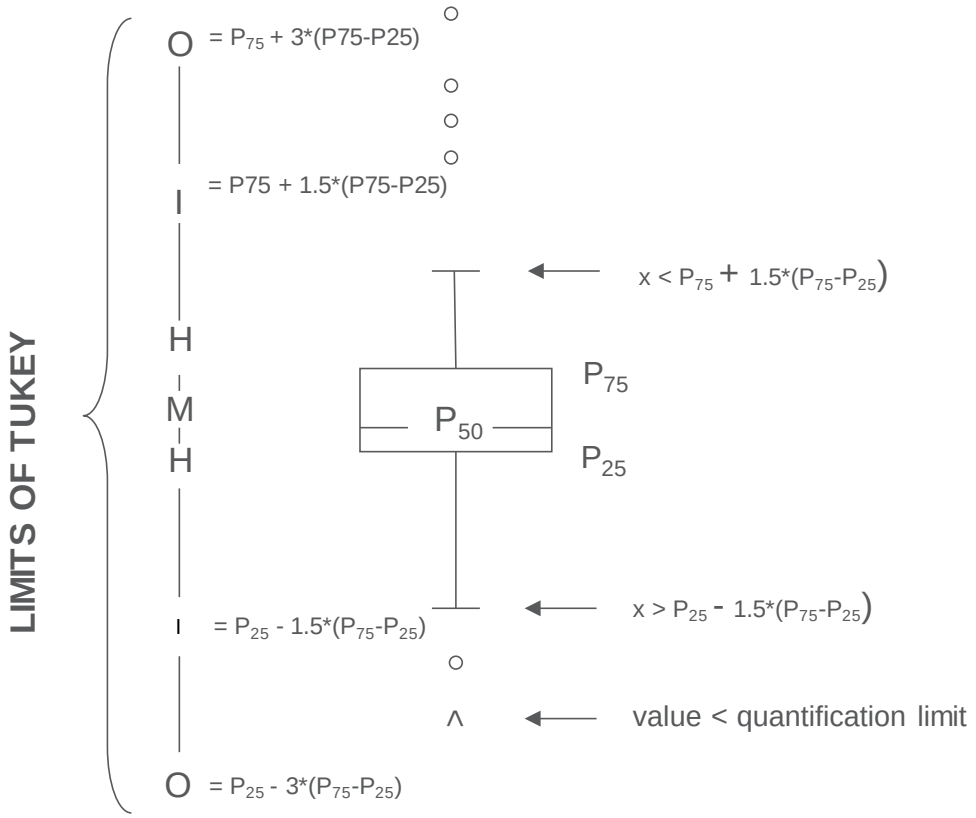
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

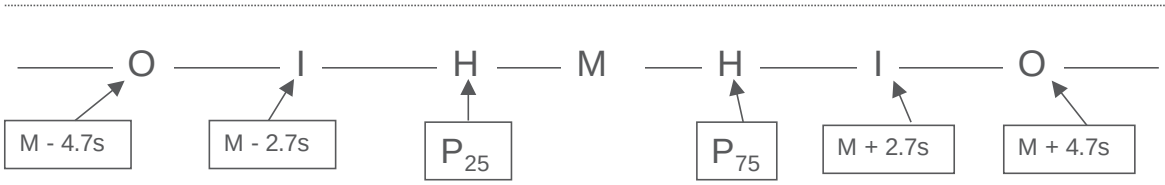
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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





Corresponding limits in case of normal distribution

END