BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

BOVINE TUBERCULOSIS (BT)

PROFICIENCY TEST 2022/11

CORRECTED VERSION

Sciensano/PT VET BT/3-E/CV

Biological health risks
Quality of laboratories
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A draft version of this report was submitted to the experts on: 12/01/2023.

Corrections were made on page 10 and 17.

- Page 10: The sentence "*As a results, it can be concluded that the method from ID.VET is a suitable option for antibody detection against Bovine Tuberculosis in serum of cattle"* has been removed from the conclusion.
- Page 17: An additional explanation has been added to the table (indicated in blue).

A new section has been added to the report on page 18-20 (indicated in blue).

- Additional calculations for the INFg test were added

This report replaces the previous version of the global report of 19/01/2023.

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

YnVa2851 Digitally signed by YnVa2851 Date: 2023.02.13 10:49:09 +01'00'

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All the reports are also available on our webpage:

- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
- FR: https://www.sciensano.be/fr/evaluation-externe-de-la-qualite/sante-animale-pt-vet
- 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

This PT was divided into two different parts: serology and ELISA gamma interferon testing:

- The aim of the **serology part** was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies against Bovine Tuberculosis in serum of cattle.
- The aim of the ELISA **gamma interferon part** was to perform the detection of INF gamma in the context of the diagnosis of Bovine Tuberculosis in cattle.

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

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

- Sciensano, department of Veterinary bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- IDEXX Technologies GmbH (Bern)

3.1.2 THE SAMPLES

The samples (freeze dried sera) were prepared by the National Reference Laboratory (NRL), Service of Veterinary bacteriology, Sciensano.

Information about the **origin** of the samples:

- Samples PS1, PS2, PS3 and PS4: these sera were collected from animals naturally infected with M. bovis.
- Samples NS1 and NS2: these sera were collected from negative cattle.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (0,2 mL) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

For the laboratory, the criteria to consider that the homogeneity is 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 6 different sera: 2 negative, 3 positive and one serum which had two possible interpretations: negative or positive.

Sample ID	Repetition	Status
PT2022BTPS1	5	POS/NEG
PT2022BTPS2	2	POS
PT2022BTPS3	3	POS
PT2022BTPS4	5	POS
PT2022BTNS1	3	NEG
PT2022BTNS2	2	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The criteria for stability is that the status of the sample in Post-PT remains the status assigned in pre-PT test. For information, the PT2022BTPS1 showed only positive results in Post-PT testing on the contrary to the pre-PT testing during which it was tested 9 times positive and 1 time negative.

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: BTSER	97504	97507	97508	97509	97544
22-1	PS1	PS1	NS2	NS2	PS3
22-2	NS2	NS1	PS4	PS1	PS4
22-3	NS1	PS4	PS4	PS3	PS3
22-4	PS4	PS2	PS4	NS1	PS1
22-5	PS1	NS2	PS4	PS4	PS2
22-6	PS1	PS1	PS1	PS1	PS4
22-7	NS1	PS1	PS4	PS3	PS4
22-8	NS2	PS3	PS2	PS4	PS4

Sample ID: BTSER	97504	97507	97508	97509	97544
22-9	PS3	PS3	NS1	NS1	PS4
22-10	PS2	NS1	NS1	PS1	PS2
22-11	PS3	PS1	PS3	PS3	PS1
22-12	PS2	NS2	NS2	PS2	PS1
22-13	PS3	PS2	PS2	NS1	PS3
22-14	PS4	PS4	NS1	PS4	NS1
22-15	NS1	NS1	PS1	PS1	NS1
22-16	PS1	PS4	PS1	PS4	NS1
22-17	PS4	PS1	PS3	PS1	NS2
22-18	PS4	PS4	PS3	NS2	NS2
22-19	PS4	PS4	PS1	PS2	PS1
22-20	PS1	PS3	PS1	PS4	PS1

3.2 Gamma interferon (plasma/serum)

3.2.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of gamma Interferon testing.
. The names of the participating laboratories are:

- Sciensano, department of Veterinary bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN

3.2.2 THE SAMPLES

The samples (freeze dried vials) were prepared by the National Reference Laboratory (NRL), Service of Veterinary bacteriology, Sciensano.

Information about the **origin** and **preparation** of the samples:

- Sample PG1: the sample was prepared by diluting 1/16 a plasma having a high concentration of IFN gamma with a IFNg negative serum
- Sample PG2: the sample was prepared by diluting 1/30 a plasma having a high concentration of IFN gamma with a IFNg negative serum
- Sample PG3: the sample was prepared by diluting 1/50 a plasma having a high concentration of IFN gamma with a IFNg negative serum
- Samples NG1 and NG2: these samples were prepared from 2 IFNg negative sera collected from Bovine tuberculosis negative cattle.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (0,2 mL) of each sample using ELISA method before the PT. The samples were considered as homogeneous.

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

3.2.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. Initially, the panel consisted of 20 different samples: 15 positive and 5 negative samples.

Sample ID	Repetition	Status
PT2022BTPG1	4	POS
PT2022BTPG2	5	POS
PT2022BTPG3	6	POS
PT2022BTNG1	2	NEG
PT2022BTNG2	3	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

The criteria for stability is that the status of the sample in Post-PT remains the status assigned in pre-PT test.

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: BTgIFN	97504	97507	97508	97509
22-1	PG2	PG3	PG2	PG3
22-2	NG2	PG3	NG2	PG3
22-3	PG1	PG1	PG3	NG2
22-4	PG3	PG2	NG2	PG2
22-5	NG2	NG1	PG2	PG3
22-6	PG1	PG1	PG2	PG1
22-7	NG1	PG3	PG1	PG2
22-8	PG2	NG1	NG1	PG3
22-9	PG2	PG2	NG2	PG2
22-10	NG1	NG2	PG3	PG3
22-11	PG2	PG3	PG3	NG2

Sample ID: BTgIFN	97504	97507	97508	97509
22-12	PG3	PG3	PG2	PG2
22-13	PG1	NG2	PG3	NG2
22-14	PG2	PG2	PG1	PG2
22-15	PG1	PG3	PG3	NG1
22-16	PG3	PG2	PG3	PG1
22-17	PG3	PG2	NG1	PG3
22-18	PG3	PG1	PG1	PG1
22-19	NG2	PG1	PG1	NG1
22-20	PG3	NG2	PG2	PG1

4 TIMELINE

Transfer of the samples from NRL to QL: 18/10/2022
Randomization of the samples by QL: 21/10/2022
Sending samples to participants: 24/10/2022

- Samples serology: cooled at 4 °C
- Samples gamma interferon: cooled at 4 °C

Deadline for submitting the results: 18/11/2022
Preliminary report: 09/01/2023

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of 6 different samples. All samples were repeated at least twice (see table below). Therefore, in total, the panel consisted of 20 samples (15 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS/NEG	5 (25)	24 POS 1 NEG
PS2	POS	2 (10)	10 POS
PS3	POS	3 (15)	15 POS
PS4	POS	5 (25)	25 POS
NS1	NEG	3 (15)	15 NEG
NS2	NEG	2 (10)	10 NEG

(POS = positive; NEG = negative)

5.1.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Indirect	IDEXX - Mycobacterium Bovis Antibody Test Kit	Short	**5**	**100**	**100**	**100**
TOTAL			**5**	**100**	**100**	**100**

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

5.1.3 CONCLUSION

In 2022, five laboratories participated in proficiency test of Bovine Tuberculosis serology (serum) organized by Sciensano. The method Mycobacterium Bovis Antibody Test Kit from IDEXX was selected by all the participants for the detection of antibodies against Bovine Tuberculosis in serum of cattle. This method is an indirect ELISA.

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.

5.2 Gamma interferon (plasma/serum)

5.2.1 RESULTS PER SAMPLE

The panel consisted of 5 different samples. All samples were repeated at least twice (see table below). Therefore, in total, the panel consisted of 20 samples (15 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
PG1	POS	4 (16)	16 POS
PG2	POS	5 (20)	20 POS
PG3	POS	6 (24)	24 POS
NG1	NEG	2 (8)	8 NEG
NG2	NEG	3 (12)	12 NEG

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Indirect	ID.VET - ID Screen ruminant IFN-g	Short	**4**	**80**	**80**	**100**
TOTAL			**4**	**80**	**80**	**100**

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

5.2.3 CONCLUSION

In 2022, four laboratories participated in proficiency test of Bovine Tuberculosis gamma interferon (serum) organized by Sciensano. The method ID Screen ruminant IFN-g from ID.VET was selected by all the participants for the detection of gamma Interferon. This method is a sandwich ELISA designed to catch the gamma interferon produced in the tested plasmas.

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 SEROLOGY (SERUM)

PT2022BTPS1

Lab number	97504	97507	97508	97509	97544
Method	IDEXX- Mycobacterium Bovis Antibody Test Kit				
OD (REP1)	0,28	0,48	0,45	0,48	0,25
OD (REP2)	0,24	0,45	0,39	0,43	0,22
OD (REP3)	0,24	0,47	0,42	0,47	0,23
OD (REP4)	0,25	0,45	0,40	0,43	0,21
OD (REP5)	0,24	0,53	0,41	0,35	0,26
Mean	0,25	0,47	0,41	0,43	0,23
SD	0,02	0,03	0,02	0,05	0,02
CV (%)	7,28	6,85	5,31	11,83	8,43

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

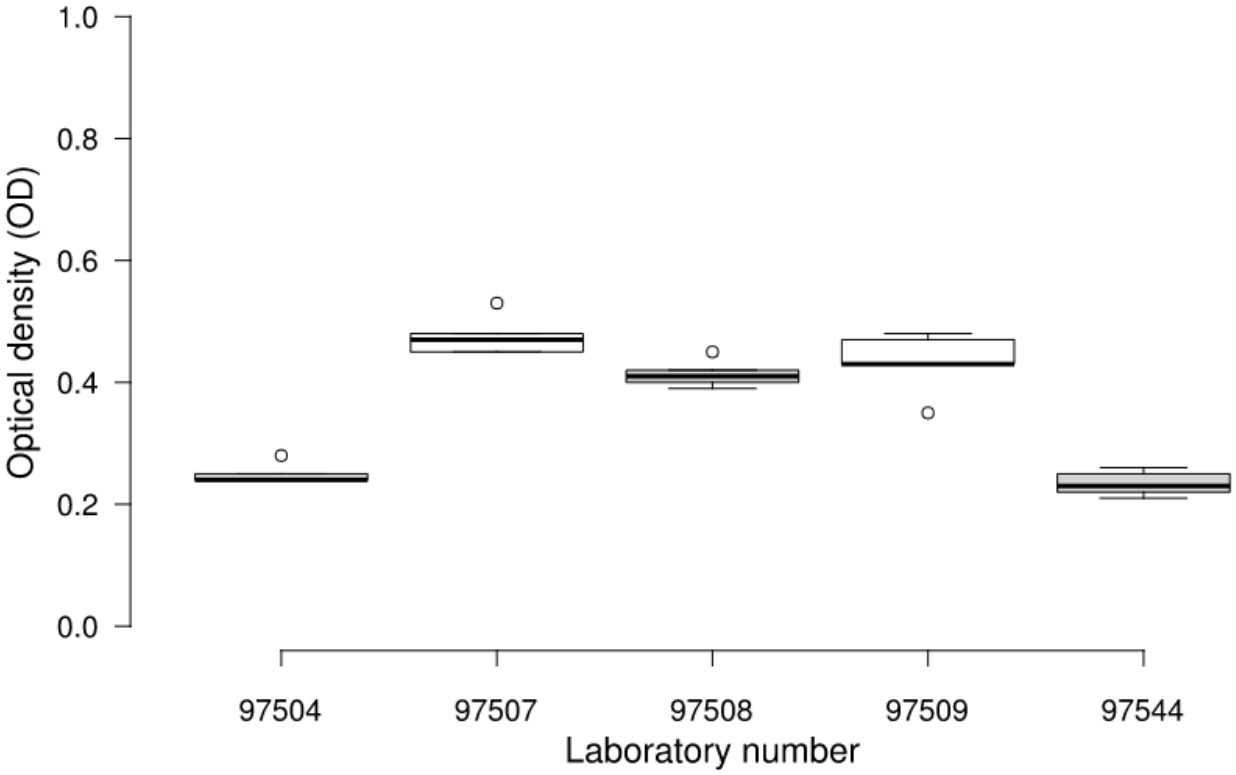


Figure 1. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97508	97509	97544
Method	IDEXX- Mycobacterium Bovis Antibody Test Kit				
OD (REP1)	0,96	1,51	1,36	1,43	0,81
OD (REP2)	0,98	1,62	1,47	1,56	0,83
Mean	0,97	1,57	1,41	1,50	0,82
SD	0,02	0,08	0,08	0,09	0,01
CV (%)	1,61	5,24	5,40	6,24	1,63

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

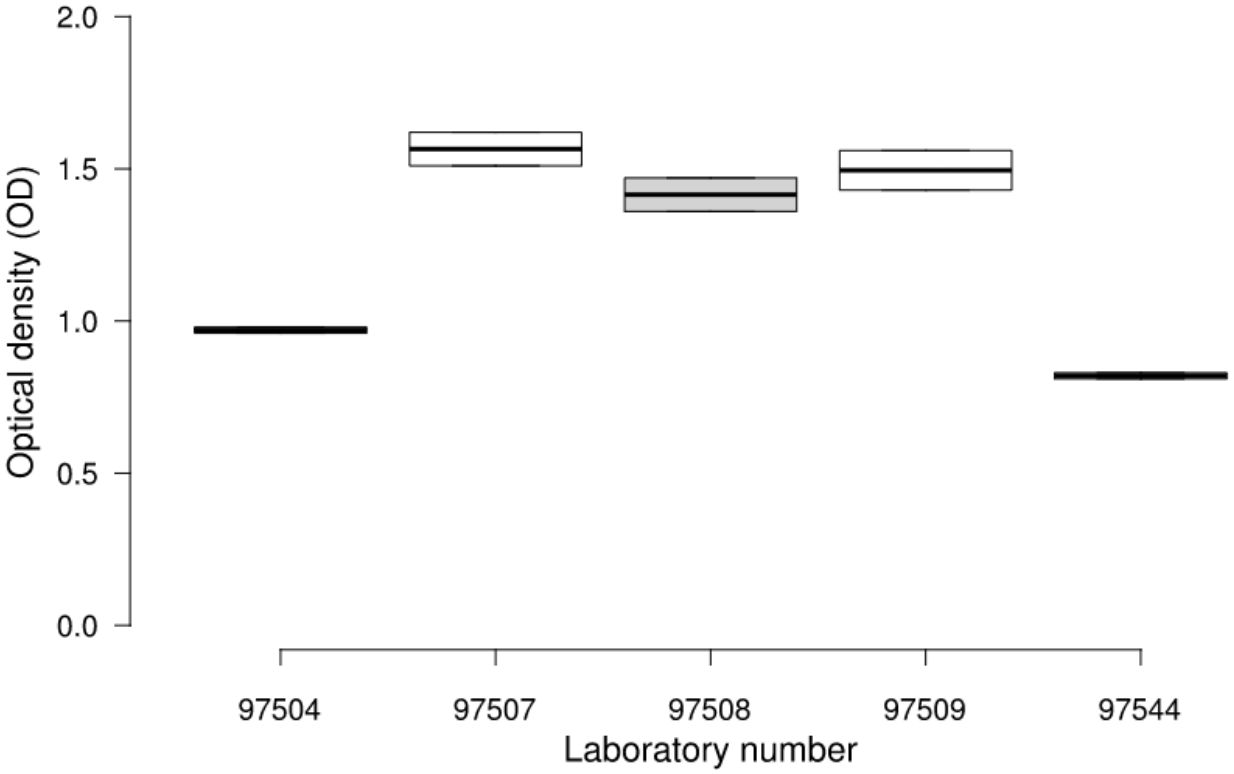


Figure 2. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97508	97509	97544
Method	IDEXX- Mycobacterium Bovis Antibody Test Kit				
OD (REP1)	1,46	2,43	1,72	2,14	1,44
OD (REP2)	1,26	2,03	1,86	2,17	1,32
OD (REP3)	1,24	2,39	1,83	2,04	1,27
Mean	1,32	2,29	1,80	2,12	1,34
SD	0,12	0,22	0,07	0,07	0,08
CV (%)	9,25	9,64	4,13	3,32	6,26

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

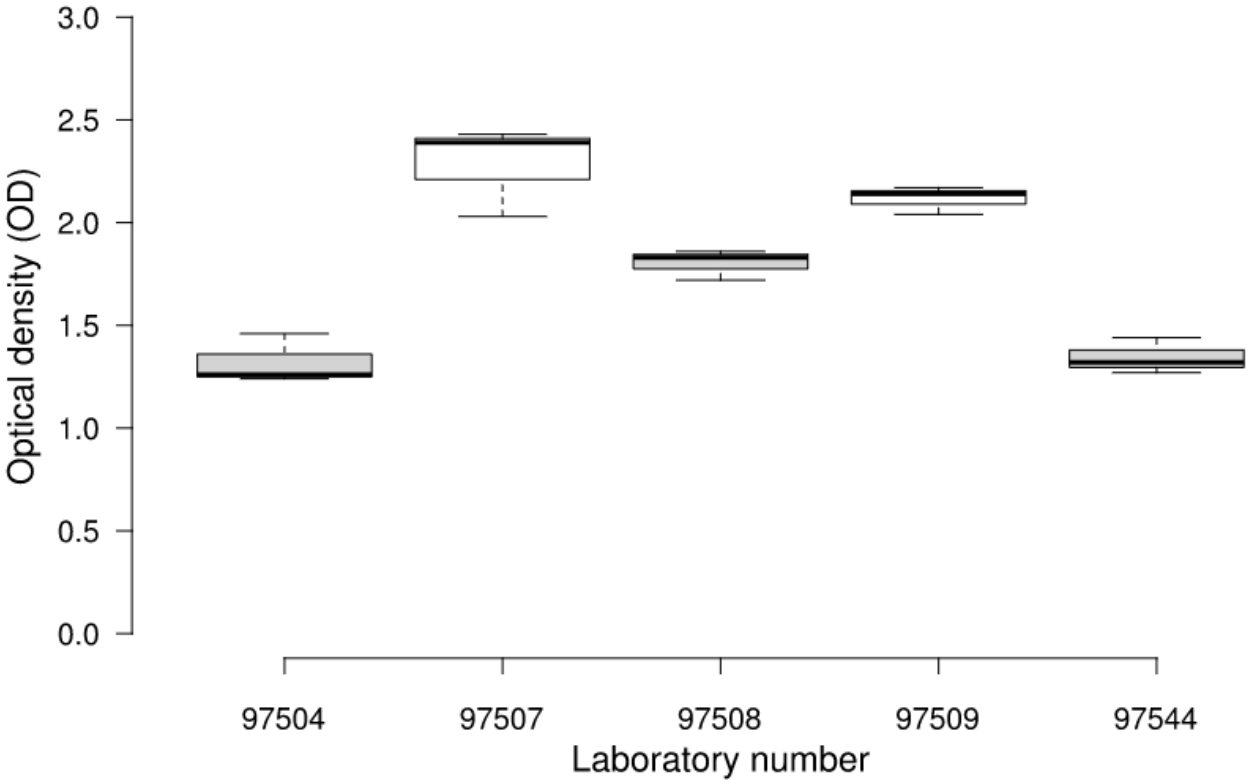


Figure 3. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97508	97509	97544
Method	IDEXX- Mycobacterium Bovis Antibody Test Kit				
OD (REP1)	0,76	0,80	0,71	0,78	0,52
OD (REP2)	0,53	0,83	0,73	0,53	0,41
OD (REP3)	0,70	1,04	0,78	0,75	0,41
OD (REP4)	0,60	0,97	0,62	0,51	0,47
OD (REP5)	0,66	1,24	0,68	0,79	0,40
Mean	0,65	0,98	0,71	0,67	0,44
SD	0,09	0,18	0,06	0,14	0,05
CV (%)	13,14	17,98	8,25	20,96	11,11

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

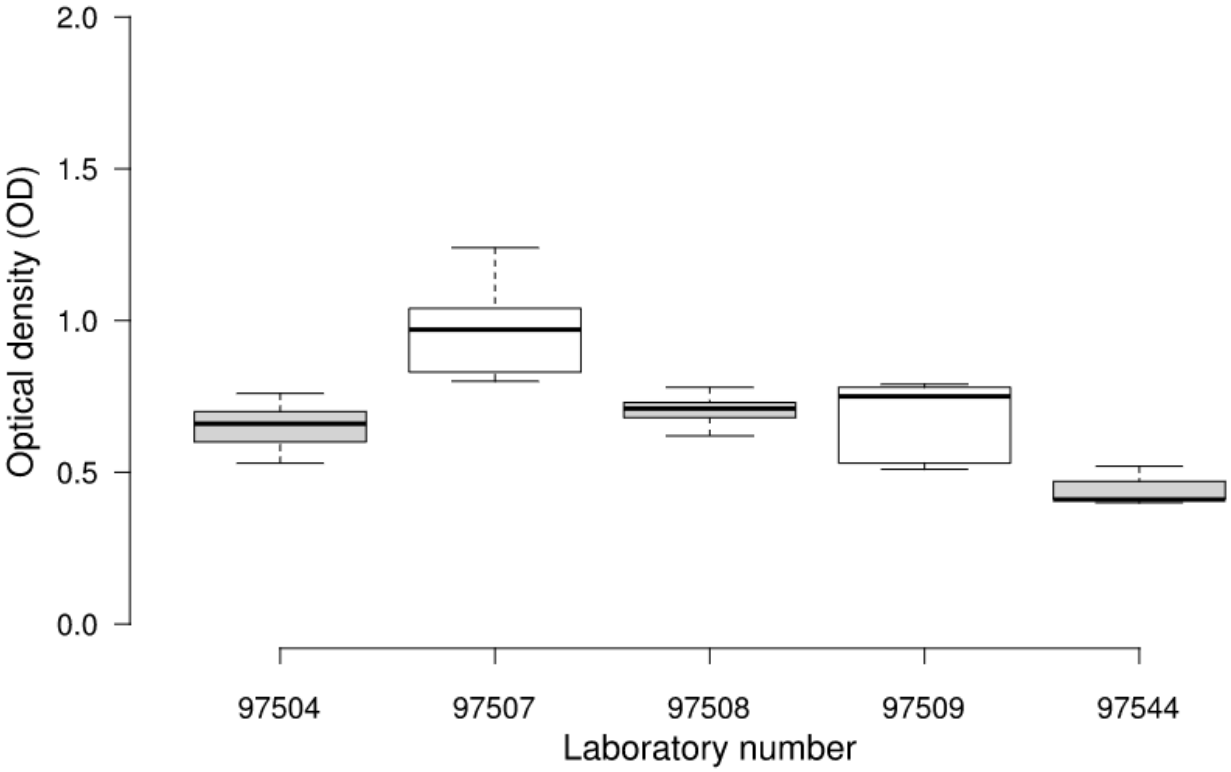


Figure 4. Distribution of the optical densities (box-plots) per laboratory.

6.1.2 GAMMA INTERFERON (PLASMA/SERUM)

PT2022BTPG1

Lab number	97504	97507	97508	97509
Method	ID.VET - ID Screen ruminant IFN-g			
OD (REP1)	1,18	1,13	1,22	1,25
OD (REP2)	1,21	1,19	1,21	1,29
OD (REP3)	1,25	1,20	1,28	1,14
OD (REP4)	1,27	1,19	1,14	1,16
Mean	1,23	1,18	1,21	1,21
SD	0,04	0,03	0,06	0,07
CV (%)	3,19	2,72	4,82	5,99

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

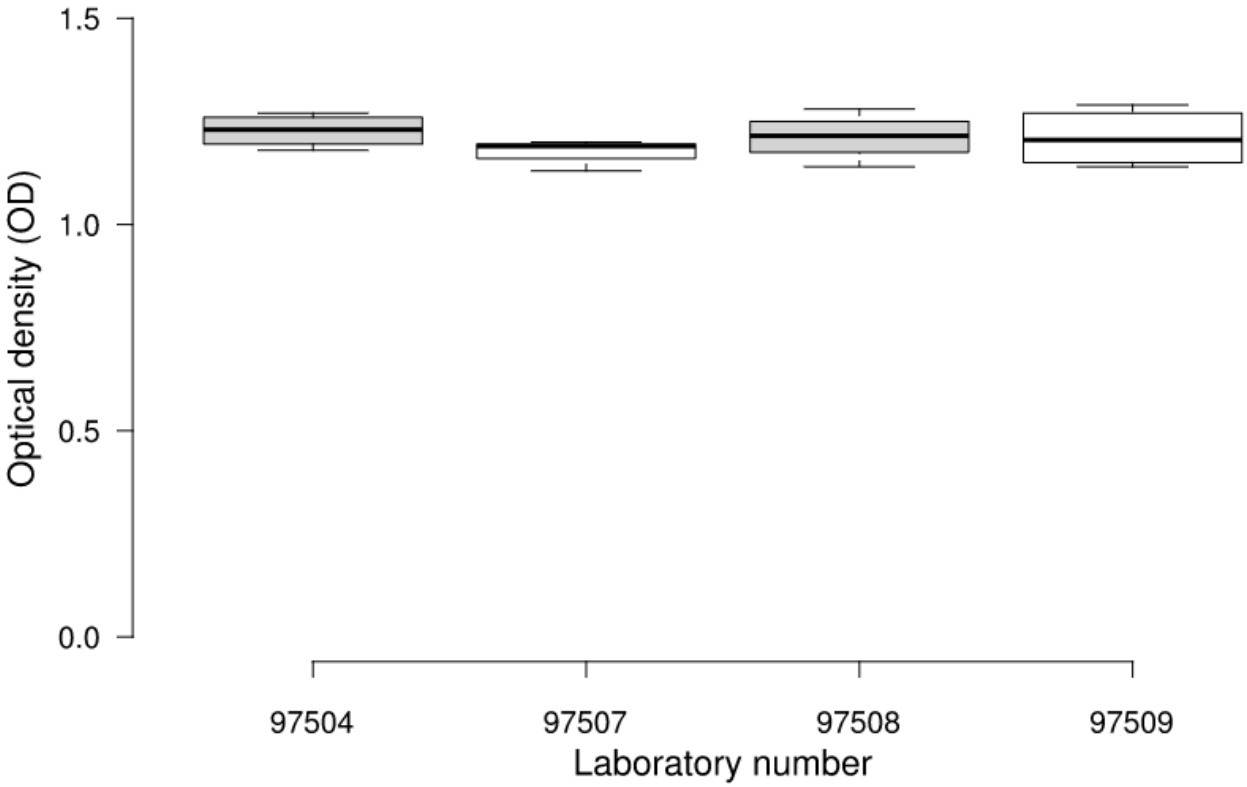


Figure 5. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97508	97509
Method	ID.VET - ID Screen ruminant IFN-g			
OD (REP1)	0,66	0,62	0,58	0,58
OD (REP2)	0,64	0,60	0,72	0,63
OD (REP3)	0,69	0,60	0,68	0,63
OD (REP4)	0,64	0,61	0,75	0,57
OD (REP5)	0,67	0,68	0,64	0,66
Mean	0,66	0,62	0,67	0,62
SD	0,02	0,03	0,07	0,04
CV (%)	2,73	5,38	9,83	6,33

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

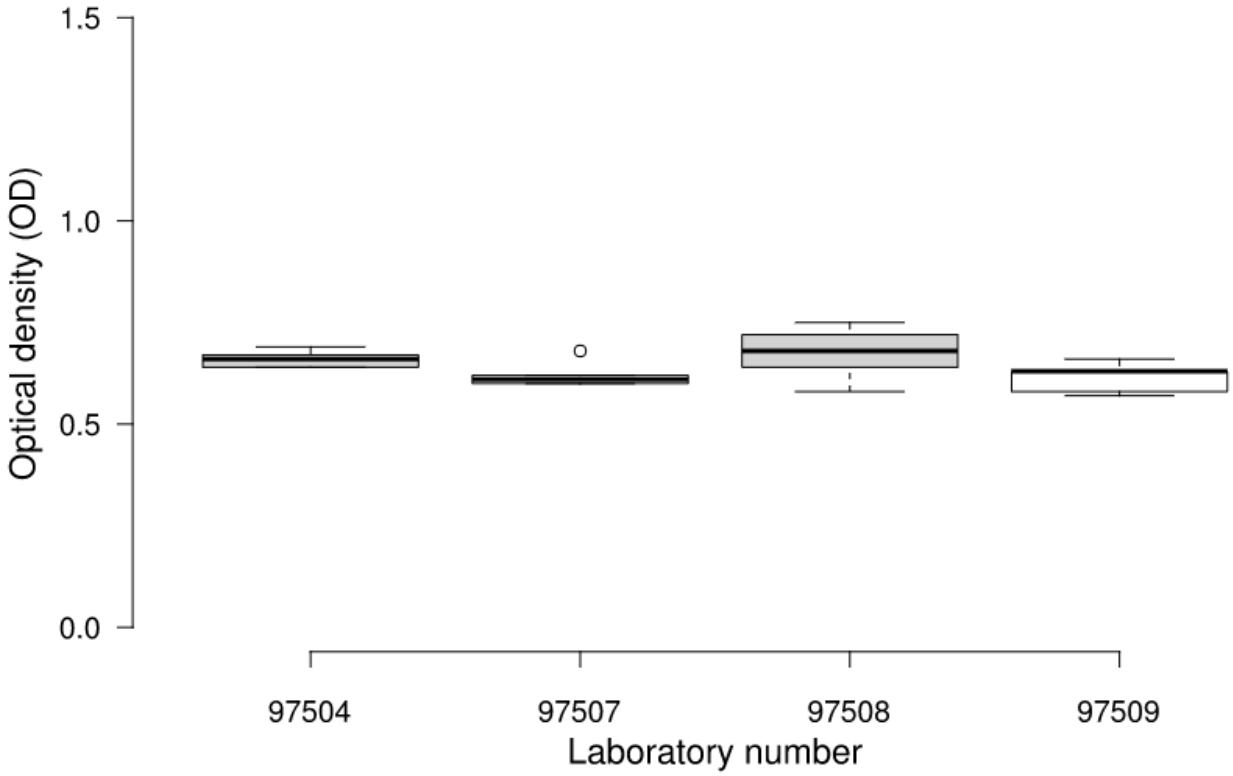


Figure 6. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97504	97507	97508	97509
Method	ID.VET - ID Screen ruminant IFN-g			
OD (REP1)	0,44	0,41	0,45	0,40
OD (REP2)	0,45	0,43	0,41	0,44
OD (REP3)	0,48	0,41	0,41	0,40
OD (REP4)	0,46	0,04*	0,42	0,42
OD (REP5)	0,48	0,43	0,41	0,42
OD (REP6)	0,45	0,40	0,42	0,43
Mean	0,46	0,35	0,42	0,42
SD	0,02	0,15	0,02	0,02
CV (%)	3,55	43,58	3,69	3,86

Numbers were rounded to 2 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation).

* = Lab 97507 entered an OD of 0.04 for sample 11 (4th repetition of sample PG3), while the normalized value is 57.37 which corresponds to an OD of 0.4 according to their formula used. Despite their interpretation being correct for this sample, they made a coding error when entering their OD.

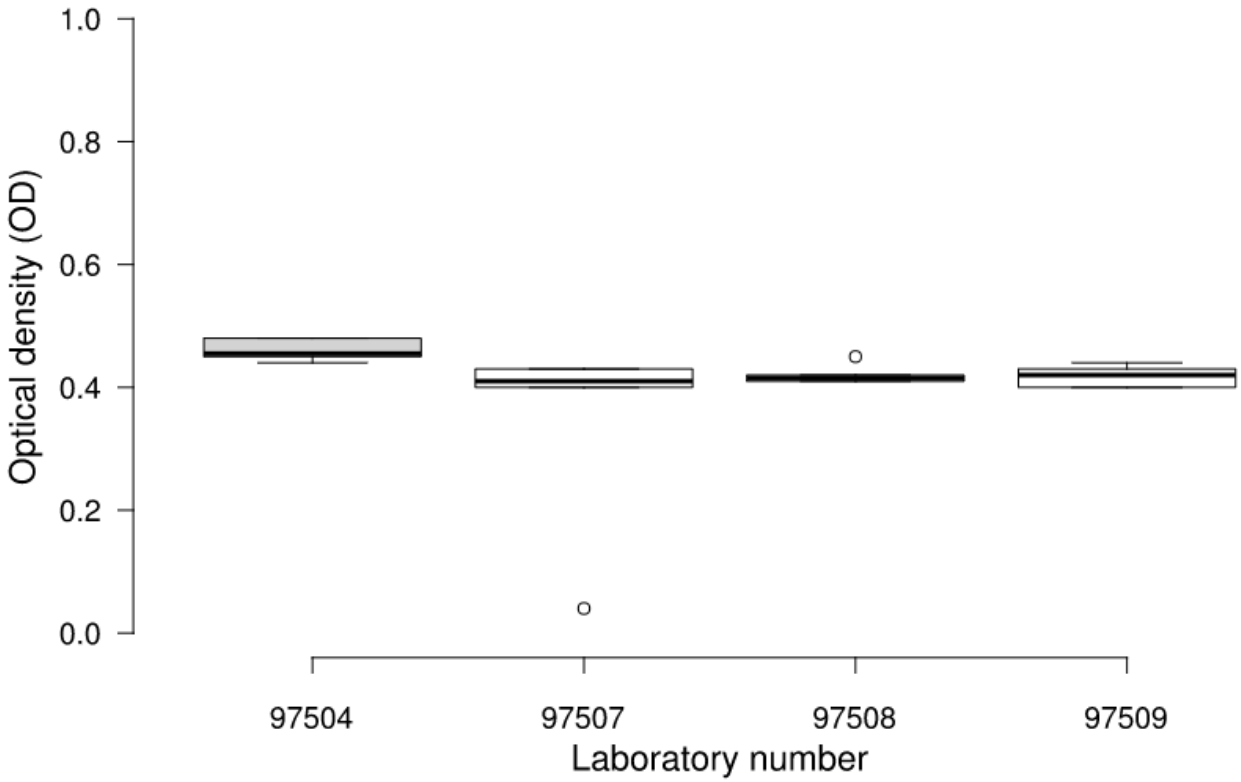


Figure 7. Distribution of the optical densities (box-plots) per laboratory.

A new section has been added to the report and can be found down below:

6.2 Annex 2: Additional calculations for the INFg test

In this annex, we propose to make additional calculations based on the calculation used for the diagnosis of bovine tuberculosis.

Diagnosis of bovine tuberculosis = S/P IFNg bovine PPD – S/P IFNg avian PPD

- If the result of this calculation is > 35%, it is positive
- If the result of this calculation is < 35%, it is negative

To mimic that calculation with the results obtained in this PT, the samples named PG1, PG2 or PG3 were assigned as bovine PPD or avian PPD and calculations were performed.

1. **Results obtained when PG1= bovine PPD and PG2= avian PPD**

To perform the calculations, the first 4 results (expressed as S/P value) given by the laboratories were used for the PG1 and PG2. Indeed, these 2 plasmas were added at least 4 times in the panel of this PT.

97504			
PG1= bovine PPD	PG2= avian PPD	Calculations	Interpretation
110	62	48	pos
113	60	53	pos
116	64	52	pos
118	60	58	pos

97507			
PG1= bovine PPD	PG2= avian PPD	Calculations	Interpretation
160	88	72	pos
169	86	84	pos
171	85	86	pos
169	88	81	pos

97508			
PG1= bovine PPD	PG2= avian PPD	Calculations	Interpretation
129	62	68	pos
128	75	53	pos
135	71	63	pos
120	79	40	pos

97509			
PG1= bovine PPD	PG2= avian PPD	Calculations	Interpretation
125	58	67	pos
128	62	66	pos
113	63	50	pos
116	57	59	pos

2. Results obtained when PG1= bovine PPD and PG3= avian PPD

To perform the calculations, the first 4 results (expressed as S/P value) obtained by the laboratories were used for the PG1 and PG2. Indeed, these 2 plasmas were added at least 4 times in the panel of this PT.

97504			
PG1= bovine PPD	PG3= avian PPD	Calculations	Interpretation
110	41	69	pos
113	42	71	pos
116	45	71	pos
118	42	76	pos

97507			
PG1= bovine PPD	PG3= avian PPD	Calculations	Interpretation
160	59	101	pos
169	61	108	pos
171	58	113	pos
169	57	111	pos

97508			
PG1= bovine PPD	PG3= avian PPD	Calculations	Interpretation
129	47	82	pos
128	43	85	pos
135	43	92	pos
120	44	76	pos

97509			
PG1= bovine PPD	PG3= avian PPD	Calculations	Interpretation
125	40	85	pos
128	44	84	pos
113	40	73	pos
116	42	74	pos

3. Results obtained when PG2= bovine PPD and PG3= avian PPD

To perform the calculations, the first 4 results (expressed as S/P value) obtained by the laboratories were used for the PG1 and PG2. Indeed, these 2 plasmas were added at least 4 times in the panel of this PT.

97504			
PG2= bovine PPD	PG3= avian PPD	Calculations	Interpretation
62	41	21	neg
60	42	18	neg
64	45	19	neg
60	42	18	neg

97507			
PG2= bovine PPD	PG3= avian PPD	Calculations	Interpretation
88	59	29	neg
86	61	25	neg
85	58	27	neg
88	57	31	neg

97508			
PG2= bovine PPD	PG3= avian PPD	Calculations	Interpretation
62	47	14	neg
75	43	32	neg
71	43	28	neg
79	44	35	pos

97509			
PG2= bovine PPD	PG3= avian PPD	Calculations	Interpretation
58	40	18	neg
62	44	18	neg
63	40	23	neg
57	42	15	neg

Overall, the correlation between the different results of the different laboratories were very good as only 1 qualitative result was discordant.

6.3 Annex 3: Additional information

The **preliminary report** of this proficiency test is available on our website via the following link:

- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
- FR: https://www.sciensano.be/fr/evaluation-externe-de-la-qualite/sante-animale-pt-vet
- EN: https://www.sciensano.be/en/external-quality-assessment/animal-health-pt-vet

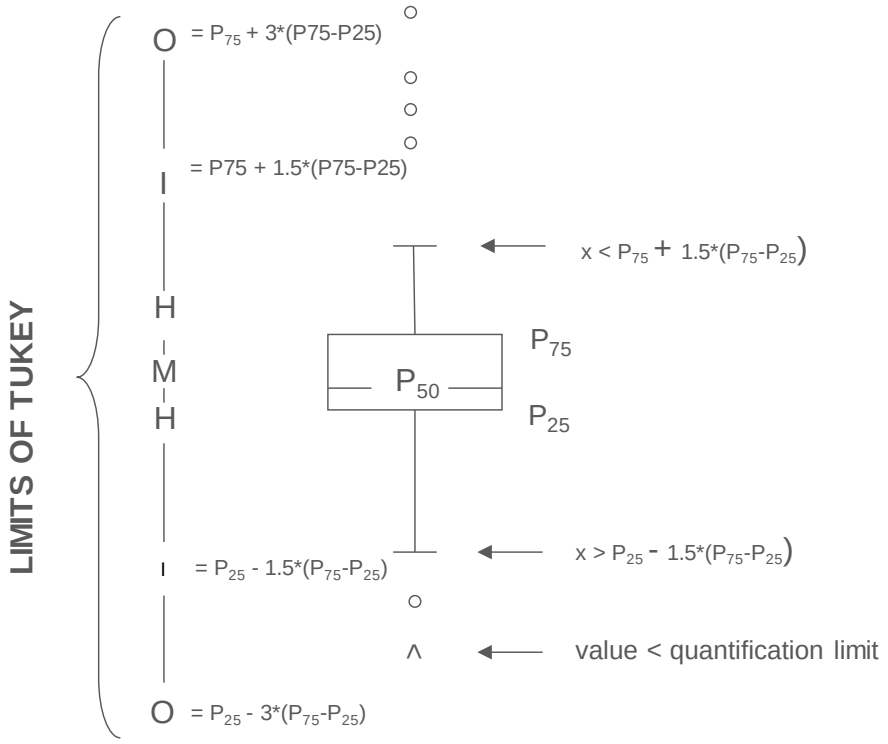
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

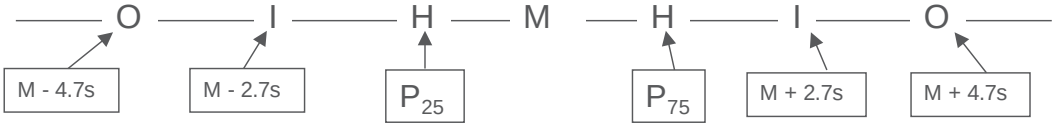
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

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