

**BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES**

COMMITTEE OF EXPERTS

**EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS**

DEFINITIVE GLOBAL REPORT

VETERINARY MEDECINE

AUJESZKY'S DISEASE (AUJ)

PROFICIENCY TEST 2022/7

CORRECTED VERSION

Sciensano/PT VET AUJ/2-E-CV

Biological health risks
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A draft version of this report was submitted to the experts on: 18/11/2022.

The changes in the corrected report are indicated in blue. Changes were made on pages 4, 6, 7, 8 and 9.

This report replaces the previous version of the global report of 01/12/2022.

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

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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

The aim of this PT was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies to Aujeszky's disease (AUJ) virus in serum (gB/gE) of [pigs](#).

3 MATERIALS AND METHODS

3.1 Serology on serum gB

3.1.1 THE PARTICIPANTS

Six laboratories participated in the proficiency test of AUJ serology on serum gB. The names of the participating laboratories are:

- Sciensano, department of Enzootic, vector-borne and bee diseases
- ARSIA
- DGZ
- ANSES-Ploufragan-Unité Virologie Immunologie Porcines_LNR Aujeszky
- IDEXX Diavet (Bäch)
- LSI-Thermofisher Scientific (France)

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Service of Viral reemerging enzootic and BEE diseases, Infectious diseases in animals Directorate, Sciensano.

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

- PT2022AUJGBSERNS1, NS2 and NS3 are negative serum samples collected on three naïve animals.
- PT2022AUJGBSERPS1 is a positive serum sample prepared by the 1/64 dilution of a strong positive serum sample from a field infected animal in a negative serum.
- PT2022AUJGBSERPS2 is an undiluted positive serum sample originated from an animal experimentally challenged.
- PT2022AUJGBSERPS3 is a positive serum sample prepared by the 1/16 dilution of PT2022AUJGBSERPS2 in a negative serum.
- PT2022AUJGBSERPS4 is an undiluted positive serum sample from an experimentally infected animal.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (250 µl) of each sample using ELISA method before the PT. 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 different samples: 5 positive and 5 negative samples.

Sample ID	Repetition	Status
PT2022AUJgBSERPS1	1	POS
PT2022AUJgBSERPS2	1	POS
PT2022AUJgBSERPS3	1	POS
PT2022AUJgBSERPS4	2	POS
PT2022AUJgBSERNS1	2	NEG
PT2022AUJgBSERNS2	2	NEG
PT2022AUJgBSERNS3	1	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The samples were tested before and after the proficiency test. The results were compared and 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 ID: AUJSER gB	97505	97507	97508	97515	97521	97541
22-1	NS3	PS3	PS2	PS2	PS2	NS1
22-2	NS1	NS2	NS2	NS2	NS1	PS4
22-3	PS3	PS4	NS2	PS1	PS3	PS4
22-4	NS2	PS1	NS3	PS3	NS2	NS3
22-5	NS1	NS3	PS3	NS3	NS1	NS1
22-6	PS4	NS2	PS4	NS1	PS4	PS2
22-7	NS2	PS2	PS4	NS1	NS2	NS2
22-8	PS4	NS1	NS1	PS4	NS3	NS2
22-9	PS2	NS1	PS1	PS4	PS4	PS3
22-10	PS1	PS4	NS1	NS2	PS1	PS1

3.2 Serology on serum gE

3.2.1 THE PARTICIPANTS

Nine laboratories participated in the proficiency test of AUJ serology on serum gE. The names of the participating laboratories are:

- Sciensano, department of Enzootic, vector-borne and bee diseases
- ARSIA
- DGZ
- LAVETAN
- ANSES-Ploufragan-Unité Virologie Immunologie Porcines_LNR Aujeszky
- Laboratoire de médecine vétérinaire de l'état (LMVE)
- IDEXX Diavet (Bäch)
- Poulpharm
- LSI-Thermofisher Scientific (France)

3.2.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL), Service of Viral reemerging enzootic and BEE diseases, Infectious diseases in animals Directorate, Sciensano.

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

- PT2022AUJGESERNS1 and NS2 are negative serum samples collected on two naïve animals.
- PT2022AUJGESERPS1 and PS3 are respectively positive and weak positive serum samples prepared by the 1/32 and 1/64 dilution of a strong positive serum sample in a negative serum. The strong positive serum is originated from an animal experimentally vaccinated with NIA3 vaccine strain and challenged.
- PT2022AUJGESERPS2 is a positive serum sample prepared by the 1/8 dilution of a strong positive serum sample from an infected animal in a negative serum
- PT2022AUJGESERPS4 is an undiluted positive serum sample from an experimentally infected animal.

3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 10 aliquots (250 µl) of each sample using ELISA method before the PT. 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 different of samples: 6 positive and 4 negative samples.

Sample ID	Repetition	Status
PT2022AUJgESERPS1	1	POS
PT2022AUJgESERPS2	1	POS
PT2022AUJgESERPS3	2	POS
PT2022AUJgESERPS4	2	POS
PT2022AUJgESERNNS1	2	NEG
PT2022AUJgESERNNS2	2	NEG

(POS = positive; NEG = negative)

3.2.5 STABILITY

The samples were tested before and after the proficiency test. The results were compared and 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 ID: AUJSER gE	97505	97507	97508	97509	97515	97516	97521	97540	97541
22-1	PS4	PS4	PS4	PS2	PS4	PS4	PS4	PS4	NS2
22-2	NS1	PS2	NS1	NS1	NS1	NS1	PS3	NS1	NS2
22-3	PS3	PS4	NS2	NS1	NS1	PS3	PS3	NS1	PS4
22-4	NS2	PS1	PS3	NS2	PS3	PS2	NS1	PS3	PS4
22-5	PS1	PS3	NS1	PS3	PS2	NS2	NS2	NS2	PS2
22-6	PS4	NS2	PS3	PS4	PS3	PS1	NS1	PS1	PS3
22-7	NS1	NS1	PS4	PS4	NS2	PS4	PS4	PS4	PS3
22-8	PS2	PS3	PS2	PS3	PS4	NS1	NS2	PS2	PS1
22-9	PS3	NS1	NS2	NS2	PS1	NS2	PS1	NS2	NS1
22-10	NS2	NS2	PS1	PS1	NS2	PS3	PS2	PS3	NS1

4 TIMELINE

Transfer of the samples from NRL to QL: 25/05/2022

Randomization of the samples by QL: 02/06/2022

Sending samples (cooled at 4 °C) to participants: 07/06/2022

Deadline for submitting the results: 01/07/2022

Preliminary report: 06/09/2022

5 RESULTS

5.1 Serology on serum gB

5.1.1 RESULTS PER SAMPLE

The panel consisted of 7 different samples. Samples PS4, NS1 and NS2 were repeated twice. Therefore, in total, the panel consisted of 10 samples (5 positive and 5 negative samples).

Sample ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	1 (6)	4 POS 1 NEG 1 NI
PS2	POS	1 (6)	6 POS
PS3	POS	1 (6)	6 NEG
PS4	POS	2 (12)	12 POS
NS1	NEG	2 (12)	12 NEG
NS2	NEG	2 (12)	12 NEG
NS3	NEG	1 (6)	6 NEG

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

5.1.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Competition	Thermofisher Scientific - PrioCHECK® PRV gB	Short	4	40	39	98
ELISA Indirect	Idexx - Pseudorabies Virus gB Antibody Test Kit	Long	2	20	20	100
TOTAL			6	60	58	97

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

5.1.3 CONCLUSION

In 2022, six laboratories participated in proficiency test of Aujeszky disease (serum gB) organized by Sciensano. Three methods, PrioCHECK® PRV gB from ThermoFisher Scientific, Pseudorabies Virus gB Antibody Test Kit from idexx and PRV/ADV gB Ab from idexx, were selected by the laboratories for the detection of antibodies to the Aujeszky disease virus gB antigen. Two methods fall under the ELISA blocking (competitive) format and one under the indirect format.

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. Despite the fact that two laboratories gave incorrect answers for the PS1 sample, all the laboratories achieved a satisfactory performance (> 90%) for the detection of AUJgB-specific antibodies in serum samples.

5.2 Serology on serum gE

5.2.1 RESULTS PER SAMPLE

The panel consisted of 6 different samples. Samples PS3, PS4, NS1 and NS2 were repeated twice. Therefore, in total, the panel consisted of 10 samples (6 positive and 4 negative samples).

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 ID	Status	Number of repetitions (total results)	Observed result
PS1	POS	1 (10)	10 POS
PS2	POS	1 (10)	10 POS
PS3	POS	2 (20)	20 POS
PS4	POS	2 (20)	20 POS
NS1	NEG	2 (20)	20 NEG
NS2	NEG	2 (20)	20 NEG

(POS = positive; NEG = negative)

5.2.2 USED METHOD

Method		Short or long incubation protocol	N	NR	NCR	%
ELISA Competition	Idexx - PRV/ADV gl Ab (= PRV/ADV gE)	Short	6	60	60	100
ELISA Competition	Idexx - PRV/ADV gl Ab (= PRV/ADV gE)	Long	1	10	10	100
ELISA Competition	Idexx - PRV/ADV gl Ab (= PRV/ADV gE)	Not applicable	2	20	20	100
ELISA Competition	Thermofisher Scientific - PrioCHECK PRV gE 2.0	Long	1	10	10	100
TOTAL			10	100	100	100

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

5.2.3 CONCLUSION

In 2022, nine laboratories participated in proficiency test of Aujeszky disease (serum gE) organized by Sciensano. Two methods, PRV/ADV gl Ab from Idexx and PrioCHECK PRV gE 2.0 from Thermofisher Scientific, were selected by the laboratories for the detection of antibodies to the Aujeszky disease virus gl antigen (gE). Both methods fall under the ELISA blocking (competitive) format. A distinction was made in the 'PRV/ADV gl Ab' method as the incubation protocol was different or not applicable. One laboratory entered 2 datasets making a total of 10 datasets. In conclusion, both methods achieved a 100% correctness, which implies that 100 correct results were submitted.

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 ON SERUM GB

PT2022AUJgBSERPS4

Lab number	97505	97507	97508	97515	97521	97541
Method	M ₁	M ₁	M ₁	M ₂	M ₃	M ₁
OD (REP1)	0,2	0,1	0,1	0,1	0,1	0,1
OD (REP2)	0,1	0,1	0,1	0,1	0,1	0,1
Mean	0,2	0,1	0,1	0,1	0,1	0,1
SD	0,0	0,0	0,0	0,0	0,0	0,0
CV (%)	2,8	5,2	1,6	1,3	11,9	9,5

Numbers were rounded to 1 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation, M₁ = ThermoFisher Scientific - PrioCHECK® PRV gB; M₂ = Idexx - Pseudorabies Virus gB Antibody Test Kit; M₃ = Idexx - PRV/ADV gB Ab)

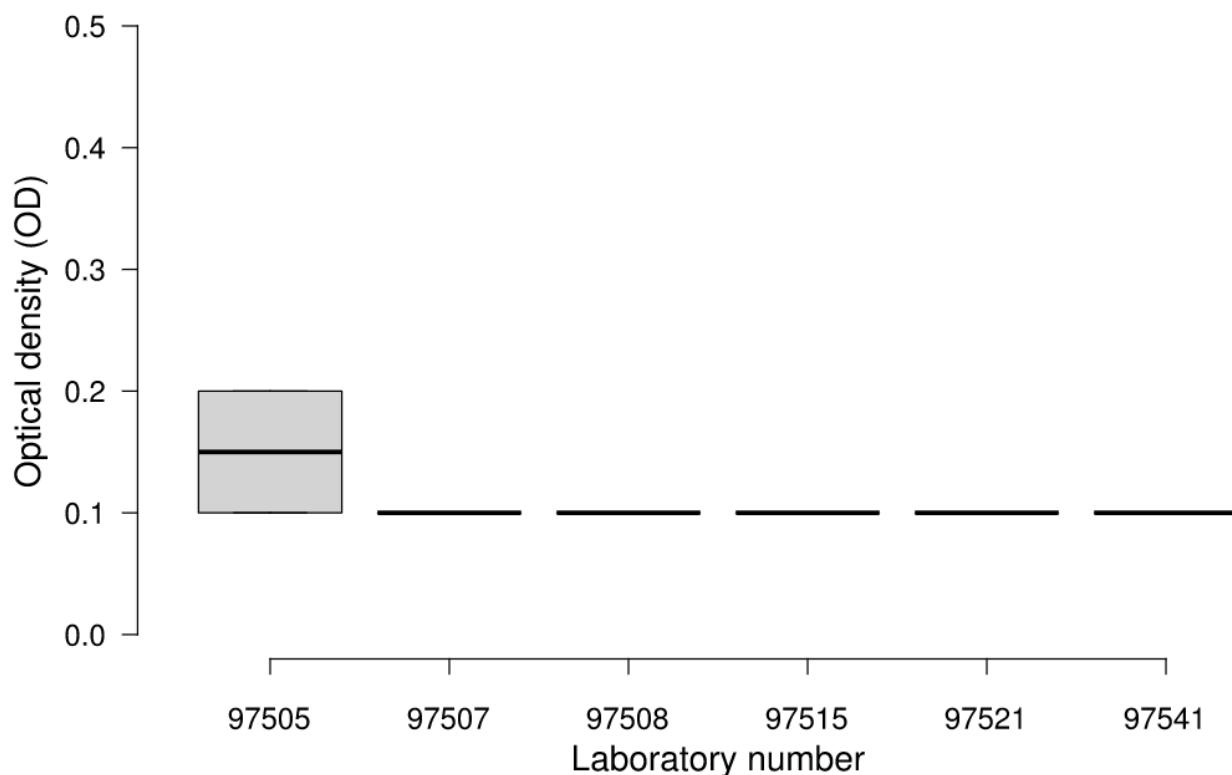


Figure 1. Distribution of the optical densities (box-plots) per laboratory.

6.1.2 SEROLOGY ON SERUM GE

PT2022AUJgESERPS3

Lab number	97505	97507	97508	97509	97515	97516	97521 (1)*	97521 (2)*	97540	97541
Method	M ₁	M ₁	M ₁	M ₃	M ₂	M ₃	M ₁	M ₁	M ₁	M ₄
OD (REP1)	0,6	0,5	0,5	0,8	0,3	0,5	0,5	0,5	0,5	0,9
OD (REP2)	0,5	0,6	0,5	0,7	0,3	0,5	0,5	0,5	0,6	0,9
Mean	0,5	0,5	0,5	0,8	0,3	0,5	0,5	0,5	0,6	0,9
SD	0,0	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0
CV (%)	7,3	10,8	1,1	9,9	2,0	2,9	0,2	5,1	6,7	0,2

Numbers were rounded to 1 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation; M₁ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - short incubation; M₂ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - long incubation; M₃ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - incubation protocol not applicable; M₄ = ThermoFisher Scientific - PrioCHECK PRV gE 2.0 - long incubation).

* = Lab 97521 introduced two datasets for the same method (Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - short incubation) because the method has two presets: one for German users with results in inhibition % and one for English users in S/N. The results were the same for both presets.

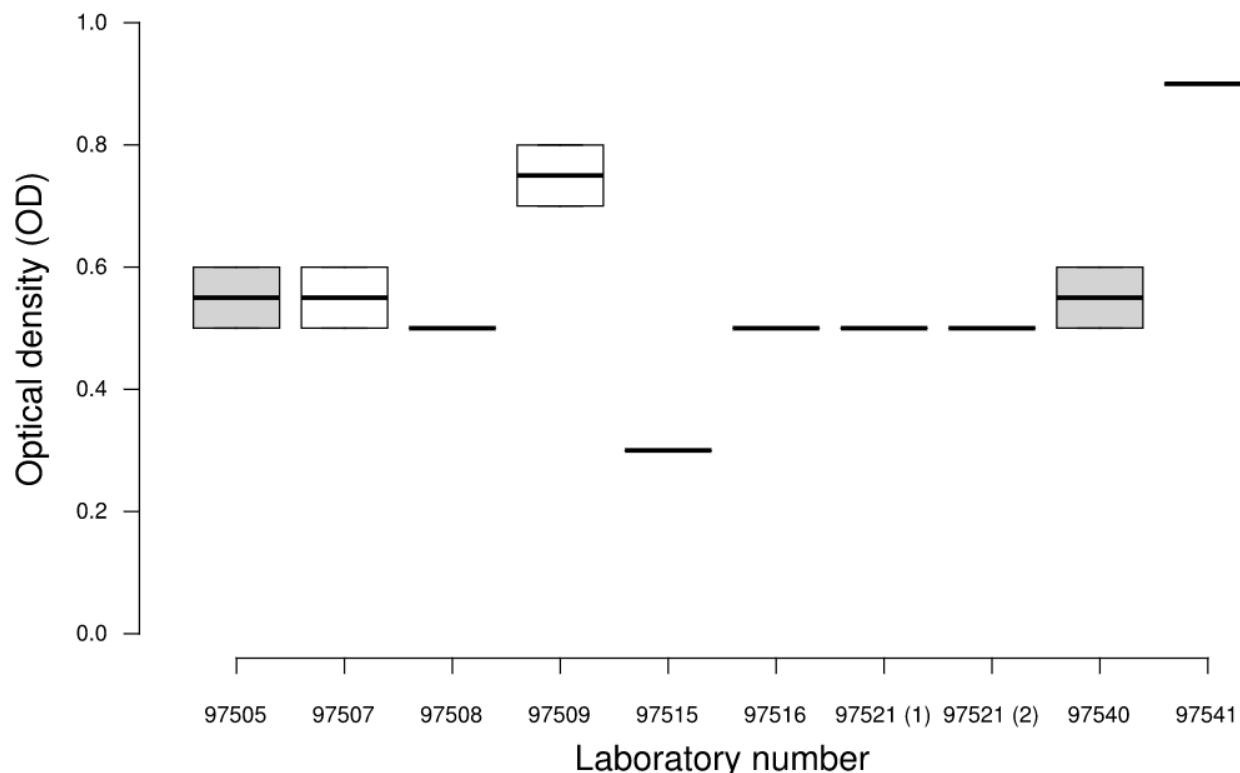


Figure 2. Distribution of the optical densities (box-plots) per laboratory.

Lab number	97505	97507	97508	97509	97515	97516	97521 (1)*	97521 (2)*	97540	97541
Method	M ₁	M ₁	M ₁	M ₃	M ₂	M ₃	M ₁	M ₁	M ₁	M ₄
OD (REP1)	0,3	0,3	0,3	0,5	0,2	0,3	0,2	0,2	0,3	0,8
OD (REP2)	0,3	0,3	0,3	0,5	0,2	0,3	0,2	0,2	0,3	0,8
Mean	0,3	0,3	0,3	0,5	0,2	0,3	0,2	0,2	0,3	0,8
SD	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
CV (%)	3,9	1,2	1,6	1,1	7,2	3,3	1,7	2,3	0,5	3,8

Numbers were rounded to 1 decimal place. (OD = optical density; REP = repetition; SD = standard deviation; CV = coefficient of variation; M₁ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - short incubation; M₂ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - long incubation; M₃ = Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - incubation protocol not applicable; M₄ = ThermoFisher Scientific - PrioCHECK PRV gE 2.0 - long incubation).

* = Lab 97521 introduced two datasets for the same method (Idexx - PRV/ADV gl Ab (= PRV/ADV gE) - short incubation) because the method has two presets: one for German users with results in inhibition % and one for English users in S/N. The results were the same for both presets.

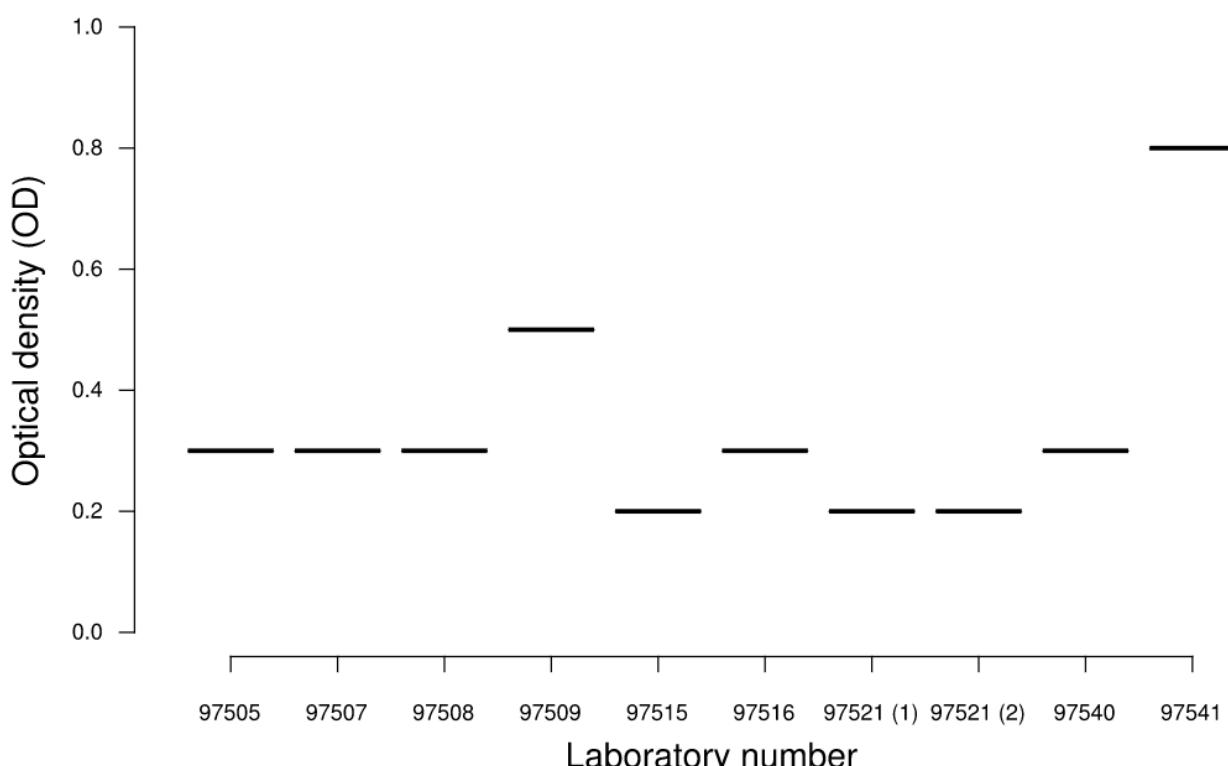


Figure 3. Distribution of the optical densities (box-plots) per laboratory.

Annex 2: 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>

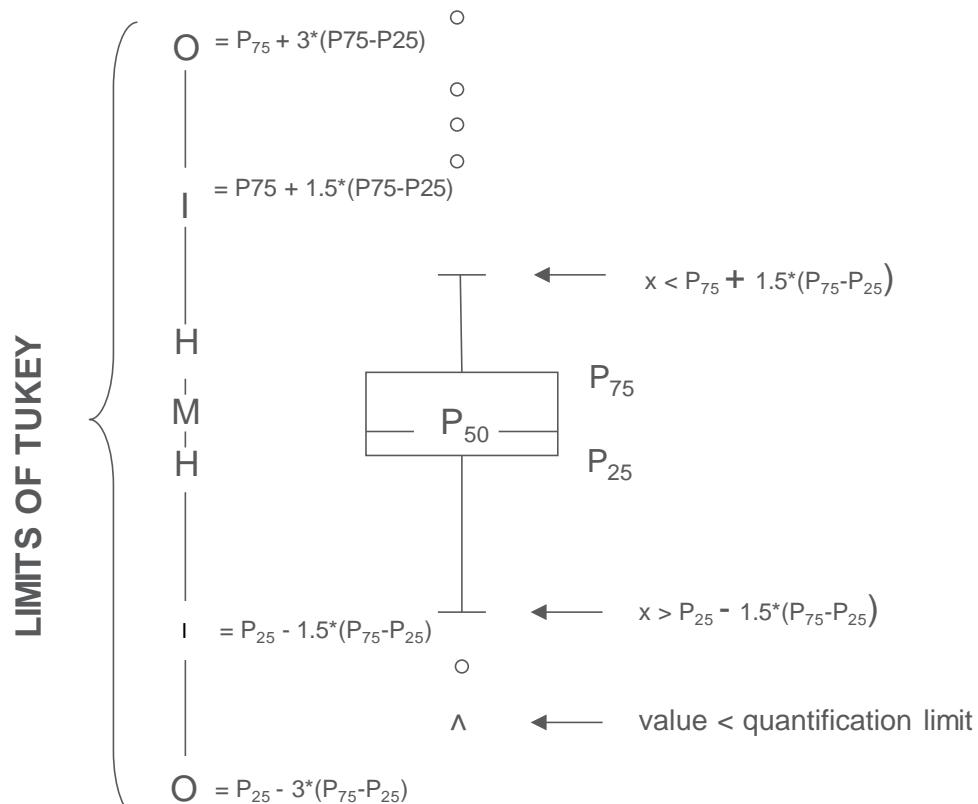
The calendar for Proficiency Testing in Veterinary diagnosis is available on our website:

- 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 (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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