BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

# DEFINITIVE GLOBAL REPORT

# VETERINARY MEDICINE

# PARATUBERCULOSIS (PARATUB)

# PROFICIENCY TEST 2023-8

**Sciensano/PT VET PARATUB/2023-8/E**
Biological health risks
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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 Paratuberculosis (serology) was to evaluate the ability of the participating laboratories to detect the absence or presence of antibodies against *Mycobacterium avium subsp.* in serum and milk of cattle, using ELISA.

# 3 MATERIALS AND METHODS

## 3.1 Serology (serum)

### 3.1.1 THE PARTICIPANTS

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

- Sciensano, department of Veterinary Bacteriology
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- LAVETAN
- LNCR / ACSEDIATE
- Biosellal
- Laboratoire de Médecine Vétérinaire de l'Etat du Grand-Duché de Luxembourg (LMVE)
- INDICAL BIOSCIENCE GmbH
- Poulpharm

### 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 200 µL 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 to rest the sample for another 10 minutes before using it.

Information about the origin and preparation of the samples:

- PT2023PT_PS1 was a positive serum sample collected from naturally infected animal (RT-PCR positive in organs). The serum was diluted 1/16 in negative serum.
- PT2023PT_PS2 was a positive serum sample collected from naturally infected animal (RT-PCR positive in organs), same animal as PS1. The serum was diluted 1/25 in negative serum.
- PT2023PT_PS3 was a positive serum sample collected from naturally infected animal (RT-PCR positive in organs), another animal as PS1 and PS2. The serum was diluted 1/16 in negative serum.
- PT2023PT_NS1 was a negative serum sample collected from field negative animal.
- PT2023PT_NS2 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 three aliquots (0,2 mL) of each sample using ELISA. The samples were considered as homogeneous.

### 3.1.4 TARGET VALUES

The panel consisted of five different samples. However, repetitions were included in both positive and negative samples. Therefore, the panel included 20 samples in total.

| Sample content | Repetition | Expected result |
|---|---|---|
| **PT2023PT_PS1** | 3 | POS |
| **PT2023PT_PS2** | 5 | POS |
| **PT2023PT_PS3** | 5 | POS |
| **PT2023PT_NS1** | 4 | NEG |
| **PT2023PT_NS2** | 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:

| PT2023PT_ | 97504 | 97507 | 97508 | 97509 | 97510 |
|---|---|---|---|---|---|
| PS1 (1) | PTSERUM23-4 | PTSERUM23-4 | PTSERUM23-3 | PTSERUM23-9 | PTSERUM23-6 |
| PS1 (2) | PTSERUM23-7 | PTSERUM23-14 | PTSERUM23-18 | PTSERUM23-12 | PTSERUM23-7 |
| PS1 (3) | PTSERUM23-19 | PTSERUM23-19 | PTSERUM23-20 | PTSERUM23-20 | PTSERUM23-19 |
| PS2 (1) | PTSERUM23-3 | PTSERUM23-1 | PTSERUM23-4 | PTSERUM23-5 | PTSERUM23-3 |
| PS2 (2) | PTSERUM23-8 | PTSERUM23-3 | PTSERUM23-6 | PTSERUM23-6 | PTSERUM23-8 |
| PS2 (3) | PTSERUM23-10 | PTSERUM23-7 | PTSERUM23-8 | PTSERUM23-7 | PTSERUM23-9 |
| PS2 (4) | PTSERUM23-13 | PTSERUM23-17 | PTSERUM23-13 | PTSERUM23-14 | PTSERUM23-11 |
| PS2 (5) | PTSERUM23-18 | PTSERUM23-18 | PTSERUM23-15 | PTSERUM23-17 | PTSERUM23-16 |
| PS3 (1) | PTSERUM23-1 | PTSERUM23-2 | PTSERUM23-1 | PTSERUM23-1 | PTSERUM23-1 |
| PS3 (2) | PTSERUM23-9 | PTSERUM23-5 | PTSERUM23-5 | PTSERUM23-8 | PTSERUM23-2 |
| PS3 (3) | PTSERUM23-11 | PTSERUM23-9 | PTSERUM23-7 | PTSERUM23-13 | PTSERUM23-5 |
| PS3 (4) | PTSERUM23-16 | PTSERUM23-10 | PTSERUM23-10 | PTSERUM23-18 | PTSERUM23-14 |
| PS3 (5) | PTSERUM23-20 | PTSERUM23-15 | PTSERUM23-12 | PTSERUM23-19 | PTSERUM23-17 |
| NS1 (1) | PTSERUM23-6 | PTSERUM23-6 | PTSERUM23-14 | PTSERUM23-2 | PTSERUM23-4 |
| NS1 (2) | PTSERUM23-14 | PTSERUM23-8 | PTSERUM23-16 | PTSERUM23-4 | PTSERUM23-15 |
| NS1 (3) | PTSERUM23-15 | PTSERUM23-11 | PTSERUM23-17 | PTSERUM23-11 | PTSERUM23-18 |
| NS1 (4) | PTSERUM23-17 | PTSERUM23-20 | PTSERUM23-19 | PTSERUM23-16 | PTSERUM23-20 |
| NS2 (1) | PTSERUM23-2 | PTSERUM23-12 | PTSERUM23-2 | PTSERUM23-3 | PTSERUM23-10 |
| NS2 (2) | PTSERUM23-5 | PTSERUM23-13 | PTSERUM23-9 | PTSERUM23-10 | PTSERUM23-12 |
| NS2 (3) | PTSERUM23-12 | PTSERUM23-16 | PTSERUM23-11 | PTSERUM23-15 | PTSERUM23-13 |

| PT2023PT_ | 97514 | 97516 | 97532 | 97540 |
|---|---|---|---|---|
| PS1 (1) | PTSERUM23-6 | PTSERUM23-4 | PTSERUM23-3 | PTSERUM23-9 |
| PS1 (2) | PTSERUM23-14 | PTSERUM23-5 | PTSERUM23-8 | PTSERUM23-11 |
| PS1 (3) | PTSERUM23-16 | PTSERUM23-14 | PTSERUM23-9 | PTSERUM23-20 |
| PS2 (1) | PTSERUM23-2 | PTSERUM23-1 | PTSERUM23-1 | PTSERUM23-3 |
| PS2 (2) | PTSERUM23-9 | PTSERUM23-2 | PTSERUM23-5 | PTSERUM23-4 |
| PS2 (3) | PTSERUM23-11 | PTSERUM23-7 | PTSERUM23-14 | PTSERUM23-8 |
| PS2 (4) | PTSERUM23-15 | PTSERUM23-11 | PTSERUM23-17 | PTSERUM23-16 |

| | | | | |
|---|---|---|---|---|
| **PS2 (5)** | PTSERUM23-19 | PTSERUM23-17 | PTSERUM23-20 | PTSERUM23-18 |
| **PS3 (1)** | PTSERUM23-7 | PTSERUM23-12 | PTSERUM23-2 | PTSERUM23-1 |
| **PS3 (2)** | PTSERUM23-10 | PTSERUM23-13 | PTSERUM23-7 | PTSERUM23-10 |
| **PS3 (3)** | PTSERUM23-12 | PTSERUM23-15 | PTSERUM23-11 | PTSERUM23-12 |
| **PS3 (4)** | PTSERUM23-17 | PTSERUM23-16 | PTSERUM23-15 | PTSERUM23-15 |
| **PS3 (5)** | PTSERUM23-20 | PTSERUM23-19 | PTSERUM23-19 | PTSERUM23-19 |
| **NS1 (1)** | PTSERUM23-3 | PTSERUM23-3 | PTSERUM23-4 | PTSERUM23-5 |
| **NS1 (2)** | PTSERUM23-5 | PTSERUM23-8 | PTSERUM23-12 | PTSERUM23-7 |
| **NS1 (3)** | PTSERUM23-8 | PTSERUM23-10 | PTSERUM23-13 | PTSERUM23-14 |
| **NS1 (4)** | PTSERUM23-18 | PTSERUM23-20 | PTSERUM23-16 | PTSERUM23-17 |
| **NS2 (1)** | PTSERUM23-1 | PTSERUM23-6 | PTSERUM23-6 | PTSERUM23-2 |
| **NS2 (2)** | PTSERUM23-4 | PTSERUM23-9 | PTSERUM23-10 | PTSERUM23-6 |
| **NS2 (3)** | PTSERUM23-13 | PTSERUM23-18 | PTSERUM23-18 | PTSERUM23-13 |

## 3.2 Serology (milk)

### 3.2.1 THE PARTICIPANTS

Seven laboratories participated in the proficiency test of Paratuberculosis on milk. The names of the participating laboratories are:

- Sciensano, department of Veterinary Bacteriology
- LAVETAN
- MCC-Vlaanderen
- Comité du Lait
- Biosellal
- Laboratoire de Médecine Vétérinaire de l'Etat du Grand-Duché de Luxembourg (LMVE)
- INDICAL BIOSCIENCE GmbH

### 3.2.2 THE SAMPLES

The samples (lyophilized milk) were prepared by the National Reference Laboratory (NRL), department of Veterinary Bacteriology, Sciensano. Participants were instructed to reconstitute the milk with 500 µL 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 to rest the sample for another 10 minutes before using it.

Information about the origin and preparation of the samples:

- PT2023PT_PM1 was a positive milk sample collected from naturally infected animal, RT-PCR positive in faeces, undiluted.
- PT2023PT_PM2 was a positive milk sample prepared by diluting a positive serum in negative commercial milk, 1/150. The serum was collected from naturally infected animal, positive in RT-PCR in organs.
- PT2023PT_PM3 was a positive milk sample was prepared by diluting a positive serum in negative commercial milk, 1/120. The serum was collected from naturally infected animal, positive in RT-PCR in organs.
- PT2023PT_NM1 was a negative milk sample collected from field negative animal from a herd historically negative for paratuberculosis.
- PT2023PT_NM2 was a negative milk sample collected from field negative animal from a herd historically negative for paratuberculosis.

### 3.2.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on three aliquots (0,5 mL) of each sample using ELISA. The samples were considered as homogeneous.

### 3.2.4 TARGET VALUES

The panel consisted of five different samples. However, repetitions were included in both positive and negative samples. Therefore, the panel included 20 samples in total.

| Sample content | Repetition | Expected result |
|---|---|---|
| **PT2023PT_PM1** | 3 | POS |
| **PT2023PT_PM2** | 5 | POS |
| **PT2023PT_PM3** | 5 | POS |
| **PT2023PT_NM1** | 4 | NEG |
| **PT2023PT_NM2** | 3 | NEG |

*(POS = positive; NEG = negative)*

### 3.2.5 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.

### 3.2.6 RANDOMISATION AND PANEL COMPOSITION

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

| PT2023PT_ | 97504 | 97509 | 97511 | 97512 | 97514 |
|---|---|---|---|---|---|
| **PM1 (1)** | PTMILK23-4 | PTMILK23-1 | PTMILK23-2 | PTMILK23-5 | PTMILK23-17 |
| **PM1 (2)** | PTMILK23-5 | PTMILK23-13 | PTMILK23-3 | PTMILK23-12 | PTMILK23-19 |
| **PM1 (3)** | PTMILK23-7 | PTMILK23-15 | PTMILK23-8 | PTMILK23-13 | PTMILK23-20 |
| **PM2 (1)** | PTMILK23-3 | PTMILK23-3 | PTMILK23-11 | PTMILK23-1 | PTMILK23-1 |
| **PM2 (2)** | PTMILK23-8 | PTMILK23-4 | PTMILK23-12 | PTMILK23-4 | PTMILK23-8 |
| **PM2 (3)** | PTMILK23-13 | PTMILK23-5 | PTMILK23-13 | PTMILK23-10 | PTMILK23-10 |
| **PM2 (4)** | PTMILK23-15 | PTMILK23-14 | PTMILK23-15 | PTMILK23-16 | PTMILK23-13 |
| **PM2 (4)** | PTMILK23-18 | PTMILK23-18 | PTMILK23-16 | PTMILK23-17 | PTMILK23-18 |
| **PM3 (1)** | PTMILK23-2 | PTMILK23-6 | PTMILK23-1 | PTMILK23-2 | PTMILK23-4 |
| **PM3 (2)** | PTMILK23-9 | PTMILK23-7 | PTMILK23-5 | PTMILK23-8 | PTMILK23-12 |
| **PM3 (3)** | PTMILK23-14 | PTMILK23-8 | PTMILK23-7 | PTMILK23-15 | PTMILK23-14 |
| **PM3 (4)** | PTMILK23-16 | PTMILK23-12 | PTMILK23-18 | PTMILK23-18 | PTMILK23-15 |
| **PM3 (4)** | PTMILK23-17 | PTMILK23-20 | PTMILK23-20 | PTMILK23-20 | PTMILK23-16 |
| **NM1 (1)** | PTMILK23-6 | PTMILK23-10 | PTMILK23-4 | PTMILK23-3 | PTMILK23-3 |
| **NM1 (2)** | PTMILK23-10 | PTMILK23-16 | PTMILK23-6 | PTMILK23-9 | PTMILK23-5 |
| **NM1 (3)** | PTMILK23-11 | PTMILK23-17 | PTMILK23-9 | PTMILK23-11 | PTMILK23-7 |

| PT2023PT_ | 97504 | 97509 | 97511 | 97512 | 97514 |
|---|---|---|---|---|---|
| NM1 (4) | PTMILK23-20 | PTMILK23-19 | PTMILK23-10 | PTMILK23-19 | PTMILK23-9 |
| NM2 (1) | PTMILK23-1 | PTMILK23-2 | PTMILK23-14 | PTMILK23-6 | PTMILK23-2 |
| NM2 (2) | PTMILK23-12 | PTMILK23-9 | PTMILK23-17 | PTMILK23-7 | PTMILK23-6 |
| NM2 (3) | PTMILK23-19 | PTMILK23-11 | PTMILK23-19 | PTMILK23-14 | PTMILK23-11 |

| PT2023PT_ | 97516 | 97532 |
|---|---|---|
| PM1 (1) | PTMILK23-2 | PTMILK23-1 |
| PM1 (2) | PTMILK23-8 | PTMILK23-18 |
| PM1 (3) | PTMILK23-17 | PTMILK23-20 |
| PM2 (1) | PTMILK23-5 | PTMILK23-8 |
| PM2 (2) | PTMILK23-12 | PTMILK23-10 |
| PM2 (3) | PTMILK23-16 | PTMILK23-12 |
| PM2 (4) | PTMILK23-18 | PTMILK23-13 |
| PM2 (4) | PTMILK23-20 | PTMILK23-17 |
| PM3 (1) | PTMILK23-4 | PTMILK23-6 |
| PM3 (2) | PTMILK23-6 | PTMILK23-7 |
| PM3 (3) | PTMILK23-9 | PTMILK23-11 |
| PM3 (4) | PTMILK23-13 | PTMILK23-15 |
| PM3 (4) | PTMILK23-14 | PTMILK23-16 |
| NM1 (1) | PTMILK23-1 | PTMILK23-2 |
| NM1 (2) | PTMILK23-3 | PTMILK23-4 |
| NM1 (3) | PTMILK23-7 | PTMILK23-14 |
| NM1 (4) | PTMILK23-19 | PTMILK23-19 |
| NM2 (1) | PTMILK23-10 | PTMILK23-3 |
| NM2 (2) | PTMILK23-11 | PTMILK23-5 |
| NM2 (3) | PTMILK23-15 | PTMILK23-9 |

# 4 TIMELINE

Transfer of the samples from NRL to QL: 21/08/2023
Randomization of the samples by QL: 25/08/2023
Sending samples to participants: in the week of 28/08/2023

- Samples serum and milk: refrigerated at 4 °C

Deadline for submitting the results: 15/09/2023
Individual report to the participants: 17/11/2023

# 5 RESULTS

## 5.1 Serology (serum)

### 5.1.1 RESULTS PER SAMPLE

The panel consisted of five different samples. However, repetitions were included in both positive and negative samples. Therefore, the panel included 20 samples in total.

Two 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 | Status | Number of repetitions (total results) | Observed result |
|---|---|---|---|
| **PS1** | POS | 3 (33) | 33 POS |
| **PS2** | POS | 5 (55) | 55 POS |
| **PS3** | POS | 5 (55) | 51 POS<br>4 NEG |
| **NS1** | NEG | 4 (44) | 44 NEG |
| **NS2** | NEG | 3 (33) | 33 NEG |

*(POS = positive; NEG = negative)*

### 5.1.2 USED METHOD

| Method | Name producer | Name kit | N | NR | NCR | % |
|---|---|---|---|---|---|---|
| ELISA Indirect | ID.VET | ID Screen® Paratuberculosis Indirect | **5** | **100** | **100** | **100** |
| ELISA Indirect | IDEXX | Paratuberculosis Screening Ab Test | **4** | **80** | **80** | **100** |
| ELISA Indirect | *Not known* | *Not known* | **2** | **40** | **36** | **90** |
| **TOTAL** | | | **11** | **220** | **216** | **98** |

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

### 5.1.3 CONCLUSION

In 2023, nine laboratories participated in proficiency test of Paratuberculosis 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 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. Eight laboratories succeeded in achieving the maximum score (100%) for this test. One laboratory has a score of 80% as the sample PS3 was misinterpreted four out of five times. However, an overall score of 98% was achieved.

## 5.2 Serology (milk)

### 5.2.1 RESULTS PER SAMPLE

The panel consisted of five different samples. However, repetitions were included in both positive and negative samples. Therefore, the panel included 20 samples in total.

| Sample ID | Status | Number of repetitions (total results) | Observed result |
|---|---|---|---|
| **PM1** | POS | 3 (21) | 21 POS |
| **PM2** | POS | 5 (35) | 35 POS |
| **PM3** | POS | 5 (35) | 35 POS |
| **NM1** | NEG | 4 (28) | 28 NEG |
| **NM2** | NEG | 3 (21) | 21 NEG |

*(POS = positive; NEG = negative)*

### 5.2.2 USED METHOD

| Method | Name producer | Name kit | N | NR | NCR | % |
|---|---|---|---|---|---|---|
| ELISA Indirect | IDEXX | Paratuberculosis screening Ab test | **5** | **100** | **100** | **100** |
| ELISA Indirect | *Not known* | *Not known* | **2** | **40** | **40** | **100** |
| **TOTAL** | | | **7** | **140** | **140** | **100** |

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

### 5.2.3 CONCLUSION

In 2023, seven laboratories participated in proficiency test of Paratuberculosis serology (milk) 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 SEROLOGY (SERUM)

PT2023PT_PS1

| Lab number | 97504 | 97507 | 97508 (1) | 97508 (2) | 97509 | 97510 (1) |
|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_2$ | $M_1$ |
| S/P (REP1) | 305,32 | 294,29 | 280,03 | 103,75 | 105,77 | 146,30 |
| S/P (REP2) | 318,85 | 215,24 | 259,25 | 102,57 | 98,73 | 183,90 |
| S/P (REP3) | 308,20 | 290,48 | 277,41 | 127,96 | 104,43 | 197,20 |
| Mean | 310,79 | 266,67 | 272,23 | 111,43 | 102,98 | 175,80 |
| SD | 7,12 | 44,58 | 11,32 | 14,33 | 3,74 | 26,40 |
| CV (%) | 2,29 | 16,72 | 4,16 | 12,86 | 3,63 | 15,02 |

| Lab number | 97510 (2) | 97514 | 97516 | 97532 | 97540 |
|---|---|---|---|---|---|
| Method | $M_2$ | $M_3$ | $M_2$ | $M_4$ | $M_1$ |
| S/P (REP1) | 92,52 | 48,42 | 97,10 | 141,70 | 286,00 |
| S/P (REP2) | 99,48 | 59,45 | 102,20 | 153,30 | 307,00 |
| S/P (REP3) | 77,73 | 55,56 | 91,60 | 137,50 | 311,00 |
| Mean | 89,91 | 54,48 | 96,97 | 144,17 | 301,33 |
| SD | 11,11 | 5,59 | 5,30 | 8,18 | 13,43 |
| CV (%) | 12,35 | 10,26 | 5,47 | 5,68 | 4,46 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = ID.VET - ID Screen® Paratuberculosis Indirect; $M_2$ = IDEXX - Paratuberculosis Screening Ab Test; $M_3$ = not known; $M_4$ =not known).*

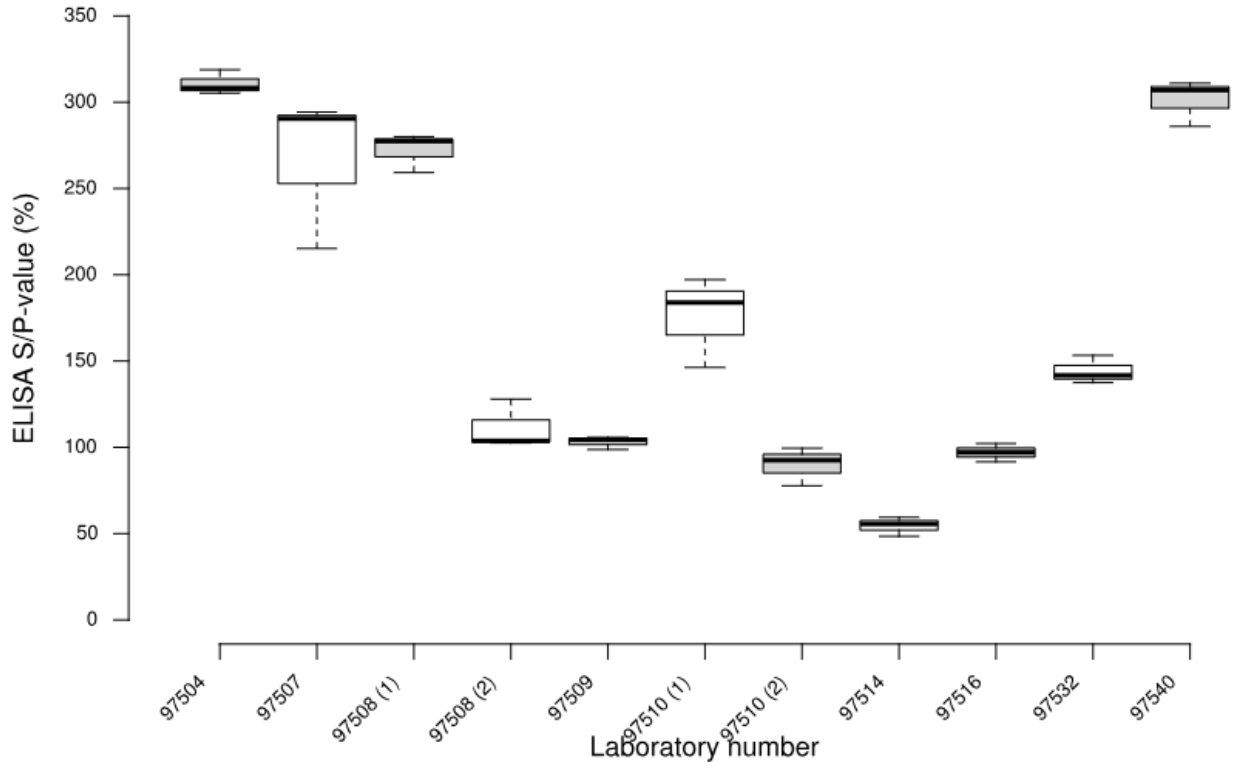



*Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.*

| Lab number | 97504 | 97507 | 97508 (1) | 97508 (2) | 97509 | 97510 (1) |
|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_2$ | $M_1$ |
| S/P (REP1) | 237,96 | 270,48 | 242,47 | 102,91 | 100,77 | 212,00 |
| S/P (REP2) | 272,43 | 241,91 | 202,46 | 80,64 | 88,811 | 160,20 |
| S/P (REP3) | 261,87 | 265,71 | 212,68 | 90,51 | 86,49 | 138,20 |
| S/P (REP4) | 225,83 | 266,67 | 204,99 | 79,038 | 83,67 | 193,20 |
| S/P (REP5) | 275,39 | 255,24 | 202,13 | 83,0030 | 81,70 | 156,90 |
| Mean | 254,69 | 260,00 | 212,95 | 87,22 | 88,29 | 172,10 |
| SD | 21,84 | 11,59 | 17,045 | 9,81 | 7,48 | 29,83 |
| CV (%) | 8,57 | 4,46 | 8,0045 | 11,25 | 8,48 | 17,33 |

| Lab number | 97510 (2) | 97514 | 97516 | 97532 | 97540 |
|---|---|---|---|---|---|
| Method | $M_2$ | $M_3$ | $M_2$ | $M_4$ | $M_1$ |
| S/P (REP1) | 63,20 | 38,70 | 89,60 | 107,70 | 242,00 |
| S/P (REP2) | 73,97 | 33,91 | 77,20 | 106,50 | 245,00 |
| S/P (REP3) | 70,49 | 38,70 | 66,10 | 104,70 | 231,00 |
| S/P (REP4) | 70,33 | 41,69 | 84,70 | 110,90 | 231,00 |
| S/P (REP5) | 75,010 | 36,78 | 73,00 | 110,50 | 227,00 |
| Mean | 70,60 | 37,95 | 78,12 | 108,060 | 235,20 |
| SD | 4,63 | 2,86 | 9,31 | 2,64 | 7,82 |
| CV (%) | 6,55 | 7,54 | 11,92 | 2,44 | 3,33 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = ID.VET - ID Screen® Paratuberculosis Indirect; $M_2$ = IDEXX - Paratuberculosis Screening Ab Test; $M_3$ = not known; $M_4$ =not known).*

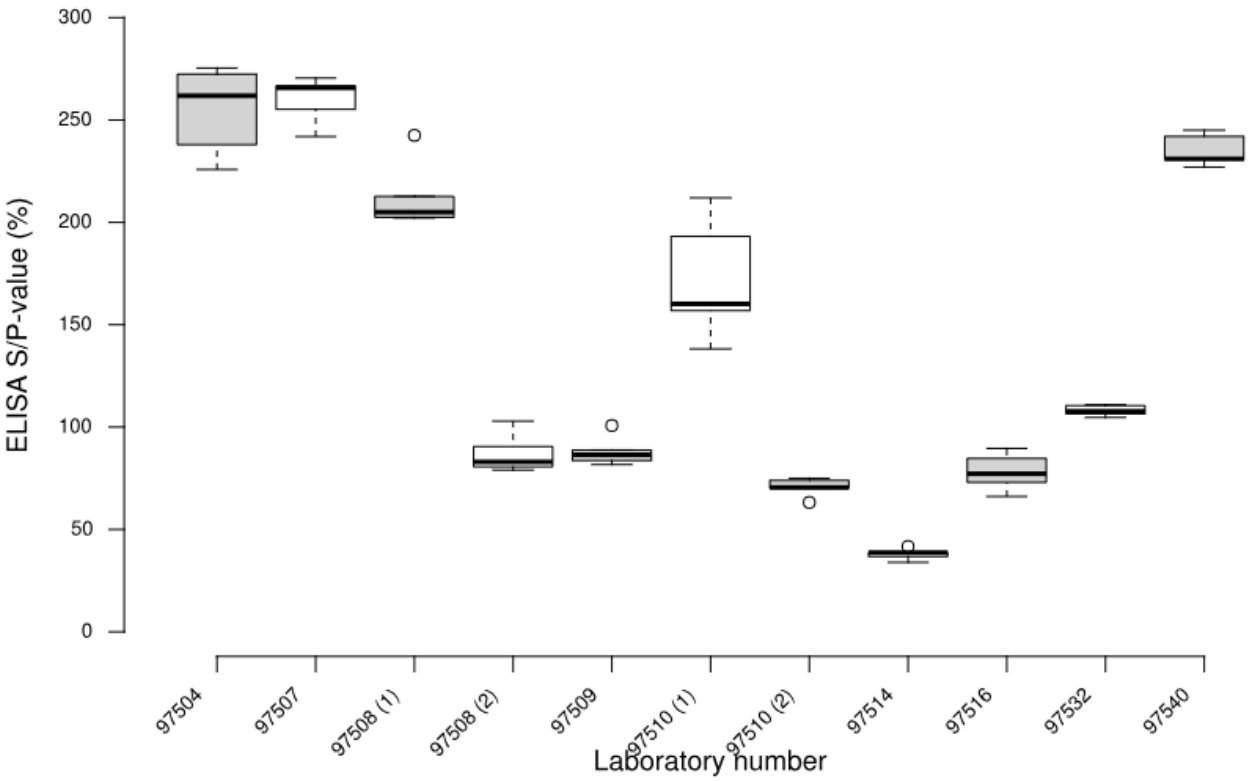



*Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.*

| Lab number | 97504 | 97507 | 97508 (1) | 97508 (2) | 97509 | 97510 (1) |
|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_2$ | $M_1$ |
| S/P (REP1) | 121,29 | 109,52 | 120,21 | 81,57 | 90,64 | 117,20 |
| S/P (REP2) | 120,51 | 108,57 | 122,42 | 77,86 | 81,28 | 117,30 |
| S/P (REP3) | 106,81 | 119,05 | 117,19 | 76,17 | 84,45 | 116,00 |
| S/P (REP4) | 116,14 | 117,14 | 128,23 | 71,19 | 74,60 | 112,60 |
| S/P (REP5) | 102,18 | 113,33 | 140,84 | 76,85 | 70,80 | 104,60 |
| Mean | 113,39 | 113,52 | 125,78 | 76,73 | 80,35 | 113,54 |
| SD | 8,51 | 4,59 | 9,34 | 3,73 | 7,87 | 5,35 |
| CV (%) | 7,50 | 4,04 | 7,42 | 4,86 | 9,80 | 4,71 |

| Lab number | 97510 (2) | 97514 | 97516 | 97532 | 97540 |
|---|---|---|---|---|---|
| Method | $M_2$ | $M_3$ | $M_2$ | $M_4$ | $M_1$ |
| S/P (REP1) | 71,58 | 83,80 | 69,80 | 30,70 | 101,00 |
| S/P (REP2) | 65,92 | 82,46 | 63,80 | 29,00 | 95,00 |
| S/P (REP3) | 72,99 | 79,28 | 71,50 | 26,70 | 105,00 |
| S/P (REP4) | 75,99 | 84,30 | 74,40 | 27,10 | 103,00 |
| S/P (REP5) | 77,29 | 72,27 | 70,70 | 26,50 | 101,00 |
| Mean | 72,75 | 80,42 | 70,04 | 28,00 | 101,00 |
| SD | 4,45 | 4,96 | 3,89 | 1,81 | 3,74 |
| CV (%) | 6,12 | 6,16 | 5,56 | 6,45 | 3,71 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = ID.VET - ID Screen® Paratuberculosis Indirect; $M_2$ = IDEXX - Paratuberculosis Screening Ab Test; $M_3$ = not known; $M_4$ =not known).*

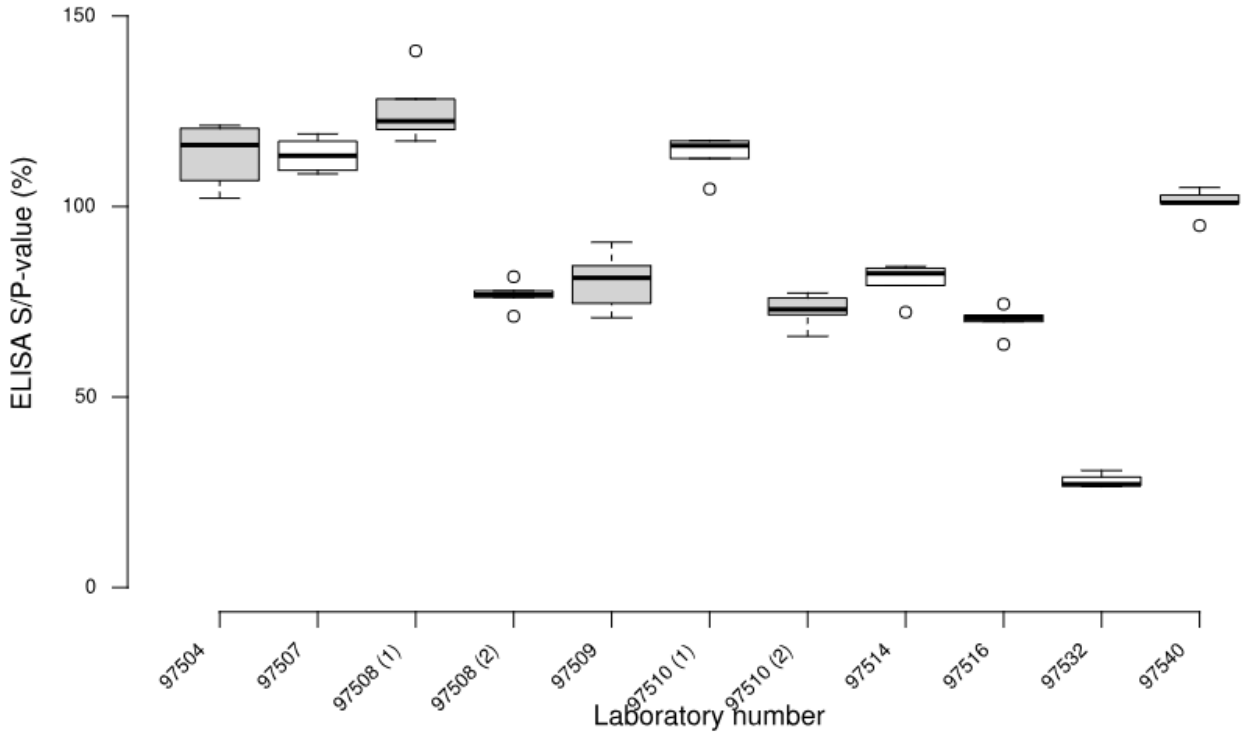



*Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.*

### 6.1.2 SEROLOGY (MILK)

PT2023PT_PM1

| Lab number | 97504 | 97509 | 97511 | 97512 | 97514 | 97516 | 97532 |
|---|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_1$ | $M_3$ |
| S/P (REP1) | 129,058 | 113,85 | 111,16 | 107,11 | 161,34 | 130,00 | 67,20 |
| S/P (REP2) | 125,53 | 113,048 | 109,40 | 97,98 | 168,40 | 146,80 | 64,20 |
| S/P (REP3) | 124,35 | 114,94 | 113,67 | 99,51 | 159,19 | 129,90 | 77,70 |
| Mean | 126,31 | 113,94 | 111,41 | 101,53 | 162,98 | 135,57 | 69,70 |
| SD | 2,45 | 0,95 | 2,14 | 4,89 | 4,82 | 9,73 | 7,09 |
| CV (%) | 1,94 | 0,83 | 1,92 | 4,82 | 2,96 | 7,18 | 10,17 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = IDEXX - Paratuberculosis screening Ab test; $M_2$ = not known; $M_3$ =not known).*

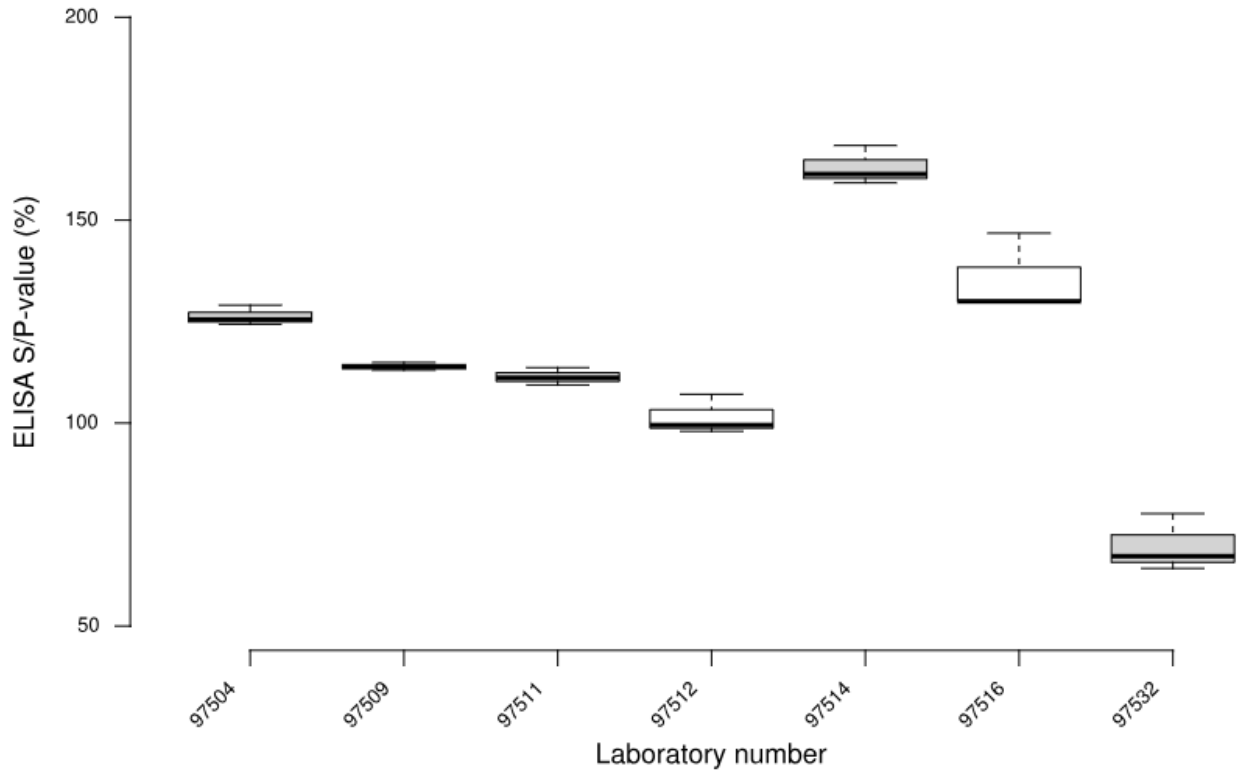



*Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.*

| Lab number | 97504 | 97509 | 97511 | 97512 | 97514 | 97516 | 97532 |
|---|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_1$ | $M_3$ |
| S/P (REP1) | 59,25 | 66,93 | 65,00 | 58,088 | 51,52 | 78,50 | 46,00 |
| S/P (REP2) | 63,20 | 68,43 | 67,63 | 55,70 | 45,28 | 70,10 | 39,80 |
| S/P (REP3) | 64,97 | 67,43 | 64,31 | 58,64 | 42,87 | 68,00 | 41,50 |
| S/P (REP4) | 62,027 | 62,95 | 62,93 | 52,33 | 40,025 | 71,90 | 44,90 |
| S/P (REP5) | 62,53 | 63,15 | 65,60 | 53,86 | 41,47 | 72,50 | 40,80 |
| Mean | 62,40 | 65,78 | 65,09 | 55,72 | 44,23 | 72,20 | 42,60 |
| SD | 2,08 | 2,55 | 1,73 | 2,70 | 4,51 | 3,93 | 2,70 |
| CV (%) | 3,34 | 3,88 | 2,66 | 4,84 | 10,20 | 5,45 | 6,34 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = IDEXX - Paratuberculosis screening Ab test; $M_2$ = not known; $M_3$ =not known).*

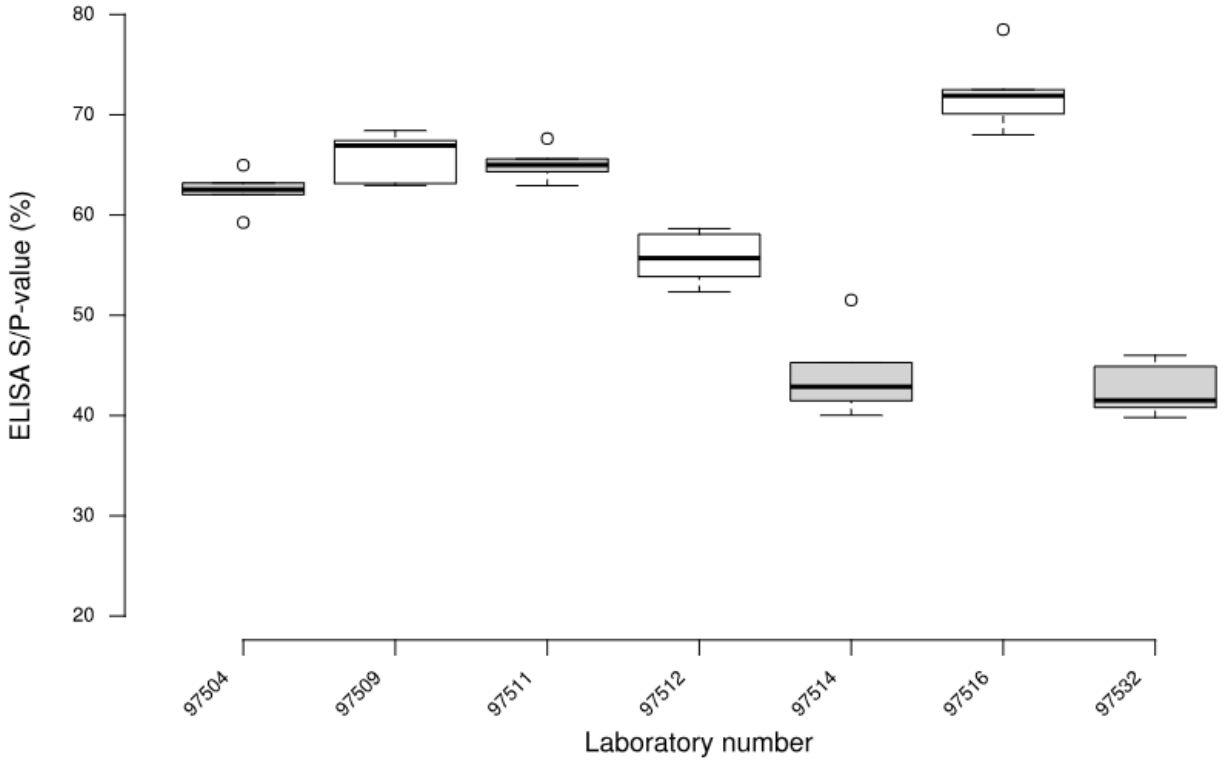



*Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.*

| Lab number | 97504 | 97509 | 97511 | 97512 | 97514 | 97516 | 97532 |
|---|---|---|---|---|---|---|---|
| Method | $M_1$ | $M_1$ | $M_1$ | $M_1$ | $M_2$ | $M_1$ | $M_3$ |
| S/P (REP1) | 66,91 | 73,90 | 71,64 | 55,70 | 52,057 | 89,50 | 91,5 |
| S/P (REP2) | 68,92 | 84,56 | 76,19 | 53,74 | 47,76 | 77,30 | 98,3 |
| S/P (REP3) | 62,70 | 70,12 | 74,89 | 54,90 | 47,12 | 77,10 | 91,8 |
| S/P (REP4) | 67,66 | 67,43 | 69,013 | 50,31 | 50,58 | 88,20 | 97,6 |
| S/P (REP5) | 71,11 | 75,90 | 78,081 | 54,23 | 47,49 | 82,00 | 102,5 |
| Mean | 67,46 | 74,38 | 73,96 | 53,77 | 49,00 | 82,82 | 96,34 |
| SD | 3,10 | 6,57 | 3,63 | 2,07 | 2,19 | 5,86 | 4,67 |
| CV (%) | 4,60 | 8,83 | 4,91 | 3,86 | 4,47 | 7,08 | 4,85 |

*Numbers were rounded to two significant decimal places. (REP = repetition; SD = standard deviation; CV = coefficient of variation; $M_1$ = IDEXX - Paratuberculosis screening Ab test; $M_2$ = not known; $M_3$ =not known).*

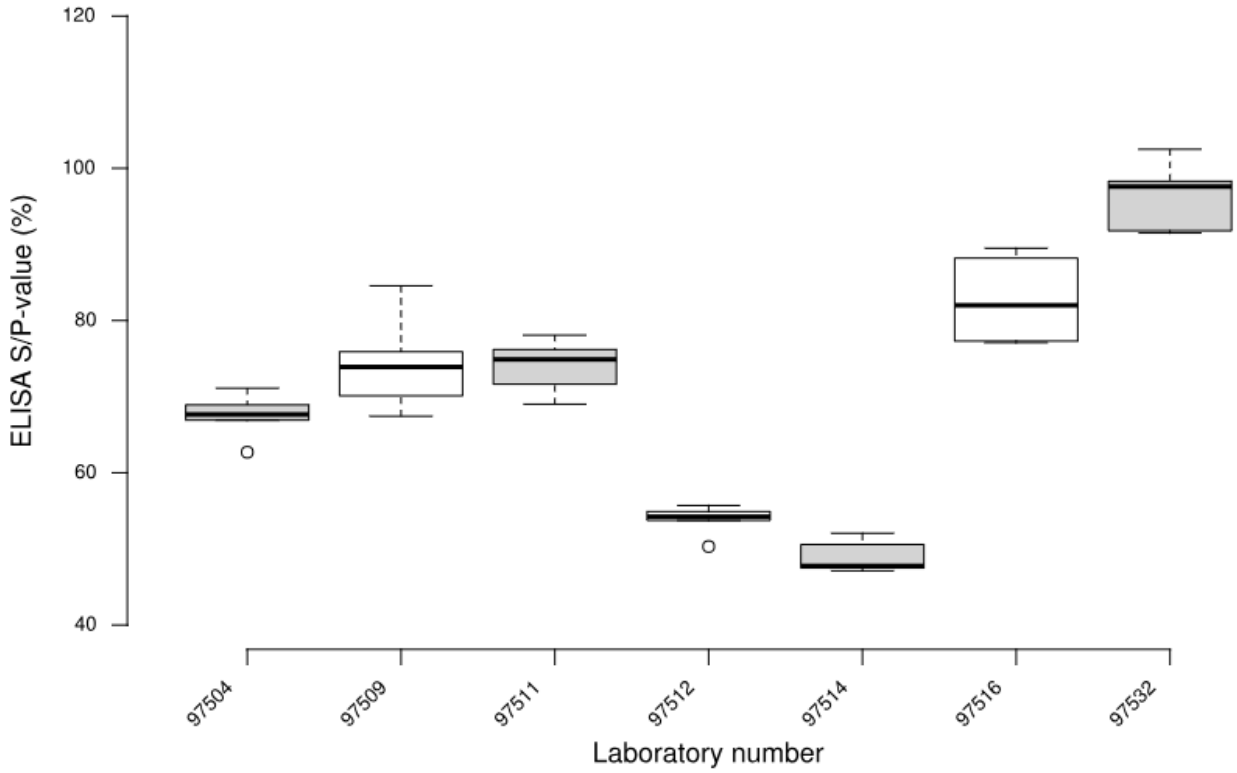



*Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.*

## 6.2 Annex 2: Additional information

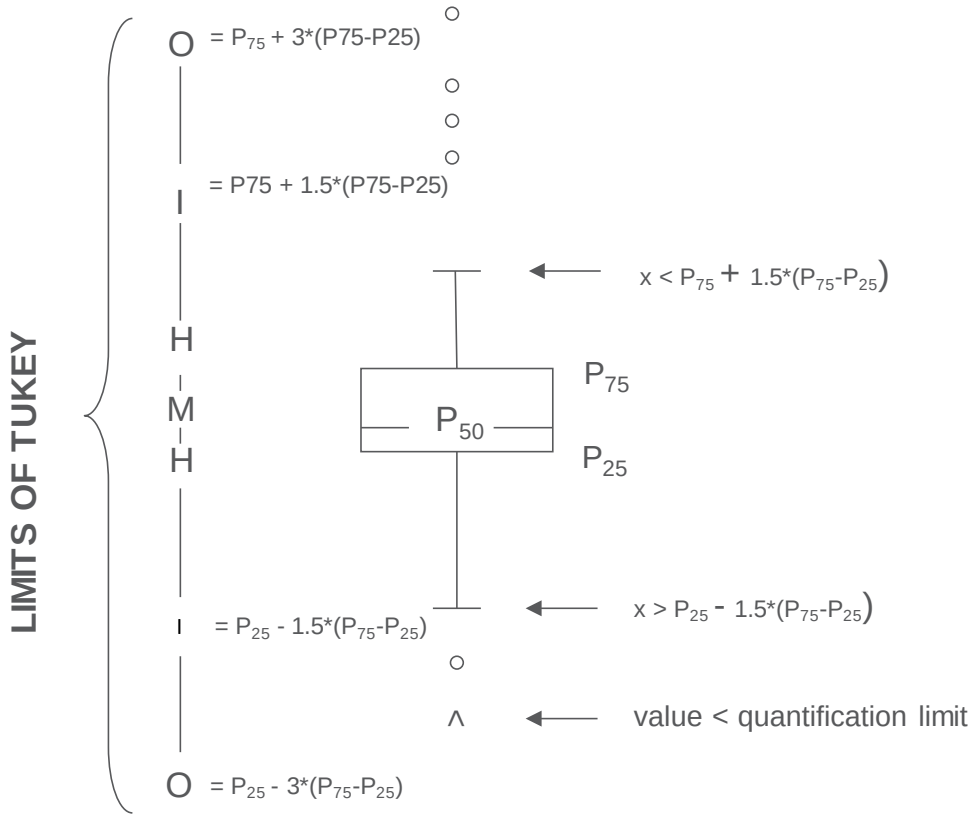
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

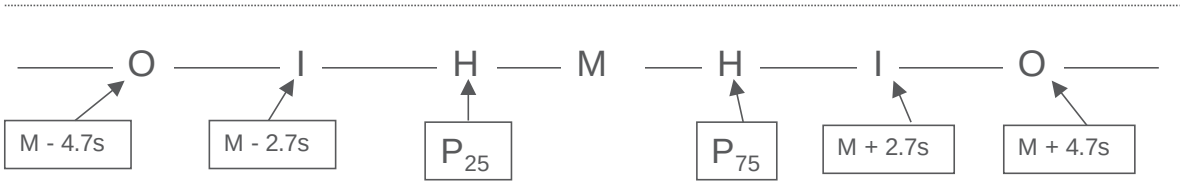
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

### 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**