

**BIOLOGICAL HEALTH RISKS  
QUALITY OF LABORATORIES**

**COMMITTEE OF EXPERTS**

**EXTERNAL QUALITY ASSESSMENT  
IN VETERINARY DIAGNOSIS**

**DEFINITIVE GLOBAL REPORT**

**VETERINARY MEDECINE**

**LEPTOSPIROSIS (LEPT)**

**PROFICIENCY TEST 2022/12**

**Sciensano/PT VET LEPT/2-E**

Biological health risks  
Quality of laboratories  
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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 pathogenic *Leptospira* spp. bacteria in organs.

## 3 MATERIALS AND METHODS

### 3.1 Bacteriology (organs)

#### 3.1.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Leptospirosis bacteriology on organs. The names of the participating laboratories are:

- Sciensano, Veterinary bacteriology service
- ARSIA
- Dierengezondheidszorg Vlaanderen (DGZ)
- IDVET (France)

#### 3.1.2 THE SAMPLES

The samples (frozen smashed organs) were prepared by the National Reference Laboratory (NRL), Service of Veterinary bacteriology, Sciensano.

Information about the origin of the samples:

- Sample OP1 and OP2: abortion material spiked with two different dilutions of *Leptospira* spp.
- Sample ON1: abortion material

#### 3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on 4 aliquots (0,5 mL) of each sample using RT-qPCR 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 different of samples: 4 positive and 1 negative sample.

| Sample ID    | Repetition | Status |
|--------------|------------|--------|
| PT2022LEPOP1 | 2          | POS    |
| PT2022LEPOP2 | 2          | POS    |
| PT2022LEPON1 | 1          | 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. 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: LEPBAC | 97504 | 97507 | 97508 | 97522 |
|-------------------|-------|-------|-------|-------|
| 22-1              | OP2   | OP1   | OP2   | OP2   |
| 22-2              | OP1   | OP2   | OP2   | OP1   |
| 22-3              | ON1   | OP2   | OP1   | OP1   |
| 22-4              | OP1   | OP1   | ON1   | OP2   |
| 22-5              | OP2   | ON1   | OP1   | ON1   |

## 4 TIMELINE

Randomization of the samples by QL: 07/11/2022

Sending samples to participants: 08/11/2022

- Samples bacteriology: frozen at -20 °C

Deadline for submitting the results: 02/12/2022

Preliminary report: 09/01/2023

## 5 RESULTS

### 5.1 Bacteriology (organs)

#### 5.1.1 RESULTS PER SAMPLE

The panel consisted of 3 different samples. Positive samples OP1 and OP2 were repeated twice. Therefore, in total, the panel consisted of 5 samples (4 positive and 1 negative sample).

Unfortunately, one laboratory did not submit its results even after sending a reminder to them. Therefore, the table below shows the results of three laboratories instead of four.

| Sample ID | Status | Number of repetitions (total results) | Observed result |
|-----------|--------|---------------------------------------|-----------------|
| OP1       | POS    | 2 (6)                                 | 6 POS           |
| OP2       | POS    | 2 (6)                                 | 6 POS           |
| ON1       | NEG    | 1 (3)                                 | 3 NEG           |

(POS = positive; NEG = negative)

#### 5.1.2 USED METHOD

| Manufacturer extraction protocol / kit | Name extraction protocol / kit | RT-qPCR protocol / kit                  | N        | NR        | NCR       | %          |
|--|--------------------------------|---|----------|-----------|-----------|------------|
| ThermoFisher Scientific                | Kit MagMax                     | Homemade (SOP/BAC/ANA16)                | 1        | 10        | 10        | 100        |
| Indical                                | IndiMag Pathogen Kit           | Thermofisher Vetmax SARP kit            | 1        | 10        | 10        | 100        |
| Indical                                | IndiMag Pathogen Kit           | Ingenetix - BactoReal Kit Leptospirosis | 1        | 10        | 10        | 100        |
| <b>TOTAL</b>                           |                                |   | <b>3</b> | <b>30</b> | <b>30</b> | <b>100</b> |

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

#### 5.1.3 CONCLUSION

In 2022, three laboratories participated in proficiency test of Leptospirosis bacteriology (organs) organized by Sciensano. Unfortunately, the fourth laboratory that was registered for this PT did not submit their results, even not after a reminder of the deadline via mail.

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. Different methods, from ThermoFisher Scientific and Indical were selected by the participants for the detection of pathogenic *Leptospira* spp. bacteria in organs. 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/](http://shiny.chemgrid.org/boxplotr/)

### 6.1 Annex 1: Quantitative results

#### 6.1.1 BACTERIOLOGY (ORGANS)

PT2022LEPOP1

| Lab number | 97504          | 97507          | 97508          | 97522 |
|------------|----------------|----------------|----------------|-------|
| Method     | M <sub>1</sub> | M <sub>2</sub> | M <sub>3</sub> | /     |
| Ct (REP1)  | 27,86          | 26,78          | 27,72          | /     |
| Ct (REP2)  | 28,09          | 26,69          | 27,58          | /     |
| Mean       | 27,98          | 26,74          | 27,65          | /     |
| SD         | 0,16           | 0,06           | 0,10           | /     |
| CV (%)     | 0,58           | 0,24           | 0,36           | /     |

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M<sub>1</sub> = ThermoFisher Scientific-Kit MagMax-Homemade; M<sub>2</sub> = Indical-IndiMag Pathogen Kit-Thermofisher Vetmax SARP kit; M<sub>3</sub> = Indical-IndiMag Pathogen Kit-Ingenetix - BactoReal Kit Leptospirosis).

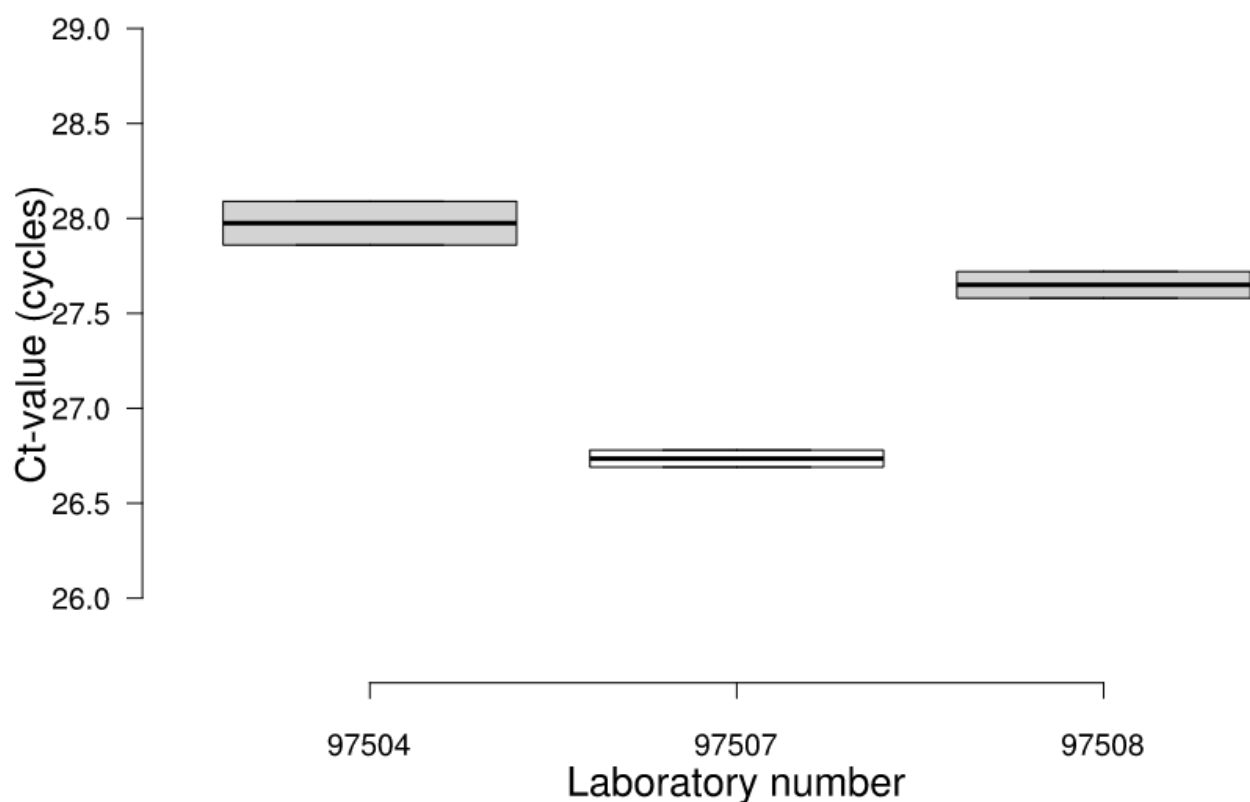


Figure 1. Distribution of the Ct-values (box-plots) per laboratory.

| Lab number | 97504          | 97507          | 97508          | 97522 |
|------------|----------------|----------------|----------------|-------|
| Method     | M <sub>1</sub> | M <sub>2</sub> | M <sub>3</sub> | /     |
| Ct (REP1)  | 30,24          | 26,63          | 30,21          | /     |
| Ct (REP2)  | 30,53          | 28,24          | 30,20          | /     |
| Mean       | 30,39          | 27,44          | 30,21          | /     |
| SD         | 0,21           | 1,14           | 0,01           | /     |
| CV (%)     | 0,67           | 4,15           | 0,02           | /     |

Numbers were rounded to 2 decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M<sub>1</sub> = ThermoFisher Scientific-Kit MagMax-Homemade; M<sub>2</sub> = Indical-IndiMag Pathogen Kit-Thermofisher Vetmax SARP kit; M<sub>3</sub> = Indical-IndiMag Pathogen Kit-Ingenetix - BactoReal Kit Leptospirosis).

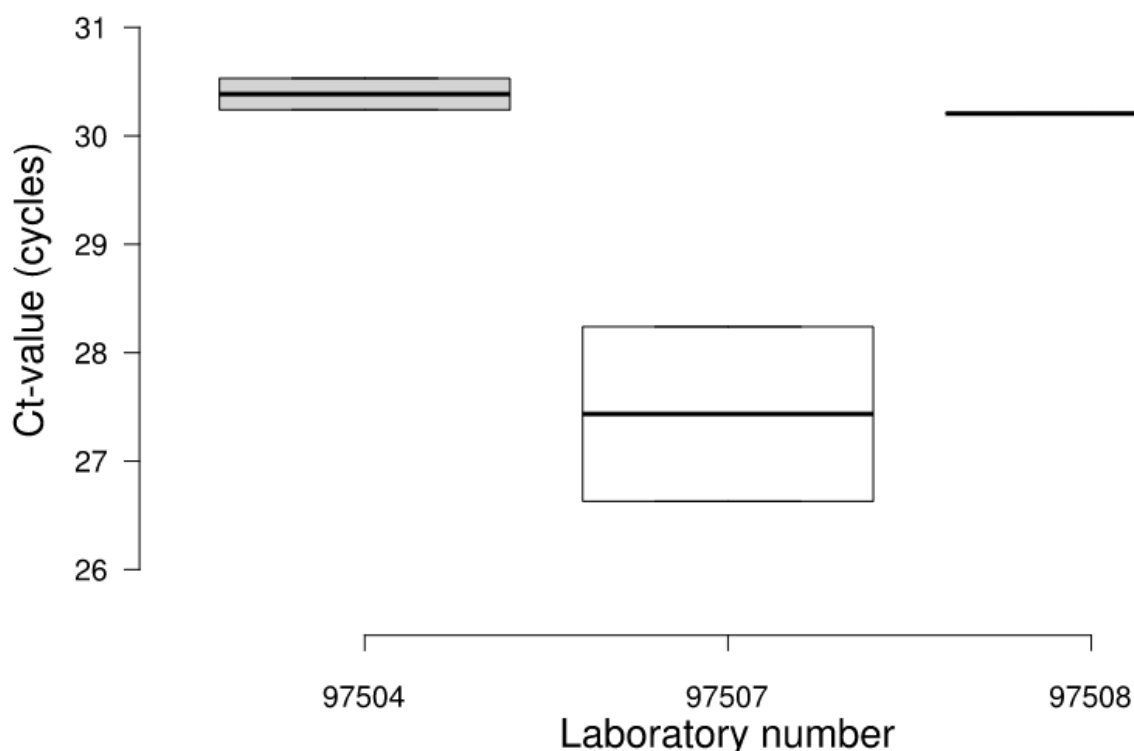


Figure 2. Distribution of the Ct-values (box-plots) per laboratory.

## 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:

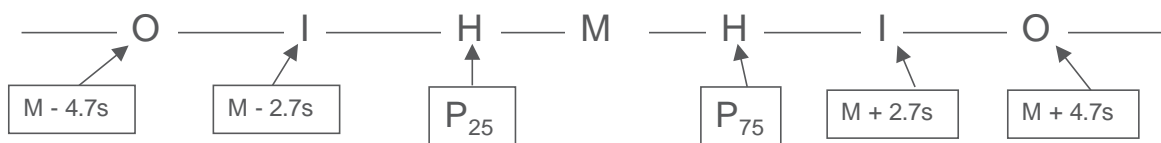
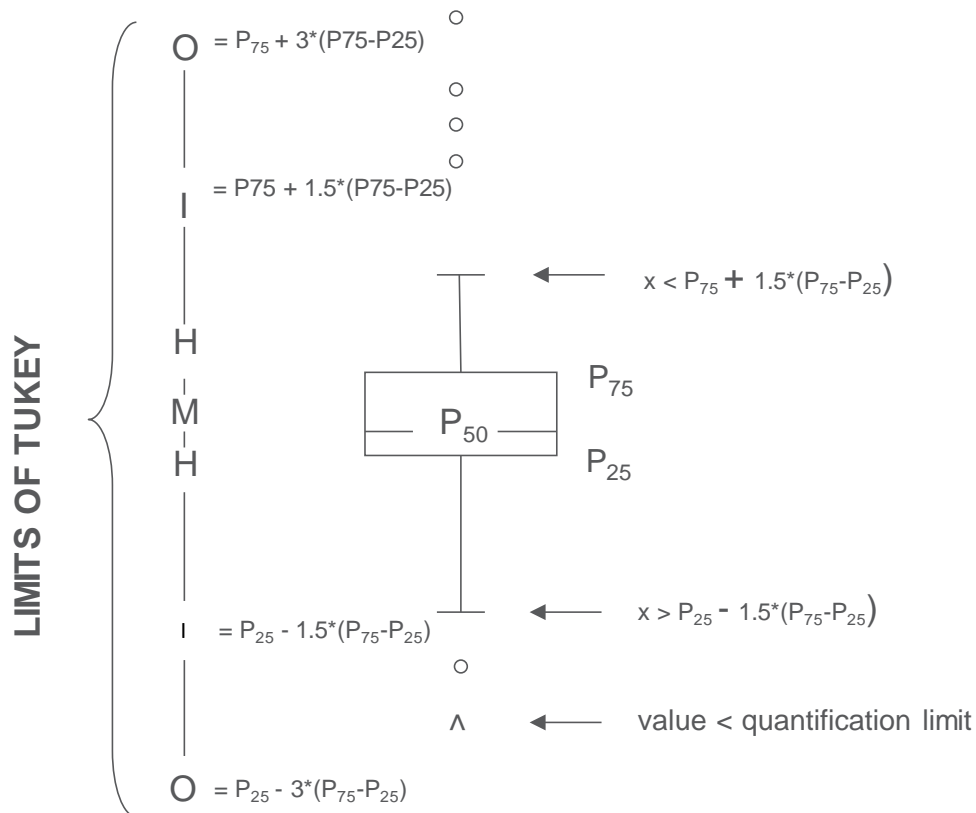
- NL: <https://www.sciensano.be/fr/biblio/eke-kalender-2023>
- FR: <https://www.sciensano.be/en/biblio/calendrier-eeq-2023>
- EN: <https://www.sciensano.be/en/biblio/eqa-calendar-2023>



**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**

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**END**

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