BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2024-10

LEPTOSPIROSIS (LEP)

Sciensano/PT-program LEP/2024-10/E

Biological health risks
Quality of laboratories
Rue Juliette Wytsmanstraat, 14
1050 Brussels | Belgium

www.sciensano.be

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NATIONAL REFERENCE LABORATORY

Sciensano					
Secretariat		Phone:	02/642.55.22	Fax:	02/642.56.45
		E-mail	ql_secretariat@sciensano.be		
Ynse Van de Maele	Coordinator	Phone:	02/642 55 24		
		E-mail:	Ynse.vandemaele@sciensano.be		
Bernard China	Alternate coordinator	Phone:	02/642 53 85		
		E-mail:	Bernard.china@sciensano.be		
Experts	**Institute**				
Marcella Mori	Sciensano – NRL Veterinary Bacteriology				
Anneleen Matthijs	Sciensano – NRL Veterinary Bacteriology				

A draft version of this report was submitted to the experts on 10/01/2025.

The experts were invited to send their comments via e-mail.

Responsibilities:
The National Reference Laboratory (NRL) of Sciensano was consulted for advice about the content of the global report, the interpretation of the results and the evaluation criteria. The responsibility for the choice of the samples used was carried out by the NRL.

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

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All the global 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

TABLE OF CONTENTS

1 INTRODUCTION 4
2 AIM 4
3 MATERIALS AND METHODS 4
3.1 Bacteriology (organs) 4
3.1.1 The participants 4
3.1.2 The samples 4
3.1.3 Homogeneity 4
3.1.4 Target values 5
3.1.5 Stability 5
3.1.6 Randomisation and panel composition 5
3.1.7 Threshold for qualification 5
4 TIMELINE 5
5 RESULTS 6
5.1 Bacteriology (organs) 6
5.1.1 Results per sample 6
5.1.2 Results per used extraction protocol/kit 6
5.1.3 Results per used PCR protocol/kit 6
5.1.4 Conclusion 6
6 ANNEXES (NOT UNDER ACCREDITATION) 7
6.1 Annex : Quantitative results 7
6.1.1 Bacteriology (organs) 7
6.2 Annex: Additional information 9

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

Three laboratories participated in the proficiency test of leptospirosis bacteriology on organ samples. The laboratory numbers of the participating laboratories are:

- 97504
- 97507
- 97514

3.1.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of ' Veterinary Bacteriology' in the department of 'Infectious diseases in animals Directorate', prepared the frozen smashed organ samples. Participants were instructed to store the samples at -20°C until the analysis was carried out.

Information about the origin and preparation of the samples:

- Samples OP1 and OP2: smashed kidneys spiked with two different dilutions of *Leptospira* spp.
- Sample ON1: smashed kidneys free of *Leptospira spp.*

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (500 µL each) of each sample, both before and after the PT, via qPCR. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2024LEPBAC_OP1	POS
PT2024LEPBAC_OP2	POS
PT2024LEPBAC_ON1	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The stability of the samples was confirmed by comparing the pre-PT results with those obtained by the NRL after the PT. The samples were deemed 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 content: PT2024 LEPBAC_	97504	97507	97514
OP1 (1)	LEPBAC24-1	LEPBAC24-1	LEPBAC24-1
OP1 (2)	LEPBAC24-3	LEPBAC24-4	LEPBAC24-5
OP2 (1)	LEPBAC24-2	LEPBAC24-2	LEPBAC24-2
OP2 (2)	LEPBAC24-5	LEPBAC24-3	LEPBAC24-4
ON1	LEPBAC24-4	LEPBAC24-5	LEPBAC24-3

3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the five reference samples is 100%.

4 TIMELINE

Transfer of the samples from NRL to QL: 26/11/2024
Randomisation of the samples by QL: 26/11/2024
Sending of samples to participants: 26/11/2024
Deadline for submitting the results: 16/12/2024
Individual report to the participants: 20/12/2024

5 RESULTS

5.1 Bacteriology (organs)

5.1.1 RESULTS PER SAMPLE

The panel consisted of three different samples. However, the positive samples OP1 and OP2 were replicated twice. Therefore, the panel included five samples in total.

* Quantitative data can be found in section 6.1, “Annex: Quantitative results”.

Sample content	Expected results	Total results	Observed results*
OP1	POS	6	6 POS
OP2	POS	6	6 POS
ON1	NEG	3	2 NEG / 1 POS

(POS = positive; NEG = negative)

5.1.2 RESULTS PER USED EXTRACTION PROTOCOL/KIT

Below, the table displays the results for each used extraction protocol/kit method.

Name producer	Name kit	N	NR	NCR	%
EliTech Group	Extraction with ELITe InGenius®	1	5	4	80
Indical Bioscience	IndiMag Pathogen Kit	1	5	5	100
Biosellal	BioExtract® Column	1	5	5	100
TOTAL		3	15	14	93

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

5.1.3 RESULTS PER USED PCR PROTOCOL/KIT

Below, the table displays the results for each used PCR protocol/kit method.

Name producer	Name protocol/kit	N	NR	NCR	%
Homemade	Homemade	1	5	4	80
Thermofisher	Thermofisher Vetmax SARP kit	1	5	5	100
Biosellal	Bio-T kit® Leptospires pathogènes	1	5	5	100
TOTAL		3	15	14	93

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

5.1.4 CONCLUSION

In 2024, three laboratories participated in the proficiency test leptospirosis bacteriology (organs) organised by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if 100% of the results provided by the laboratory are in agreement with the status of the reference samples assigned by the NRL of the Scientific Directorate Infectious Diseases in Animals of Sciensano. One laboratory did not meet expectations (80%), whereas the other two achieved perfect scores of 100%. This brought the overall score to 93%.

6 ANNEXES (NOT UNDER ACCREDITATION)

This quantitative data is not under BELAC-accreditation and is solely for the information of the laboratories.

6.1 Annex : Quantitative results

Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel. The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/

6.1.1 BACTERIOLOGY (ORGANS)

Sample PT2024LEPBAC-OP1

Lab number	97504	97507	97514
Method (PCR protocol/kit)	M_1	M_2	M_3
Cut-off	45	45	40
	45	45	40
Ct-value (1)	21,83	24,64	21,19
Ct-value (2)	21,88	24,89	21,24
Mean	21,86	24,77	21,22
SD	0,035	0,18	0,035
CV (%)	0,16	0,71	0,17

Values were rounded to two significant decimal place. (SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = Thermofisher - Thermofisher Vetmax SARP kit; M_3 = Biosellal - Bio-T kit® Leptospires pathogènes).

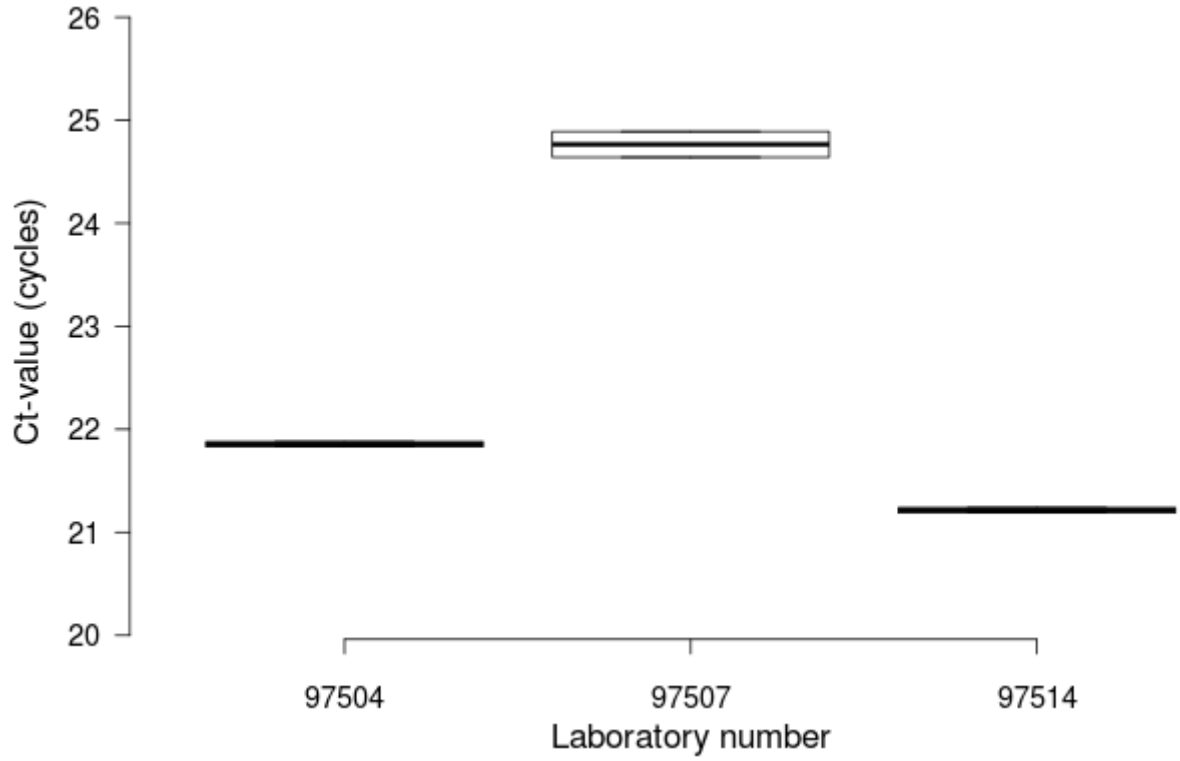


Figure 1. Distribution of the Ct-values (cycles) (box-plots) per laboratory.

Sample PT2024LEPBAC-OP2

Lab number	97504	97507	97514
Method (PCR protocol/kit)	M_1	M_2	M_3
Cut-off	45	45	40
	45	45	40
Ct-value (1)	24,26	27,28	23,57
Ct-value (2)	24,63	26,99	23,74
Mean	24,45	27,14	23,66
SD	0,26	0,21	0,12
CV (%)	1,07	0,76	0,51

Values were rounded to two significant decimal place. (SD = standard deviation; CV = coefficient of variation; M_1 = Homemade; M_2 = Thermofisher - Thermofisher Vetmax SARP kit; M_3 = Biosellal - Bio-T kit® Leptospires pathogènes).

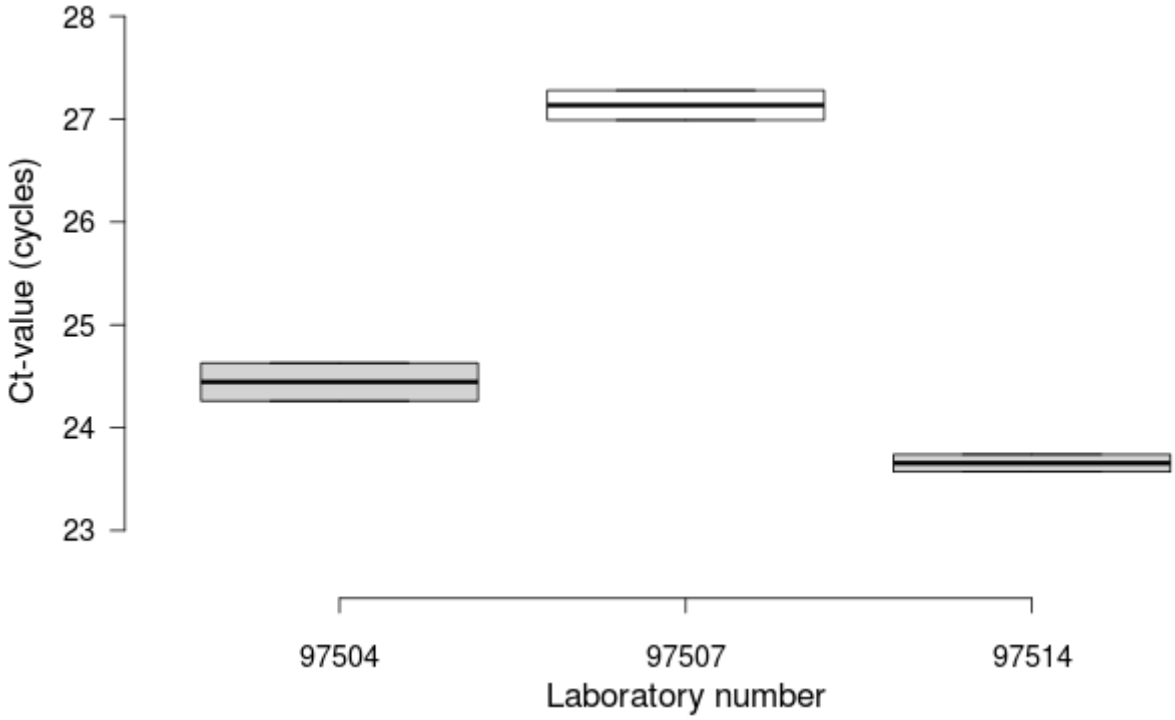


Figure 2. Distribution of the Ct-values (cycles) (box-plots) per laboratory.

6.2 Annex: Additional information

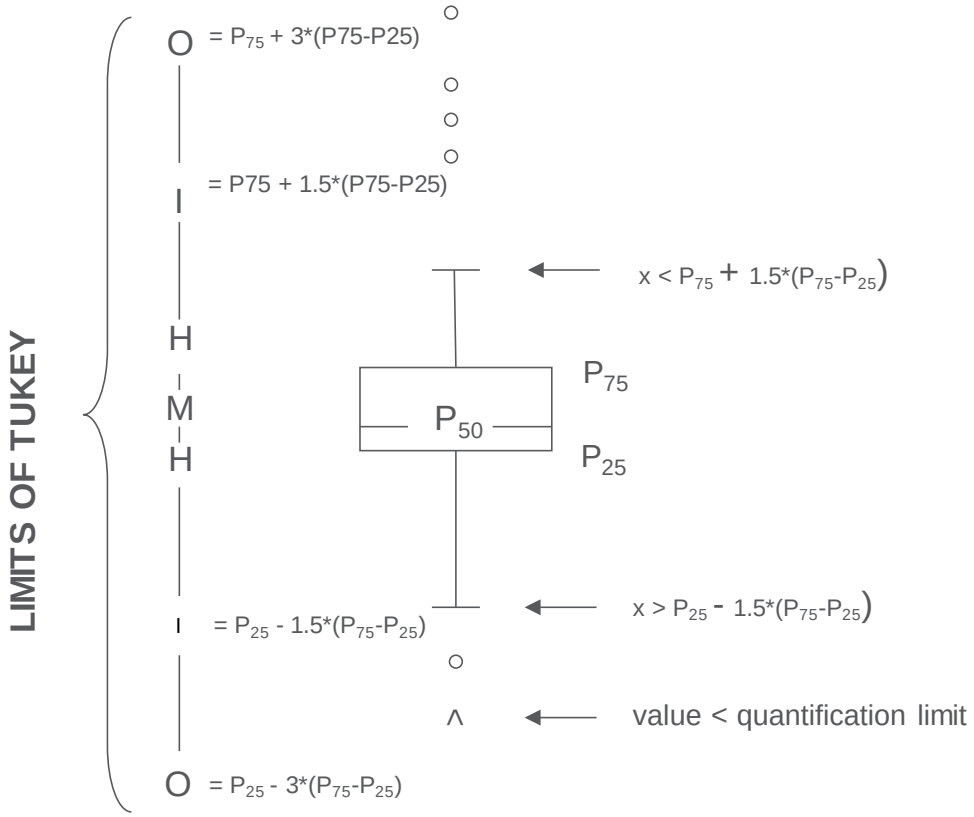
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

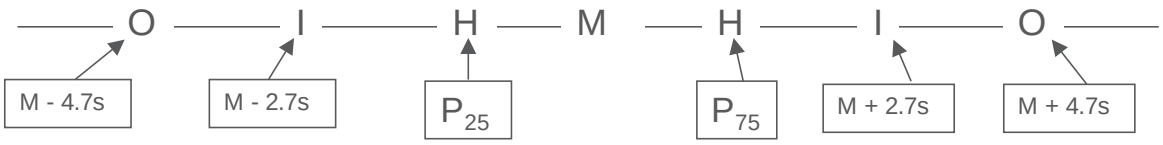
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2024
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2024
- EN: https://www.sciensano.be/nl/biblio/eqa-calendar-2024

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