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172-PT

PROFICIENCY TESTING 2017

LEPTOSPIRA (LEP)

Detection of LEP-specific antigens in organs

by Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

CODA-CERVA-UCCLE

DATE BEGIN PT: 12 DECEMBER 2017

DATE REPORT: 13 MARCH 2018

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure PRO/2.5/01 'Beheer van de proficiency testen op het CODA-CERVA-Ukkel/Gestion des essais d'aptitude au CODA-CERVA-Uccle', which is summarized in the 'Manual for the participant'.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of LEP-specific antigens in organs samples by RT-qPCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference organs samples were tested by means of RT-qPCR. The procedure for the RT-qPCR assay must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. Reference samples

Five reference samples (four organs and one urine sample) of bovine, horse and cat origin, either free from detectable LEP-specific antigens (n=3; coded 'PT2017LEPBACON1', 'PT2017LEPBACON2' and 'PT2017LEPBACON3') or containing detectable LEP-specific antigens (n=2; coded 'PT2017LEPBACOP1' and 'PT2017LEPBACOP2') were used.

In total, 15 aliquots were distributed to 3 participating laboratories. All participants received one aliquot of each reference sample. The identification numbers of the reference samples were randomized for all participants (Table 4).

For each reference sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference samples was based on (i) the historical background of the animals and (ii) the results obtained by an in-house developed RT-qPCR assays (pre-verification). The pre-verification tests consisted of two extraction protocols, SOP/BAC/PRE/04 (in-house) and the QIAGEN DNeasy Blood & Tissue Kits. All extracted DNAs were subsequently analyzed using an in-house developed RT-qPCR assay (SOP/BAC/ANA/16). The preparation of the positive samples and the pre-verification tests were performed in triplicate the same day of shipment due to the requirement of fresh conditions for this assay. The stability of the LEP-specific antigens in positive samples stored at 5±3°C was formerly defined and guaranteed to be optimal within a timeframe of ten days following preparation (see Annexe 3).

The reference organ samples were obtained from inactivated (internal procedure) kidneys or urine of animals neither infected with nor vaccinated for *Leptospira spp*. PT2017LEPBACON1 was an inactivated cat urine sample, PT2017LEPBACON2 was an inactivated catlle kidney sample (this matrix was used to prepare spiked positive samples), and PT2017LEPBACON3 was an inactivated horse kidney sample. PT2017LEPBACOP1 and PT2017LEPBACOP2 represented the naïve inactivated cattle kidney sample spiked with serial dilutions (1/60 and 1/6000, respectively) of a confluent culture at optical density of 0.19 of *L. interrogans* serogroup Sejroe serovar Hardjo type Prajitno strain Hardjoprajitno.

Taken together, the reference sample PT2017LEPBACON1, PT2017LEPBACON2 and PT2017LEPBACON3 were considered as negative sample and the reference samples PT2017LEPBACOP1 and PT2017LEPBACOP2 were considered as positive samples.

A homogeneity check on the aliquoted reference samples had been performed as in the context of PTs under the procedure PRO/2.5/01. Indeed, 3 aliquots of each reference sample were analysed using the in-house developed RT-qPCR assay (SOP/BAC/ANA16), hereby obtaining the same qualitative result for all 3 aliquots of the same reference sample. Consequently, all reference samples were considered as reliable samples in order to evaluate the ability of the participating laboratories to correctly identify the absence or presence of LEP-specific antigens in organs (and urine).

In addition, all reference samples were tested three times after the PT in order to confirm their stability and status (post-verification) using the in-house developed RT-qPCR assay (SOP/BAC/ANA16).

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

Results provided by the participating laboratories are categorized as *success* when the reported result matches with the assigned status or *failure* when the reported result does not match with the assigned status.

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 5 aliquots of reference samples used for this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 5 aliquots of reference samples is 100%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the CODA-CERVA-Uccle.

IV.1. Transfer and start of the analyses of the reference samples

The reference samples were sent at 5±3°C to the participating laboratories by national courier on the 12^{ve} of December 2017 (15 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 12^{ve} and 20th of December 2017 (Table 1).

IV.2. Dates at which results were returned to the CODA-CERVA-Uccle

Results were submitted to the CODA-CERVA-Uccle between the 15th and the 21st of December 2017 (Table 1). All participants hereby respected the deadline of 22th of December 2017 for submission of the results.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of extraction	Start of RT-qPCR analysis	Submission of the results (Excel file)
LAB1	12/12/2017	19/12/2017	20/12/2017	21/12/2017
LAB2	12/12/2017	12/12/2017	12/12/2017	15/12/2017
LAB3	12/12/2017	13/12/2017	13/12/2017	21/12/2017

IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Two out of 3 participating laboratories (LAB2 and LAB3) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 1 aliquot (80% of agreement) (Table 2).

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference samples assigned by the LEP reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 5 aliquots of reference samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	2	3
failure	1 (20)	0 (0)	0 (0)
success	4 (80)	5 (100)	5 (100)

IV.4.2. Variability among participating laboratories

No variability between LAB2 and LAB3 could be observed since these laboratories correctly identified all reference samples. In contrast, LAB1 misclassified 1 out of 5 aliquots of the reference samples [POS instead of NEG for 1 organ (urine) sample].

For each participating laboratory, the obtained results and the assigned statuses for the reference samples are shown in Table 3.

Raw RT-qPCR data and a quantitative data analysis (box plots) showing the Ct or Cp values per *Leptospira* positive sample are shown for educational purposes in Annex 1 and Annex 2, respectively. Stability data of the *Leptospira* antigens at storage of 5±3°C are provided in Annex 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference samples (SAMPLE), the external identification of the reference samples (LABPOSIT), and the status assigned by the LEP reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017LEPBACON2	NEG	NEG	1
2	1	2	PT2017LEPBACOP1	POS	POS	1
3	1	3	PT2017LEPBACON1	NEG	POS	0
4	1	4	PT2017LEPBACOP2	POS	POS	1
5	1	5	PT2017LEPBACON3	NEG	NEG	1
6	2	1	PT2017LEPBACON3	NEG	NEG	1
7	2	2	PT2017LEPBACON1	NEG	NEG	1
8	2	3	PT2017LEPBACON2	NEG	NEG	1
9	2	4	PT2017LEPBACOP1	POS	POS	1
10	2	5	PT2017LEPBACOP2	POS	POS	1
11	3	1	PT2017LEPBACON2	NEG	NEG	1
12	3	2	PT2017LEPBACOP1	POS	POS	1
13	3	3	PT2017LEPBACON1	NEG	NEG	1
14	3	4	PT2017LEPBACOP2	POS	POS	1
15	3	5	PT2017LEPBACON3	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference organ samples of cat, bovine and horse origin for the detection of LEP-specific antigens by RT-qPCR.

Two out of 3 participating laboratories (LAB2 and LAB3) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 1 out of 5 aliquots (80% of agreement) (Table 2).

LEP extraction kits were from different producers: one from Qiagen and two from Thermofisher Scientific. LEP RT-qPCR kits were from different producers: one In house/Home made kit and two from Thermofisher Scientific.

LAB1 was contacted and explained that the provided volume of the missqualified sample was not in agreement with that indicated in their procedure. This inconsistancy might be the source of the missclassification.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 100% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by CODA-CERVA-Uccle (see III.3.3.). Consequently, 2 out of 3 participating laboratories (LAB2 and LAB3) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 1 out of 5 aliquots (80% of agreement).

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, Belgium)

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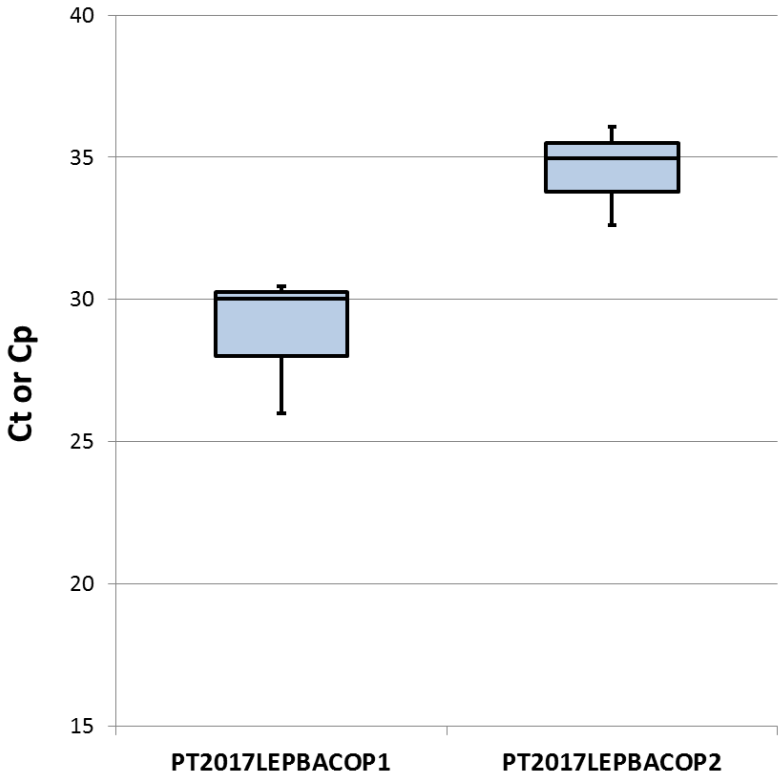
Annex 1:

Raw Ct or Cp data obtained with the RT-qPCR assays

LabNR				1	2	3
Sample	Ct or Cp value	1	PT2017LEPBACON1	35,95	45	-
		2	PT2017LEPBACON2	46	45	-
		3	PT2017LEPBACON3	46	45	-
		4	PT2017LEPBACOP1	30,03	25,98	30,47
		5	PT2017LEPBACOP2	34,96	32,6	36,06
	Conclusion	1	PT2017LEPBACON1	POS	NEG	NEG
		2	PT2017LEPBACON2	NEG	NEG	NEG
		3	PT2017LEPBACON3	NEG	NEG	NEG
		4	PT2017LEPBACOP1	POS	POS	POS
		5	PT2017LEPBACOP2	POS	POS	POS

Annex 2: Quantitative data analysis

Box plot distribution of Ct or Cp values of *Leptospira* positive samples among the participating laboratories



Annex 3:

Stability data of *Leptospira* antigens in positive samples stored various days at 5±3°C following preparation