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172-PT

PROFICIENCY TESTING 2017

Q-FEVER (QFV)

Detection of QFV-specific antigens in milk and organs

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

CODA-CERVA-UCCLE

In collaboration with NRC Coxiella burnetii (bacteriology)

DATE BEGIN PT: 13 NOVEMBER 2017

DATE REPORT: 23 FEBRUARY 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 QFV-specific antigens in milk and organs samples of bovidae origin by RT-qPCR.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference milk and 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 organs samples of bovine origin, either free from detectable QFV-specific antigens (n=1; coded 'PT2017QFVBACON1') or containing detectable QFV-specific antigens (n=4; coded 'PT2017QFVBACOP1', 'PT2017QFVBACOP2', 'PT2017QFVBACOP3' and 'PT2017QFVBACOD1') and 5 reference milk samples of small ruminants origin, either free from detectable QFV-specific antigens (n=3; coded 'PT2017QFVBACMN1', 'PT2017QFVBACMN2' and 'PT2017QFVBACMN3') or containing detectable QFV-specific antigens (n=2; coded 'PT2017QFVBACMP1' and 'PT2017QFVBACMP2') were used.

In total, 50 aliquots were distributed to 5 participating laboratories. All participants received 10 aliquots of reference samples. The identification numbers of the reference samples were randomized for all participants (Table 3).

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/farm and (ii) the results obtained by analytical tests (pre-verification). The pre-verification tests consisted of two extraction protocols, SOP/BAC/PRE/03 (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/15), the ADIAVET™ *Coxiella* real time (Adiagene) and the VetMAX™ *C. burnetii* Relative Quant Kit (Thermo Fisher). Totally, all samples were characterized by a combination of two different extraction protocols and three RT-qPCR assays. For these reference samples, the same qualitative results were obtained with all assays used.

The reference organ samples were obtained from an inactivated (internal procedure) spleen derived from an animal neither infected with nor vaccinated for *C.burnetii*. PT2017QFVBACON1 was the naïve original inactivated organ sample. PT2017QFVBACOP1, PT2017QFVBACOP2, PT2017QFVBACOP3 and PT2017QFVBACOD1 represented the naïve original inactivated organ sample spiked with serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) of confluent inactivated cell culture infected with an avirulent (phase II) *C.burnetii* strain (RSA 439). The estimated concentrations of *C.burnetii* bacteria in the spiked samples were of 1,03E+05, 1,03E+04, 1,03E+03 and 1,03E+02 genomic equivalent (GE)/ml, respectively, the latter sample providing a Ct around the limit of detection.

Taken together, the reference organ sample PT2017QFVBACON1 was considered as negative sample, the reference organ samples PT2017QFVBACOP1, PT2017QFVBACOP2 and PT2017QFVBACOP3 were considered as positive samples. The reference organ sample PT2017QFVBACOD1 was considered as doubtfull sample; positive, non-interpretable (doubtful) or negative results will be acceptable.

The reference milk samples were obtained from bulk tank milks of five different sheep/goat farms participating to the trimestrial screening and resulting free of infection since 2013 (PT2017QFVBACMN1, PT2017QFVBACMN2 and PT2017QFVBACMN3) or naturally infected (PT2017QFVBACMP1 and PT2017QFVBACMP2) with *C.burnetii*. Milks, infected or not, were inactivated (internal procedure) and lyophilized.

Taken together, the reference milk sample PT2017QFVBACMN1, PT2017QFVBACMN2 and PT2017QFVBACMN3 were considered as negative sample, the reference milk samples PT2017QFVBACMP1 and PT2017QFVBACMP2 were considered as positive samples.

A homogeneity check on the aliquoted reference organ and milk samples had been performed as in the context of PTs under the procedure PRO/2.5/01. Indeed, 10 aliquots of each reference sample were analysed using the in-house developed RT-qPCR assay (SOP/BAC/ANA/15), hereby obtaining the same qualitative result for all 10 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 QFV-specific antigens in organs and milk. In addition, all reference samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed RT-qPCR assay (SOP/BAC/ANA/15).

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 10 aliquots of reference samples used for either PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 10 aliquots of reference samples used for either PT is at least 90%.

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 milk samples were sent lyophilized, whereas the reference organ samples were sent frozen (dry ice) to each of the participating laboratories by national courier on the 13th of November 2017. All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 15th and 21st of November 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 27th of November and the 1st of December 2017 (Table 1). All participants hereby respected the deadline of 1st of December 2017 for submission of the results.
LAB3 received 10 aliquots of the reference samples but did not registered for this PT and LAB2 did not participate.

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 analysis	Submission of the results (Excel file)
LAB1	13/11/2017	16/11/2017	01/12/2017
LAB3	13/11/2017	/	/
LAB4	13/11/2017	21/11/2017	27/11/2017
LAB5	13/11/2017	15/11/2017	29/11/2017

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 (LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 6 aliquots (40% 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 QFV reference laboratory / NRC Coxiella burnetii of CODA-CERVA-Uccle. All participating laboratories received 10 aliquots of reference samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**4**	**5**
failure	**6 (60)**	0 (0)	0 (0)
success	**4 (40)**	10 (100)	10 (100)

IV.4.2. Variability among participating laboratories

No variability between LAB4 and LAB5 could be observed since these laboratories correctly identified all reference samples. In contrast, LAB1 misclassified 6 out of 10 aliquots of the reference samples (4x NEG instead of POS for 2x organ samples and 2x milk samples and 2x POS instead of NEG for 1 organ sample and 1 milk 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 *C.burnetii* positive sample are shown for educational purposes in Annex 1 and Annex 2.

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 QFV reference laboratory / NRC Coxiella burnetii of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive; DBS: doubtfull.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017QFVBACMN3	NEG	NEG	1
2	1	2	PT2017QFVBACOP2	POS	POS	1
3	1	3	PT2017QFVBACMN2	NEG	NEG	1
4	1	4	PT2017QFVBACON1	**NEG**	**POS**	0
5	1	5	PT2017QFVBACMN1	**NEG**	**POS**	0
6	1	6	PT2017QFVBACOP3	**POS**	**NEG**	0
7	1	7	PT2017QFVBACMP2	**POS**	**NEG**	0
8	1	8	PT2017QFVBACOP1	**POS**	**NEG**	0
9	1	9	PT2017QFVBACOD1	DBS	POS	1
10	1	10	PT2017QFVBACMP1	**POS**	**NEG**	0
11	4	1	PT2017QFVBACMP2	POS	POS	1
12	4	2	PT2017QFVBACMN3	NEG	NEG	1
13	4	3	PT2017QFVBACMP1	POS	POS	1
14	4	4	PT2017QFVBACOP3	POS	POS	1
15	4	5	PT2017QFVBACOP1	POS	POS	1
16	4	6	PT2017QFVBACOP2	POS	POS	1
17	4	7	PT2017QFVBACOD1	DBS	NEG	1
18	4	8	PT2017QFVBACMN1	NEG	NEG	1
19	4	9	PT2017QFVBACON1	NEG	NEG	1
20	4	10	PT2017QFVBACMN2	NEG	NEG	1
21	5	1	PT2017QFVBACMN3	NEG	NEG	1
22	5	2	PT2017QFVBACOP2	POS	POS	1
23	5	3	PT2017QFVBACMN2	NEG	NEG	1
24	5	4	PT2017QFVBACON1	NEG	NEG	1
25	5	5	PT2017QFVBACMN1	NEG	NEG	1
26	5	6	PT2017QFVBACOP3	POS	POS	1
27	5	7	PT2017QFVBACMP2	POS	POS	1
28	5	8	PT2017QFVBACOP1	POS	POS	1
29	5	9	PT2017QFVBACOD1	DBS	POS	1
30	5	10	PT2017QFVBACMP1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference organ and milk samples for the detection of QFV-specific antigens by RT-qPCR.

QFV extraction kits were from different producers: one from Applied biosystems (Life technologies) and two from Qiagen. QFV Rt-PCR kits were from different producers: one from Thermofisher Scientific and two In house/Home made kits.

Two out of 3 participating laboratories (LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 6 out of 10 aliquots (40% of agreement) (Table 2). LAB1 was contacted and explained that encoding of the samples was reshuffled at the dispatching level, where samples were kept separated following their matrix. With the corrected assignment to the samples, LAB1 misclassified 3 out of 10 aliquots (70% of agreement).

VI. Conclusions

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 samples assigned by CODA-CERVA-Uccle (see III.3.3.). Consequently, 2 out of 3 participating laboratories (LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference samples (100% of agreement), whereas LAB1 misclassified 6 out of 10 aliquots (40% of agreement). LAB1 reached a higher agreement (70% of agreement) when taking into account additional information and clarification requested to the laboratories after submission of the PT data. However, these were not sufficient to achieve a satisfactory performance.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

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

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

AZ Sint-Jan Brugge-Oostende AV (Bruges, Belgium)

Hôpital Erasme Laboratoire de microbiologie (Brussel, Belgium)

CODA-CERVA

Annex 1: Raw Ct or Cp data obtained with the RT-qPCR assays

		LabNR		1	1 (V2)	4	5
Sample	Ct or Cp value	1	PT2017QFVBACMN1	23,15	46	0	nd
		2	PT2017QFVBACMN2	46	43,69	0	nd
		3	PT2017QFVBACMN3	46	46	0	nd
		4	PT2017QFVBACMP1	46	23,15	27,8	25,68
		5	PT2017QFVBACMP2	46	25,64	29,4	25,84
		6	PT2017QFVBACON1	25,64	46	0	nd
		7	PT2017QFVBACOP1	46	33,66	27,4	27,34
		8	PT2017QFVBACOP2	39,5	46	31,2	31,46
		9	PT2017QFVBACOP3	46	46	34,6	34,66
		10	PT2017QFVBACOD1	33,66	46	0	33,92
	Conclusion	1	PT2017QFVBACMN1	POS	NEG	NEG	NEG
		2	PT2017QFVBACMN2	NEG	POS	NEG	NEG
		3	PT2017QFVBACMN3	NEG	NEG	NEG	NEG
		4	PT2017QFVBACMP1	NEG	POS	POS	POS
		5	PT2017QFVBACMP2	NEG	POS	POS	POS
		6	PT2017QFVBACON1	POS	NEG	NEG	NEG
		7	PT2017QFVBACOP1	NEG	POS	POS	POS
		8	PT2017QFVBACOP2	POS	NEG	POS	POS
		9	PT2017QFVBACOP3	NEG	NEG	POS	POS
		10	PT2017QFVBACOD1	POS	NEG	NEG	POS

Annex 2: Quantitative data analysis

Box plot distribution of Ct or Cp values of *C.burnetii* positive samples among the participating laboratories

RT-qPCR data from the participating laboratories correctly assigning the reference status to the *C.burnetii* positive sample are presented hereunder. Because the positive organ reference samples were spiked with 10 fold serial dilution of *C.burnetii* antigens, RT-qPCR slope (efficiency) of the entire process could be calculated for these samples for the laboratories correctly assigning the status to the samples. LAB 4 reached a slope of -3.6 (R^2= 0.999) corresponding to an efficiency = 89.57%; LAB5 reached a slope of -3.66 (R^2= 0.995) corresponding to an efficiency = 87.60%.