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172-PT

PROFICIENCY TESTING 2016

CLASSICAL SWINE FEVER (CSF)

Detection of CSF-specific antibodies in serum by

Enzyme-Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 13 JUNE 2016

DATE REPORT: 16 SEPTEMBER 2016

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 CSF-specific antibodies in porcine serum by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be tested by means of a CSF antibody ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 6 reference serum samples of porcine origin, either free from detectable CSF-specific antibodies (n=2; coded 'PT2016CSFSERNS1' and 'PT2016CSFSERNS2') or containing detectable CSF-specific antibodies (n=4; coded 'PT2016CSFSERPS1', 'PT2016CSFSERPS2', 'PT2016CSFSERPS3' and 'PT2016CSFSERPS4'), were used. In total, 100 aliquots were distributed to 5 participating laboratories. All participants received 20 aliquots: 5 aliquots of the reference serum samples PT2016CSFSERNS1 and PT2016CSFSERPS1, 3 aliquots of the reference serum samples PT2016CSFSERNS2 and PT2016CSFSERPS2 and 2 aliquots of the reference serum samples PT2016CSFSERPS3 and PT2016CSFSERPS4. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 3).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and/or (ii) the results obtained during pre-verification, hereby using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX and a seroneutralisation assay (SN).
The reference serum samples PT2016CSFSERNS1 and PT2016CSFSERNS2 were obtained from CSF free animals. The reference serum sample PT2016CSFSERPS1 was a 1/10 dilution of a first CSF hyperimmune serum. The reference serum sample PT2016CSFSERPS2 was obtained from a pig that became infected during an *in vivo* CSF infection experiment but additional information is missing. The reference serum sample PT2016CSFSERPS3 was obtained from a pig that became infected during an *in vivo* CSF infection experiment (contact animal; blood sample collected at 33 days post contact). The reference serum sample PT2016CSFSERPS4 was a 1/64 dilution of a second CSF hyperimmune serum. For all reference serum samples, the same qualitative result was obtained with ELISA and SN. Based on these results, the reference serum samples PT2016CSFSERNS1 and PT2016CSFSERNS2 were considered as negative sera, the reference serum samples PT2016CSFSERPS1, PT2016CSFSERPS3 and PT2016CSFSERPS4 as weak/intermediate positive sera in CSF antibody ELISA and the reference serum sample PT2016CSFSERPS2 as strong positive sera in CSF antibody ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of CSF-specific antibodies in porcine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the HerdChek CSFV Antibody ELISA Test Kit from IDEXX.

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 (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or failure when the reported result does not match with the assigned status (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for the 20 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 20 aliquots of reference samples 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 20 aliquots of the reference serum samples were sent frozen (dry ice) to each of the 5 participating laboratories by national or international courier on 13th of June 2016 (100 aliquots in total). LAB2, LAB3, LAB4 and LAB5 acknowledged receipt of the samples on the same day whereas LAB1 received the samples on 14th June 2016. All participating laboratories confirmed that the reference serum samples were still frozen upon receipt. Analyses were performed between the 15th and the 17th of June 2016 (Table 1).

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

Results from the participating laboratories were submitted to the CODA-CERVA-Uccle between 20th and 24th of June 2016 (Table 1). All participants hereby respected the deadline of 1ste of July 2016.

Table 1. Overview of the dates on which (i) the reference serum 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	14/06/2016	15/06/2016	20/06/2016
LAB2	13/06/2016	16/06/2016	21/06/2016
LAB3	13/06/2016	16/06/2016	21/06/2016
LAB4	13/06/2016	15/06/2016	24/06/2016
LAB5	13/06/2016	17/06/2016	20/06/2016

IV.3. Compliance with the procedure

LAB1, LAB3, LAB4 and LAB5 have provided a duly dated and signed copy of the results.

IV.4. Qualitative data analysis

IV.4.1. Level of agreement

Qualitative data analysis showed that all the five participating laboratories (LAB1 to LAB5) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2).
A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

Table 2. Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the CSF reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

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

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1, LAB2, LAB3, LAB4 and LAB5 since these participants correctly identified all reference serum samples.

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

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference serum samples (SAMPLE), the positions of the reference serum samples as placed in the block (LABPOSIT), and the status assigned by the CSF reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2016CSFSERNS1	NEG	NEG	1
2	1	2	PT2016CSFSERPS1	POS	POS	1
3	1	3	PT2016CSFSERNS2	NEG	NEG	1
4	1	4	PT2016CSFSERPS3	POS	POS	1
5	1	5	PT2016CSFSERPS2	POS	POS	1
6	1	6	PT2016CSFSERPS4	POS	POS	1
7	1	7	PT2016CSFSERNS2	NEG	NEG	1
8	1	8	PT2016CSFSERPS1	POS	POS	1
9	1	9	PT2016CSFSERNS1	NEG	NEG	1
10	1	10	PT2016CSFSERPS3	POS	POS	1
11	1	11	PT2016CSFSERPS2	POS	POS	1
12	1	12	PT2016CSFSERPS1	POS	POS	1
13	1	13	PT2016CSFSERNS2	NEG	NEG	1
14	1	14	PT2016CSFSERNS1	NEG	NEG	1
15	1	15	PT2016CSFSERPS1	POS	POS	1
16	1	16	PT2016CSFSERPS2	POS	POS	1
17	1	17	PT2016CSFSERPS1	POS	POS	1
18	1	18	PT2016CSFSERPS4	POS	POS	1
19	1	19	PT2016CSFSERNS1	NEG	NEG	1
20	1	20	PT2016CSFSERNS1	NEG	NEG	1
21	2	1	PT2016CSFSERNS2	NEG	NEG	1
22	2	2	PT2016CSFSERPS2	POS	POS	1
23	2	3	PT2016CSFSERNS1	NEG	NEG	1
24	2	4	PT2016CSFSERPS4	POS	POS	1
25	2	5	PT2016CSFSERPS1	POS	POS	1
26	2	6	PT2016CSFSERNS2	NEG	NEG	1
27	2	7	PT2016CSFSERPS3	POS	POS	1
28	2	8	PT2016CSFSERNS1	NEG	NEG	1
29	2	9	PT2016CSFSERPS1	POS	POS	1
30	2	10	PT2016CSFSERPS2	POS	POS	1
31	2	11	PT2016CSFSERPS4	POS	POS	1
32	2	12	PT2016CSFSERPS1	POS	POS	1
33	2	13	PT2016CSFSERNS1	NEG	NEG	1
34	2	14	PT2016CSFSERPS1	POS	POS	1
35	2	15	PT2016CSFSERPS2	POS	POS	1
36	2	16	PT2016CSFSERNS1	NEG	NEG	1
37	2	17	PT2016CSFSERPS3	POS	POS	1
38	2	18	PT2016CSFSERNS2	NEG	NEG	1
39	2	19	PT2016CSFSERNS1	NEG	NEG	1
40	2	20	PT2016CSFSERPS1	POS	POS	1

(Table 3 - continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2016CSFSERNS1	NEG	NEG	1
42	3	2	PT2016CSFSERPS1	POS	POS	1
43	3	3	PT2016CSFSERNS2	NEG	NEG	1
44	3	4	PT2016CSFSERPS3	POS	POS	1
45	3	5	PT2016CSFSERPS2	POS	POS	1
46	3	6	PT2016CSFSERPS4	POS	POS	1
47	3	7	PT2016CSFSERNS2	NEG	NEG	1
48	3	8	PT2016CSFSERPS1	POS	POS	1
49	3	9	PT2016CSFSERNS1	NEG	NEG	1
50	3	10	PT2016CSFSERPS3	POS	POS	1
51	3	11	PT2016CSFSERPS2	POS	POS	1
52	3	12	PT2016CSFSERPS1	POS	POS	1
53	3	13	PT2016CSFSERNS2	NEG	NEG	1
54	3	14	PT2016CSFSERNS1	NEG	NEG	1
55	3	15	PT2016CSFSERPS1	POS	POS	1
56	3	16	PT2016CSFSERPS2	POS	POS	1
57	3	17	PT2016CSFSERPS1	POS	POS	1
58	3	18	PT2016CSFSERPS4	POS	POS	1
59	3	19	PT2016CSFSERNS1	NEG	NEG	1
60	3	20	PT2016CSFSERNS1	NEG	NEG	1
61	4	1	PT2016CSFSERNS2	NEG	NEG	1
62	4	2	PT2016CSFSERPS2	POS	POS	1
63	4	3	PT2016CSFSERNS1	NEG	NEG	1
64	4	4	PT2016CSFSERPS4	POS	POS	1
65	4	5	PT2016CSFSERPS1	POS	POS	1
66	4	6	PT2016CSFSERNS2	NEG	NEG	1
67	4	7	PT2016CSFSERPS3	POS	POS	1
68	4	8	PT2016CSFSERNS1	NEG	NEG	1
69	4	9	PT2016CSFSERPS1	POS	POS	1
70	4	10	PT2016CSFSERPS2	POS	POS	1
71	4	11	PT2016CSFSERPS4	POS	POS	1
72	4	12	PT2016CSFSERPS1	POS	POS	1
73	4	13	PT2016CSFSERNS1	NEG	NEG	1
74	4	14	PT2016CSFSERPS1	POS	POS	1
75	4	15	PT2016CSFSERPS2	POS	POS	1
76	4	16	PT2016CSFSERNS1	NEG	NEG	1
77	4	17	PT2016CSFSERPS3	POS	POS	1
78	4	18	PT2016CSFSERNS2	NEG	NEG	1
79	4	19	PT2016CSFSERNS1	NEG	NEG	1
80	4	20	PT2016CSFSERPS1	POS	POS	1

(Table 3 - continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2016CSFSERNS1	NEG	NEG	1
82	5	2	PT2016CSFSERPS1	POS	POS	1
83	5	3	PT2016CSFSERNS2	NEG	NEG	1
84	5	4	PT2016CSFSERPS3	POS	POS	1
85	5	5	PT2016CSFSERPS2	POS	POS	1
86	5	6	PT2016CSFSERPS4	POS	POS	1
87	5	7	PT2016CSFSERNS2	NEG	NEG	1
88	5	8	PT2016CSFSERPS1	POS	POS	1
89	5	9	PT2016CSFSERNS1	NEG	NEG	1
90	5	10	PT2016CSFSERPS3	POS	POS	1
91	5	11	PT2016CSFSERPS2	POS	POS	1
92	5	12	PT2016CSFSERPS1	POS	POS	1
93	5	13	PT2016CSFSERNS2	NEG	NEG	1
94	5	14	PT2016CSFSERNS1	NEG	NEG	1
95	5	15	PT2016CSFSERPS1	POS	POS	1
96	5	16	PT2016CSFSERPS2	POS	POS	1
97	5	17	PT2016CSFSERPS1	POS	POS	1
98	5	18	PT2016CSFSERPS4	POS	POS	1
99	5	19	PT2016CSFSERNS1	NEG	NEG	1
100	5	20	PT2016CSFSERNS1	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of porcine origin for the detection of CSF-specific antibodies by ELISA.

For the detection of CSF-specific antibodies in reference serum samples, the 5 participating laboratories (LAB1, LAB2, LAB3, LAB4 and LAB5) provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement) (Table 2 and Table 3).

All participating laboratories used the same CSF antibody ELISA kit from IDEXX and the same batch E781.

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 serum samples assigned by CODA-CERVA-Uccle (see III.3.3.). Consequently, all the 5 participants achieved a satisfactory performance for the detection of CSF-specific antibodies in reference serum samples of porcine origin by ELISA.

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)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Laboratoire National de Contrôle des Reproducteurs (LNCR) / ACSEDIATE (Maisons-Alfort, France)

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

Annex 1: Quantitative data analysis

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed using the statistical software R (box plots). All quantitative data analyses were performed on the normalized data, namely the percentages blocking calculated according to the instructions of the PT provider: [1- (OD_{Sample} / mean OD $_{Negative\ Kit\ Controls}$)] x 100.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

Box plots of the percentages blocking calculated according to the instructions of the PT provider per reference serum sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

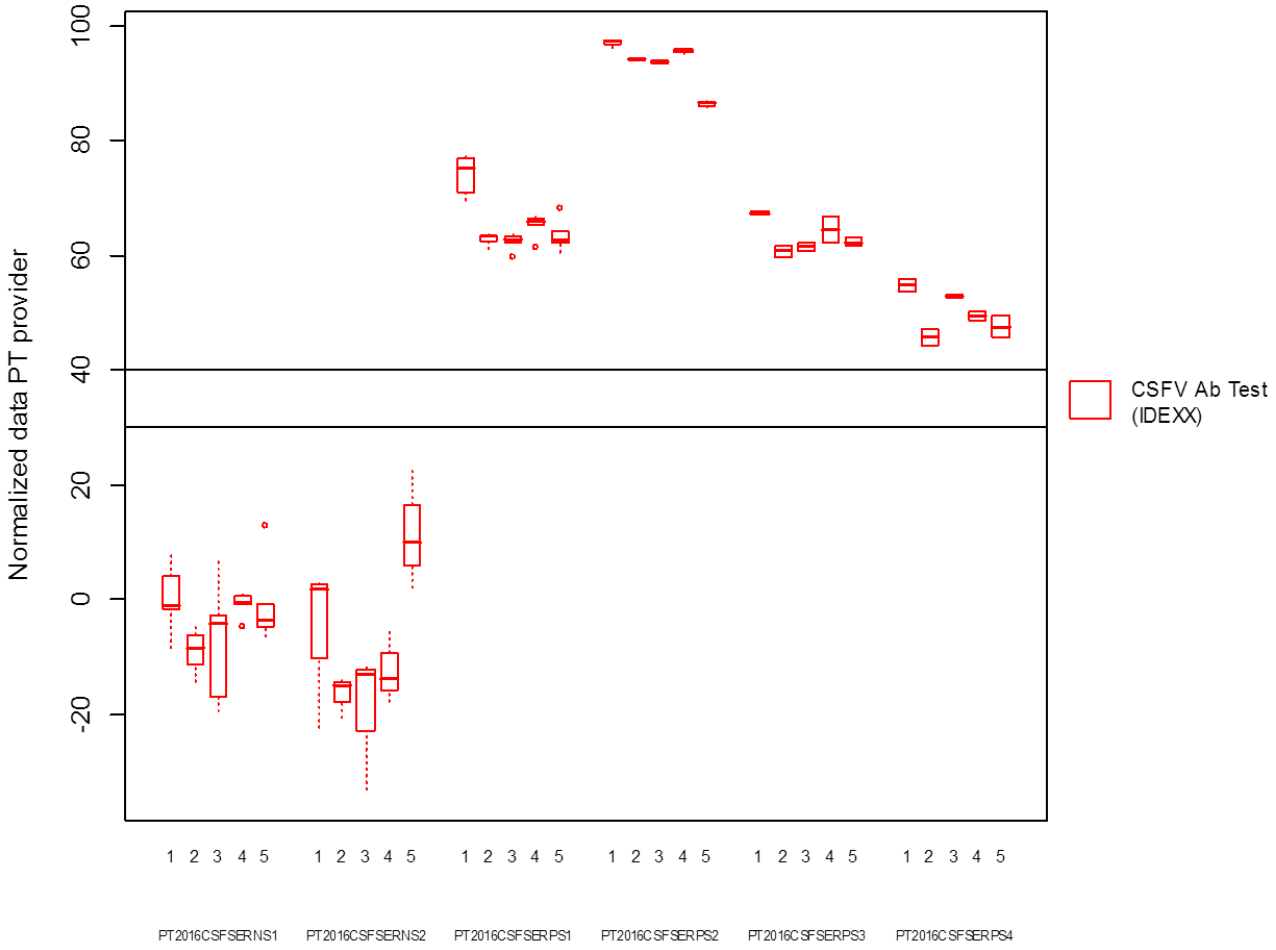

Figure 1. Box plots showing the percentage blocking calculated according to the instructions of the PT provider per reference serum sample and per participating laboratory.
Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.
All participating laboratories used the same CSF antibody ELISA kit from IDEXX and the same batch E781.
Cut-off values (30-40%) for CSF antibody ELISA kit from IDEXX are shown by horizontal lines.