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172-PT

PROFICIENCY TESTING 2012

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Detection of BSE-specific prion antigens in bovine brain tissue

by Enzyme Linked Immunosorbent Assay (ELISA)

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 01 OCTOBER 2012

DATE REPORT: 25 OCTOBER 2012

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 BSE-specific prion antigens in bovine brain tissue (obex) by ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference brain tissue samples must be tested by means of a BSE antigen ELISA. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 5 reference brain tissue samples of bovine origin, either free from detectable BSE-specific prion antigens (n=3; coded 'PT2012BSETSENBr1', 'PT2012BSETSENBr2' and 'PT2012BSETSENBr3') or containing detectable BSE-specific prion antigens (n=2; coded 'PT2012BSETSEPBr1' and 'PT2012BSETSEPBr2'), were used. In total, 80 aliquots of reference brain tissue samples were distributed to 8 participating laboratories. All participants received 2 aliquots of the 5 reference brain tissue samples, i.e. 10 aliquots. The identification numbers of the reference brain tissue samples were randomized for each participant (Table 3).

For each reference brain tissue sample, a certificate containing the status of the sample (= 'golden standard') was made by the BSE reference laboratory of the Veterinary and Agrochemical Research Center (CODA-CERVA) based on the test results obtained with the TeSeE SAP ELISA kit from Bio-Rad (pre-verification). All reference brain tissue samples were also tested once after the PT with the same ELISA kit in order to confirm their stability and status (post-verification). Consequently, these reference brain tissue samples were considered as reliable samples to evaluate the ability of laboratories to correctly identify the absence or presence of BSE-specific prion antigens in bovine brain tissue.

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* (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* (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* (i.e., the reported result matches with the assigned status) for the 10 aliquots of reference brain tissue 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 10 aliquots of reference brain tissue 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 operational unit CVD-ERA of CODA-CERVA.

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

The 10 aliquots of reference brain tissue samples were sent frozen (dry ice) to each of the 8 participating laboratories by national courier on 1st of October 2012 (80 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. LAB1, LAB2, LAB4, LAB6, LAB7 and LAB8 performed the analysis between 1st and 3rd of October 2012. LAB3 and LAB5 did not provide the date of analysis (Table 1).

IV.2. Dates at which results were returned to the operational unit CVD-ERA

Results were submitted to the operational unit CVD-ERA between 3rd and 11th of October 2012 (Table 1). All participants hereby respected the deadline of 12th of October 2012 for submission of the results.

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

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	01/10/2012	02/10/2012	04/10/2012
LAB2	01/10/2012	01/10/2012	03/10/2012
LAB3	01/10/2012	NOT PROVIDED	05/10/2012
LAB4	01/10/2012	03/10/2012	08/10/2012
LAB5	01/10/2012	NOT PROVIDED	10/10/2012
LAB6	01/10/2012	02/10/2012	09/10/2012
LAB7	01/10/2012	01/10/2012	11/10/2012
LAB8	01/10/2012	03/10/2012	05/10/2012

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

Qualitative data analysis showed that 7 out of 8 participating laboratories (LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB8) provided qualitative results that were in full agreement with the true status of the reference brain tissue samples (100% of agreement), whereas LAB7 misclassified 1 aliquot and hence obtained 90% of agreement (Table 2).

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the reference brain tissue samples assigned by the BSE reference laboratory of CODA-CERVA. All participating laboratories received 10 aliquots of reference brain tissue samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	9 (90.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1, LAB2, LAB3, LAB4, LAB5, LAB6 and LAB8 since these participants reached 100% of agreement for the detection of BSE-specific prion antigens in reference brain tissue samples. In contrast, LAB7 misclassified 1 aliquot of the positive reference brain tissue sample PT2012BSETSEPBr2 (NEG instead of POS).

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

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

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012BSETSENBr1	NEG	NEG	1
2	1	2	PT2012BSETSENBr2	NEG	NEG	1
3	1	3	PT2012BSETSENBr1	NEG	NEG	1
4	1	4	PT2012BSETSEPBr1	POS	POS	1
5	1	5	PT2012BSETSENBr2	NEG	NEG	1
6	1	6	PT2012BSETSENBr3	NEG	NEG	1
7	1	7	PT2012BSETSEPBr1	POS	POS	1
8	1	8	PT2012BSETSENBr3	NEG	NEG	1
9	1	9	PT2012BSETSEPBr2	POS	POS	1
10	1	10	PT2012BSETSEPBr2	POS	POS	1
11	2	1	PT2012BSETSEPBr2	POS	POS	1
12	2	2	PT2012BSETSENBr1	NEG	NEG	1
13	2	3	PT2012BSETSENBr2	NEG	NEG	1
14	2	4	PT2012BSETSENBr1	NEG	NEG	1
15	2	5	PT2012BSETSEPBr1	POS	POS	1
16	2	6	PT2012BSETSENBr2	NEG	NEG	1
17	2	7	PT2012BSETSENBr3	NEG	NEG	1
18	2	8	PT2012BSETSEPBr1	POS	POS	1
19	2	9	PT2012BSETSENBr3	NEG	NEG	1
20	2	10	PT2012BSETSEPBr2	POS	POS	1
21	3	1	PT2012BSETSEPBr2	POS	POS	1
22	3	2	PT2012BSETSEPBr2	POS	POS	1
23	3	3	PT2012BSETSENBr1	NEG	NEG	1
24	3	4	PT2012BSETSENBr2	NEG	NEG	1
25	3	5	PT2012BSETSENBr1	NEG	NEG	1
26	3	6	PT2012BSETSEPBr1	POS	POS	1
27	3	7	PT2012BSETSENBr2	NEG	NEG	1
28	3	8	PT2012BSETSENBr3	NEG	NEG	1
29	3	9	PT2012BSETSEPBr1	POS	POS	1
30	3	10	PT2012BSETSENBr3	NEG	NEG	1
31	4	1	PT2012BSETSENBr3	NEG	NEG	1
32	4	2	PT2012BSETSEPBr2	POS	POS	1
33	4	3	PT2012BSETSEPBr2	POS	POS	1
34	4	4	PT2012BSETSENBr1	NEG	NEG	1
35	4	5	PT2012BSETSENBr2	NEG	NEG	1
36	4	6	PT2012BSETSENBr1	NEG	NEG	1
37	4	7	PT2012BSETSEPBr1	POS	POS	1
38	4	8	PT2012BSETSENBr2	NEG	NEG	1
39	4	9	PT2012BSETSENBr3	NEG	NEG	1
40	4	10	PT2012BSETSEPBr1	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	5	1	PT2012BSETSEPBr1	POS	POS	1
42	5	2	PT2012BSETSENBr3	NEG	NEG	1
43	5	3	PT2012BSETSEPBr2	POS	POS	1
44	5	4	PT2012BSETSEPBr2	POS	POS	1
45	5	5	PT2012BSETSENBr1	NEG	NEG	1
46	5	6	PT2012BSETSENBr2	NEG	NEG	1
47	5	7	PT2012BSETSENBr1	NEG	NEG	1
48	5	8	PT2012BSETSEPBr1	POS	POS	1
49	5	9	PT2012BSETSENBr2	NEG	NEG	1
50	5	10	PT2012BSETSENBr3	NEG	NEG	1
51	6	1	PT2012BSETSENBr3	NEG	NEG	1
52	6	2	PT2012BSETSEPBr1	POS	POS	1
53	6	3	PT2012BSETSENBr3	NEG	NEG	1
54	6	4	PT2012BSETSEPBr2	POS	POS	1
55	6	5	PT2012BSETSEPBr2	POS	POS	1
56	6	6	PT2012BSETSENBr1	NEG	NEG	1
57	6	7	PT2012BSETSENBr2	NEG	NEG	1
58	6	8	PT2012BSETSENBr1	NEG	NEG	1
59	6	9	PT2012BSETSEPBr1	POS	POS	1
60	6	10	PT2012BSETSENBr2	NEG	NEG	1
61	7	1	PT2012BSETSENBr2	NEG	NEG	1
62	7	2	PT2012BSETSENBr3	NEG	NEG	1
63	7	3	PT2012BSETSEPBr1	POS	POS	1
64	7	4	PT2012BSETSENBr3	NEG	NEG	1
65	7	5	PT2012BSETSEPBr2	**POS**	**NEG**	**0**
66	7	6	PT2012BSETSEPBr2	POS	POS	1
67	7	7	PT2012BSETSENBr1	NEG	NEG	1
68	7	8	PT2012BSETSENBr2	NEG	NEG	1
69	7	9	PT2012BSETSENBr1	NEG	NEG	1
70	7	10	PT2012BSETSEPBr1	POS	POS	1
71	8	1	PT2012BSETSEPBr1	POS	POS	1
72	8	2	PT2012BSETSENBr2	NEG	NEG	1
73	8	3	PT2012BSETSENBr3	NEG	NEG	1
74	8	4	PT2012BSETSEPBr1	POS	POS	1
75	8	5	PT2012BSETSENBr3	NEG	NEG	1
76	8	6	PT2012BSETSEPBr2	POS	POS	1
77	8	7	PT2012BSETSEPBr2	POS	POS	1
78	8	8	PT2012BSETSENBr1	NEG	NEG	1
79	8	9	PT2012BSETSENBr2	NEG	NEG	1
80	8	10	PT2012BSETSENBr1	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference brain tissue samples of bovine origin for the detection of BSE-specific prion antigens by ELISA.

7 out of 8 participating laboratories provided qualitative results that were in full agreement with the true status of the reference brain tissue samples (100% of agreement). In contrast, LAB7 misclassified 1 aliquot of the positive reference brain tissue sample PT2012BSETSEPBr2 and hence reached 90% of agreement.

BSE antigen ELISA kits from 2 different producers as well as different batches from the same producer were used: Bio-Rad (4 batches: 2D1021, 2A0020, 2D1022 and 2G2023) and IDEXX (1 batch: DH709). All laboratories except LAB6 used the BSE antigen ELISA kit from Bio-Rad. Hereby, LAB1 used batch 2D1021, LAB2 and LAB3 used batch 2A0020, LAB4 and LAB7 used batch 2D1022, and LAB5 and LAB8 used batch 2G2023.

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 brain tissue samples assigned by the BSE reference laboratory of CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of BSE-specific prion antigens in reference brain tissue samples by ELISA.

Head CVD-ERA
Yves Van der Stede

Appendix

Name of the participating laboratories

Chemiphar NV (Brugge, Belgium)

Chemiphar NV (Oostkamp, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

Laboratorium ECCA (Merelbeke, Belgium)

Laboratoire Luxembourgeois de Contrôle Sanitaire (LLuCS) (Grand Duchy of Luxemburg)

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

Quality Partner SA (Herstal, Belgium)

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