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172-PT

PROFICIENCY TESTING 2015

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Detection of BSE-specific prion antigens in bovine brain tissue

by Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 12 OCTOBER 2015

DATE REPORT: 7 DECEMBER 2015

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 4 reference brain tissue samples of bovine origin, either free from detectable BSE-specific prion antigens (n=2; coded 'PT2015BSETSEBr1' and 'PT2015BSETSEBr2') or containing detectable BSE-specific prion antigens (n=2; coded 'PT2015BSETSEPr1' and 'PT2015BSETSEPr2'), were used. In total, 10 aliquots of these reference brain tissue samples were distributed to 2 participating laboratories. All participants received 5 aliquots: 1 aliquot of the reference brain tissue sample PT2015BSETSEBr1, 1 aliquot of the reference brain tissue sample PT2015BSETSEBr2, 2 aliquots of the reference brain tissue sample PT2015BSETSEPr1 and 1 aliquot of the reference brain tissue sample PT2015BSETSEPr2. 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 CODA-CERVA-Uccle based on the test results obtained during pre-verification using the TeSeE SAP ELISA kit from Bio-Rad. All reference brain tissue samples were also tested once after the PT using 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* 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 $\geq 90\%$.

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 5 aliquots of reference brain tissue samples were sent frozen (dry ice) to each of the 2 participating laboratories by national courier on 12th of October 2015 (10 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 13th and 14th of October 2015 (Table 1).

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

Results were submitted to the CODA-CERVA-Uccle between 22th and the 26th of October 2015 (Table 1). Hereby, all laboratories respected the deadline of 30th of October 2015 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 CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	12/10/2015	13/10/2015	26/10/2015
LAB2	12/10/2015	14/10/2015	22/10/2015

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 all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference brain tissue samples (100% of agreement) (Table 2).

Since the number of participants was limited to 2, no quantitative data analysis could be performed for educational purpose. Therefore only a box plot is shown in Annex 1.

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

	LABNR	
	1	2
failure	0 (0.0)	0 (0.0)
success	5 (100.0)	5 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between participating laboratories since all participants reached 100% of agreement for the detection of BSE-specific prion antigens in reference brain tissue samples.

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-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BSETSEPBr1	POS	POS	1
2	1	2	PT2015BSETSENBBr1	NEG	NEG	1
3	1	3	PT2015BSETSEPBr2	POS	POS	1
4	1	4	PT2015BSETSEPBr1	POS	POS	1
5	1	5	PT2015BSETSENBBr2	NEG	NEG	1
6	2	1	PT2015BSETSEPBr1	POS	POS	1
7	2	2	PT2015BSETSENBBr2	NEG	NEG	1
8	2	3	PT2015BSETSEPBr1	POS	POS	1
9	2	4	PT2015BSETSENBBr1	NEG	NEG	1
10	2	5	PT2015BSETSEPBr2	POS	POS	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.

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference brain tissue samples (100% of agreement) (Table 2 and Table 3). Hereby, one participant used the HerdCheck BSE Scrapie Antigen test Kit of IDEXX and the other participant used the TeSeE SAP Combi Kit of Biorad.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if $\geq 90\%$ 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-Uccle (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.

Coordinator proficiency tests

Katia Knapen



Appendix

Name of the participating laboratories

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

Laboratorium ECCA NV (Merelbeke, 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 program R (box plots). All quantitative data analyses were performed on the OD values.

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.

Since the number of participants was limited to 2, no extensive quantitative data analysis could be performed. Only box plots are shown below.

I. Box plots

Box plots of the OD values per reference brain tissue sample and per participating laboratory were made using the statistical software R and are shown in Figure 1.

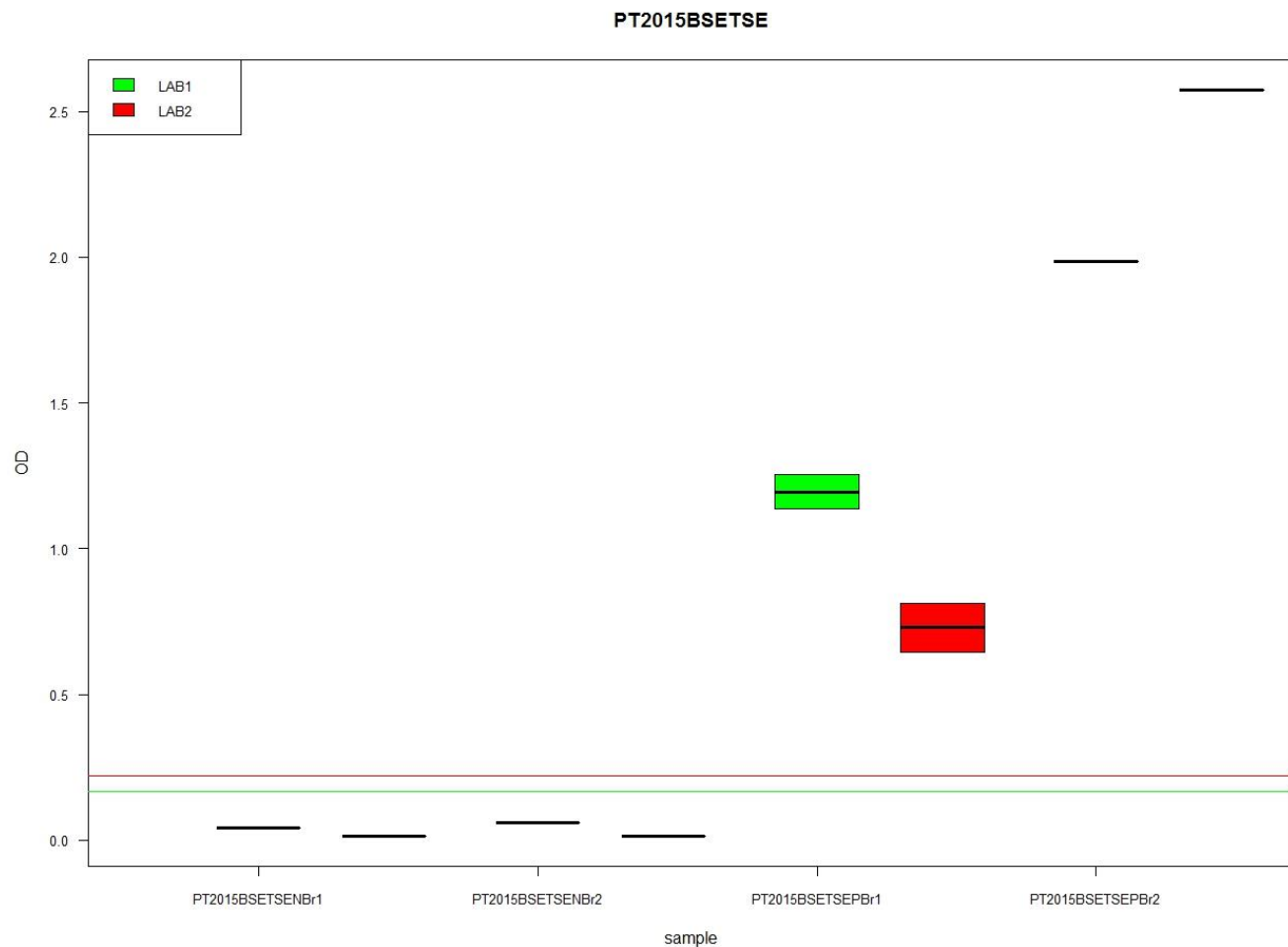


Figure 1. Box plots showing the OD values per reference brain tissue sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. The participating laboratories used different ELISA kits. The cut-off values for each ELISA kits are shown with a green and red line.