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172-PT

PROFICIENCY TESTING 2013

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: 23 SEPTEMBER 2013

DATE REPORT: 07 FEBRUARY 2014

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 2 reference brain tissue samples of bovine origin, either free from detectable BSE-specific prion antigens (n=1; coded 'PT2013BSETSENBr1') or containing detectable BSE-specific prion antigens (n=1; coded 'PT2013BSETSEPBr1'), were used. In total, 21 aliquots of these reference brain tissue samples were distributed to 3 participating laboratories. All participants received 7 aliquots: 3 aliquots of the reference brain tissue sample PT2013BSETSENBr1 and 4 aliquots of the reference brain tissue sample PT2013BSETSEPBr1. 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 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 7 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 7 aliquots of reference samples is at least 90% (in this case 100%).

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 7 aliquots of reference brain tissue samples were sent frozen (dry ice) to each of the 3 participating laboratories by national courier on 23rd of September 2013 (21 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 24th and 26th of September 2013 (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 27th of September and 7th of October 2013 (Table 1). Hereby, LAB2 did not respect the deadline of 4th of October 2013 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	23/09/2013	25/09/2013	30/09/2013
LAB2	23/09/2013	26/09/2013	**07/10/2013**
LAB3	23/09/2013	24/09/2013	27/09/2013

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).

A quantitative data analysis (including box plots) is shown for educational purposes in Annex 1 and Annex 2.

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. All participating laboratories received 7 aliquots of reference brain tissue samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	7 (100.0)	7 (100.0)	7 (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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013BSETSENBr1	NEG	NEG	1
2	1	2	PT2013BSETSEPBr1	POS	POS	1
3	1	3	PT2013BSETSEPBr1	POS	POS	1
4	1	4	PT2013BSETSENBr1	NEG	NEG	1
5	1	5	PT2013BSETSEPBr1	POS	POS	1
6	1	6	PT2013BSETSENBr1	NEG	NEG	1
7	1	7	PT2013BSETSEPBr1	POS	POS	1
8	2	1	PT2013BSETSEPBr1	POS	POS	1
9	2	2	PT2013BSETSENBr1	NEG	NEG	1
10	2	3	PT2013BSETSEPBr1	POS	POS	1
11	2	4	PT2013BSETSENBr1	NEG	NEG	1
12	2	5	PT2013BSETSEPBr1	POS	POS	1
13	2	6	PT2013BSETSENBr1	NEG	NEG	1
14	2	7	PT2013BSETSEPBr1	POS	POS	1
15	3	1	PT2013BSETSENBr1	NEG	NEG	1
16	3	2	PT2013BSETSEPBr1	POS	POS	1
17	3	3	PT2013BSETSEPBr1	POS	POS	1
18	3	4	PT2013BSETSENBr1	NEG	NEG	1
19	3	5	PT2013BSETSEPBr1	POS	POS	1
20	3	6	PT2013BSETSENBr1	NEG	NEG	1
21	3	7	PT2013BSETSEPBr1	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, all participants used the TeSeE SAP ELISA kit from Bio-Rad, but 3 different validated batches were used: 3B0048, 3D0049 and 3F0050.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% (in this case 100%) 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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

Veterinary and Agrochemical Research Center (CODA-CERVA) (Ukkel, 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 programs R (box plots) and SAS 9.2. (summary statistics). 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.

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.

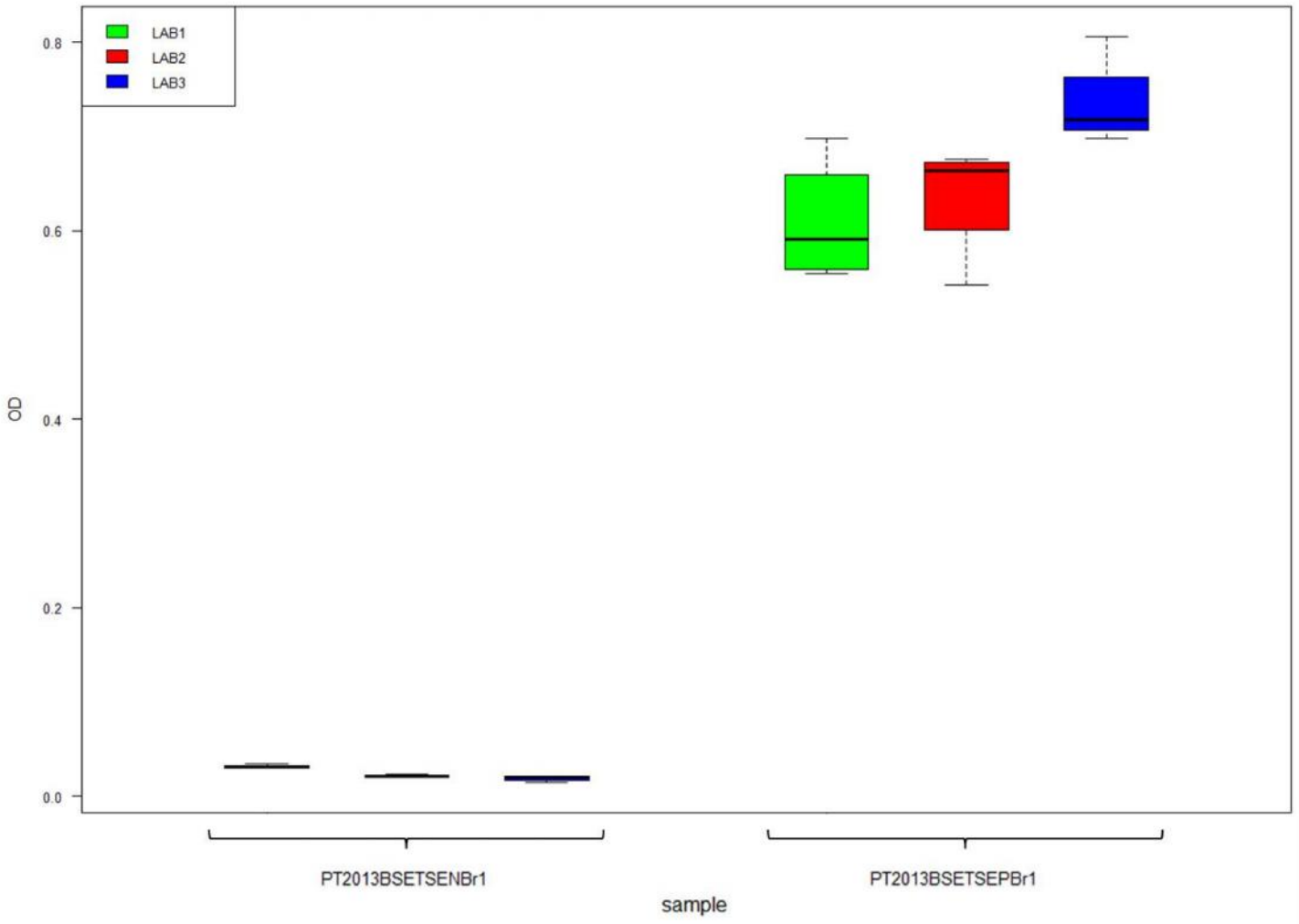

Figure 1. Box plots showing the OD values per reference brain tissue sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower (25%) and upper (75%) quartile, and possible outliers per sample and per laboratory. All participating laboratories used the same ELISA kit (3 different batches).

II. Mandel's h- and k-statistics (z-scores)

Based on ISO 5725-2 and ISO 13528, between-lab variability (reproducibility) and within-lab variability (repeatability) were estimated through Mandel's h- and k-statistics, respectively, using the statistical software SAS 9.2. Mandel's h- and k-statistics were calculated per reference brain tissue sample and per participating laboratory.

The h-statistic depends on the number of participants, whereas the k-statistic depends on both the number of participants and the number of repeats per sample. When 30 participants or more are involved in a PT, a satisfactory between-lab and within-lab consistency is obtained when the (absolute) value for the h- and k-statistic is smaller than 2. An unsatisfactory result (a corrective action is required) is reached when the (absolute) value is larger than 3. (Absolute) values between 2 and 3 indicate a questionable consistency. Importantly, in case of a smaller number of participants (which is the case in this PT), other indicator values apply for Mandel's h- and k-statistics (Table 1).

Table 1. Indicators for Mandel's h- and k-statistics at the 5% significance level in function of the number of participating laboratories (p) and the number of repeats per sample (n) as described in ISO 5725-2.

p (# labs)	h	k								
		n (# repeats)								
		2	3	4	5	6	7	8	9	10
3	1,15	1,65	1,53	1,45	1,40	1,37	1,34	1,32	1,30	1,29
4	1,42	1,76	1,59	1,50	1,44	1,40	1,37	1,35	1,33	1,31
5	1,57	1,81	1,62	1,53	1,46	1,42	1,39	1,36	1,34	1,32
6	1,66	1,85	1,64	1,54	1,48	1,43	1,40	1,37	1,35	1,33
7	1,71	1,87	1,66	1,55	1,49	1,44	1,41	1,38	1,36	1,34
8	1,75	1,88	1,67	1,56	1,50	1,45	1,41	1,38	1,36	1,34
9	1,78	1,90	1,68	1,57	1,50	1,45	1,42	1,39	1,36	1,35
10	1,80	1,90	1,68	1,57	1,50	1,46	1,42	1,39	1,37	1,35

Based on Table 1, the maximum absolute value for Mandel's h-statistic for this PT is 1,15 (p=3), whereas the maximum value for Mandel's k-statistic is 1,53 for the reference sample PT2013BSETSENBr1 (p=3 and n=3) and 1,45 for the reference sample PT2013BSETSEPBr1 (p=3 and n=4).

All participating laboratories obtained a satisfactory between-laboratory and within-laboratory consistency for all reference brain tissue samples. All participants used the same ELISA kit (3 different batches).

All data used for the calculations of Mandel's h- and k-statistics can be found in Annex 2.

III. ANOVA

Using a SAS macro encoding a general linear model (GLM) with laboratories as fixed effect and the OD values as a dependent variable, it was investigated whether statistically significant differences exist (α=0,05) between participating laboratories. Comparisons were made at the global level (all reference samples were analysed together), status level (all reference samples with the same status were analysed together) and sample level (all reference samples were analysed individually). Since comparing quantitative results between participants or methods (e.g. different kits, batches or incubation protocols) is most relevant at the status level (less variation than at a global level), we focused on the latter.

Statistically significant differences between laboratories were observed at the status (=sample) level, but not at the global level. At the status level, significant differences were observed for both the negative and positive reference brain tissue samples. For the negative reference brain tissue samples, LAB1 reported OD values that were significantly higher than those reported by LAB2 and LAB3. For the positive reference brain tissue samples, LAB3 reported OD values that were significantly higher than those reported by LAB1.

Annex 2: Calculations of Mandel's h- and k-statistics (based on OD values)

Sample	Labnr	n_i	v_i	x_i_m	x_g_m	between_ lab_coeff	STDEV _repeat	STDEV _repro	STDEV _betweenlab	h	k	cv
PT2013BSETSENBr1	1	3	0,000007	0,032	0,024	0,733	0,003	0,006	0,005	1,13	0,93	8,27
PT2013BSETSENBr1	2	3	0,000004	0,022	0,024	0,733	0,003	0,006	0,005	-0,37	0,73	9,61
PT2013BSETSENBr1	3	3	0,000013	0,019	0,024	0,733	0,003	0,006	0,005	-0,76	1,27	18,98
PT2013BSETSEPBr1	1	4	0,004358	0,609	0,660	0,331	0,060	0,073	0,042	-0,77	1,11	10,84
PT2013BSETSEPBr1	2	4	0,003940	0,637	0,660	0,331	0,060	0,073	0,042	-0,36	1,05	9,86
PT2013BSETSEPBr1	3	4	0,002335	0,735	0,660	0,331	0,060	0,073	0,042	1,13	0,81	6,57

Legend: **Labnr** = number attributed to a laboratory during the PT; **n_i** = number of replicates; **v_i** = total variability (variance) in OD values; **x_i_m** = mean of OD values; **x_g_m** = mean of OD values obtained by all laboratories; b**etween_lab_coeff** = fraction of total variability due to differences between labs for each sample; **STDEV_repeat** = repeatability standard deviation over all laboratories; **STDEV_repro** = reproducibility standard deviation over all laboratories; **STDEV_betweenlab** = between-lab standard deviation over all laboratories; **h-statistic** = between-laboratory consistency; **k-statistic** = within-laboratory consistency; **CV** = variation coefficient in %.