Rue Juliette Wytsmanstraat 14 • 1050 Brussels • Belgium
T + 32 2 642 51 11 • F + 32 2 642 50 01
www.sciensano.be

081-PT

PROFICIENCY TESTING 2019

BOVINE SPONGIFORM ENCEPHALOPATHY (BSE)

Detection of BSE-specific prion antigens in bovine brain tissue

by Enzyme Linked Immunosorbent Assay (ELISA)

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS

SCIENSANO

DATE BEGIN PT: 7 OCTOBER 2019

DATE REPORT: 13 DECEMBER 2019

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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=1; coded 'PT2019BSEELISANBr1') or containing detectable BSE-specific prion antigens (n=3; coded 'PT2019BSEELISAPBr1', 'PT2019BSEELISAPBr2' and 'PT2019BSEELISAPBr3'), were used. In total, 10 aliquots of these reference brain tissue samples were distributed to 2 participating laboratories. All participants received 2 aliquots of the reference brain tissue sample PT2019BSEELISANBr1 and 1 aliquot of the reference brain tissue samples PT2019BSEELISAPBr1, PT2019BSEELISAPBr2 and PT2019BSEELISAPBr3. 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 Scientific Directorate Infectious Diseases in Animals of Sciensano based on the test results obtained during pre-verification using the TeSeE ELISA kit from Bio-Rad. All reference brain tissue samples were also tested 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 a participating laboratory is expressed as the percentage *success* for the 5 reference samples used in 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 reference samples is at least 100%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Scientific Directorate Infectious Diseases in Animals of Sciensano.

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 7th of October 2019 (10 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed on 10th of October 2019 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submited to the Scientific Directorate Infectious Diseases in Animals of Sciensano on the 15th and 30th of October 2019 (Table 1). Only LAB1 respected the deadline of 25th of October 2019 for submission of the results.

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 Scientific Directorate Infectious Diseases in Animals of Sciensano

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	07/10/2019	10/10/2019	15/10/2019
LAB2	07/10/2019	10/10/2019	**30/10/2017**

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

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

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the brain tissue samples assigned by the BSE reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 5 aliquots of brain tissue samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR	
	1	**2**
failure	0 (0)	0 (0)
success	5 (100)	5 (100)

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 brain tissue samples (SAMPLE), the external identification of the brain tissue samples (LABPOSIT), and the status assigned by the BSE reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2019BSEELISAPBr3	POS	POS	1
2	1	2	PT2019BSEELISAPBr1	POS	POS	1
3	1	3	PT2019BSEELISANBr1	NEG	NEG	1
4	1	4	PT2019BSEELISAPBr2	POS	POS	1
5	1	5	PT2019BSEELISANBr1	NEG	NEG	1
6	2	1	PT2019BSEELISAPBr2	POS	POS	1
7	2	2	PT2019BSEELISANBr1	NEG	NEG	1
8	2	3	PT2019BSEELISAPBr1	POS	POS	1
9	2	4	PT2019BSEELISANBr1	NEG	NEG	1
10	2	5	PT2019BSEELISAPBr3	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 (batch ER361) and the other participant used the TeSeE ELISA kit of Biorad (batch 9F0074).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if 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 the Scientific Directorate Infectious Diseases in Animals of Sciensano (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 and Bernard China

Appendix

Name of the participating laboratories

Laboratorium ECCA NV (Merelbeke, Belgium)

Sciensano (Ukkel, Belgium)