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172-PT

PROFICIENCY TESTING 2014

SCRAPIE (SCR)

Genotype identification for the detection

of genetically linked susceptibility to scrapie in blood

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 14 APRIL 2014

DATE REPORT: 20 JUNE 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 genotypes related to genetically linked susceptibility to scrapie in blood of sheep origin.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference blood samples must be tested by means of real-time PCR (RT-PCR) and/or sequencing. The procedures for the RT-PCR and/or sequencing must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Ten reference blood samples of sheep origin, coded 'PT2014SCRGENB1', 'PT2014SCRGENB2', 'PT2014SCRGENB3', 'PT2014SCRGENB4', 'PT2014SCRGENB5', 'PT2014SCRGENB6', 'PT2014SCRGENB7', 'PT2014SCRGENB8', 'PT2014SCRGENB9' and 'PT2014SCRGENB10', were used. In total, 20 aliquots were distributed to 2 participating laboratories. All participants received 1 aliquot of each reference blood sample. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 3).

For each reference blood sample, a certificate containing the assigned status (= 'golden standard') was made. The genotype of the reference blood samples was assigned based on the results obtained during pre-verification tests, namely RT-PCR and Denaturing Gradient Gel Electrophoresis - Restriction Fragment Length Polymorphism (DGGE-RFLP). Hereby, the same genotype was obtained with both methods for each reference blood sample. Consequently, these reference blood samples were considered as reliable samples to use for the purpose of this PT. In addition, the reference blood samples were also tested once after the PT in order to confirm their stability and status (post-verification), using RT-PCR and DGGE-RFLP.

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 (i.e., if the genotype is correctly identified) or *failure* when the reported result does not match with the assigned status (i.e., if the genotype is not correctly identified).

III.3.2. Level of agreement

The level of agreement achieved by a participating laboratory is expressed as the percentage *success* for the 10 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 10 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 operational unit CVD-ERA of CODA-CERVA.

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

The 10 reference blood samples were sent frozen (dry ice) to each of the 2 participating laboratories by national courier on 14th of April 2014 (20 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 15th and 22nd of April 2014 (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 on 23rd and 25th of April 2014 (Table 1). All participants hereby respected the deadline of 25th of April 2014 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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	14/04/2014	22/04/2014	25/04/2014
LAB2	14/04/2014	15/04/2014	23/04/2014

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

One out of 2 participating laboratories (LAB2) reported genotypes that were in full agreement with the assigned status of the reference blood samples and thus reached 100% of agreement. In contrast, the other participating laboratory (LAB1) misclassified 1 reference blood sample and hence reached 90 % of agreement (Table 2).

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

	LABNR	
	1	**2**
failure	1 (10.0)	0 (0.0)
success	9 (90.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

Variability between the 2 participating laboratories could only be observed for reference blood sample PT2014SCRGENB5 since it was misclassified by LAB1 and correctly identified by LAB2. For each participating laboratory, the obtained results and the assigned statuses for the reference blood samples are shown in Table 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference blood samples (SAMPLE), the position of the reference blood samples as placed in the block (LABPOSIT), and the status assigned by CODA-CERVA (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014SCRGENB5	**ALRR/AFRQ**	**AFRR/ALRQ**	**0**
2	1	2	PT2014SCRGENB6	ALHQ/VLRQ	ALHQ/VLRQ	1
3	1	3	PT2014SCRGENB7	ALRR/ALRH	ALRR/ALRH	1
4	1	4	PT2014SCRGENB8	ALRR/VLRQ	ALRR/VLRQ	1
5	1	5	PT2014SCRGENB9	ALRR/ALHQ	ALRR/ALHQ	1
6	1	6	PT2014SCRGENB10	ALRR/ALRR	ALRR/ALRR	1
7	1	7	PT2014SCRGENB1	ALRH/VLRQ	ALRH/VLRQ	1
8	1	8	PT2014SCRGENB2	ALRR/ALRQ	ALRR/ALRQ	1
9	1	9	PT2014SCRGENB3	ALRQ/ALRQ	ALRQ/ALRQ	1
10	1	10	PT2014SCRGENB4	ALRQ/ALRH	ALRQ/ALRH	1
11	2	1	PT2014SCRGENB10	ALRR/ALRR	ALRR/ALRR	1
12	2	2	PT2014SCRGENB1	ALRH/VLRQ	ALRH/VLRQ	1
13	2	3	PT2014SCRGENB2	ALRR/ALRQ	ALRR/ALRQ	1
14	2	4	PT2014SCRGENB3	ALRQ/ALRQ	ALRQ/ALRQ	1
15	2	5	PT2014SCRGENB4	ALRQ/ALRH	ALRQ/ALRH	1
16	2	6	PT2014SCRGENB5	ALRR/AFRQ	ALRR/AFRQ	1
17	2	7	PT2014SCRGENB6	ALHQ/VLRQ	ALHQ/VLRQ	1
18	2	8	PT2014SCRGENB7	ALRR/ALRH	ALRR/ALRH	1
19	2	9	PT2014SCRGENB8	ALRR/VLRQ	ALRR/VLRQ	1
20	2	10	PT2014SCRGENB9	ALRR/ALHQ	ALRR/ALHQ	1

V. Discussion

The purpose of this PT was to assess the performance of the participating laboratories when analyzing reference blood samples of sheep origin by RT-PCR and/or sequencing in order to identify genotypes related to genetically linked susceptibility to scrapie.

One out of 2 participating laboratories, namely LAB2, reported genotypes that were in full agreement with the assigned status of the reference blood samples (100% of agreement). In contrast, LAB1 misclassified the reference blood sample PT2014SCRGENB5 (90% of agreement) since the genotype AFRR/ALRQ instead of ALRR/AFRQ was reported. The presence of phenylalanine (F) on codon 141 is only described for ARQ and not for ARR PRNP genotypes (codon 136, 154, 171). All participating laboratories performed RT-PCR in order to identify the genotypes.

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 blood samples assigned by CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the identification of genotypes related to genetically linked susceptibility to scrapie in reference blood samples.

Head CVD-ERA

Yves Van der Stede

Appendix

Name of the participating laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Mons, Belgium)

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