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172-PT

PROFICIENCY TESTING 2013

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: 08 APRIL 2013

DATE REPORT: 04 JUNE 2013

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 'PT2013SCRGENB1', 'PT2013SCRGENB2', 'PT2013SCRGENB3', 'PT2013SCRGENB4', 'PT2013SCRGENB5', 'PT2013SCRGENB6', 'PT2013SCRGENB7', 'PT2013SCRGENB8', 'PT2013SCRGENB9' and 'PT2013SCRGENB10', were used. In total, 30 aliquots were distributed to 3 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 during pre-verification tests and based on the results obtained by RT-PCR, Denaturing Gradient Gel Electrophoresis - Restriction Fragment Length Polymorphism (DGGE-RFLP) and sequencing, hereby obtaining each time the same result. 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, DGGE-RFLP and sequencing.

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 3 participating laboratories by national courier on 8th of April 2013 (30 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 9th and 12th of April 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 on 18th and 19th of April 2013 (Table 1). All participants hereby respected the deadline of 19th of April 2013 for submission of the results.

Because LAB1 reported the correct genotypes in an unexpected order, this participating laboratory was asked to retest the received reference blood samples. Retest confirmed their previously submitted results, suggesting an error during randomization of the samples by the operational unit CVD-ERA. Therefore, a new set of the same reference blood samples was provided to LAB1 on 6th of May 2013. Analysis of these samples and submission of the results occurred on 7th and 17th of May 2013, respectively (Table 1). LAB1 hereby respected the deadline of 17th of May 2013 for submission of the results for this new set of reference blood samples.

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 ▲	08/04/2013 (06/05/2013)	09/04/2013 (07/05/2013)	19/04/2013 (17/05/2013)
LAB2	08/04/2013	10/04/2013	18/04/2013
LAB3	08/04/2013	12/04/2013	18/04/2013

Legend: ▲ Due to suspicion of an error during randomization of the reference blood samples, a new set of samples was provided to LAB1. The dates corresponding to these newly provided reference blood samples are shown between brackets.

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 3 participating laboratories reported genotypes that were in full agreement with the assigned status of the reference blood samples and hence reached 100% 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**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

No variability could be observed between the 3 participating laboratories since they identified all reference blood samples correctly.

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	PT2013SCRGENB5	ALRR/ALRR	ALRR/ALRR	1
2	1	2	PT2013SCRGENB6	VLRQ/VLRQ	VLRQ/VLRQ	1
3	1	3	PT2013SCRGENB7	ALRQ/ALRQ	ALRQ/ALRQ	1
4	1	4	PT2013SCRGENB8	ALRR/ALRH	ALRR/ALRH	1
5	1	5	PT2013SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
6	1	6	PT2013SCRGENB10	ALRR/ALHQ	ALRR/ALHQ	1
7	1	7	PT2013SCRGENB1	ALRR/VLRQ	ALRR/VLRQ	1
8	1	8	PT2013SCRGENB2	ALRR/ALRH	ALRR/ALRH	1
9	1	9	PT2013SCRGENB3	ALRR/ALRQ	ALRR/ALRQ	1
10	1	10	PT2013SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
11	2	1	PT2013SCRGENB3	ALRR/ALRQ	ALRR/ALRQ	1
12	2	2	PT2013SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
13	2	3	PT2013SCRGENB5	ALRR/ALRR	ALRR/ALRR	1
14	2	4	PT2013SCRGENB6	VLRQ/VLRQ	VLRQ/VLRQ	1
15	2	5	PT2013SCRGENB7	ALRQ/ALRQ	ALRQ/ALRQ	1
16	2	6	PT2013SCRGENB8	ALRR/ALRH	ALRR/ALRH	1
17	2	7	PT2013SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
18	2	8	PT2013SCRGENB10	ALRR/ALHQ	ALRR/ALHQ	1
19	2	9	PT2013SCRGENB1	ALRR/VLRQ	ALRR/VLRQ	1
20	2	10	PT2013SCRGENB2	ALRR/ALRH	ALRR/ALRH	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2013SCRGENB1	ALRR/VLRQ	ALRR/VLRQ	1
22	3	2	PT2013SCRGENB2	ALRR/ALRH	ALRR/ALRH	1
23	3	3	PT2013SCRGENB3	ALRR/ALRQ	ALRR/ALRQ	1
24	3	4	PT2013SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
25	3	5	PT2013SCRGENB5	ALRR/ALRR	ALRR/ALRR	1
26	3	6	PT2013SCRGENB6	VLRQ/VLRQ	VLRQ/VLRQ	1
27	3	7	PT2013SCRGENB7	ALRQ/ALRQ	ALRQ/ALRQ	1
28	3	8	PT2013SCRGENB8	ALRR/ALRH	ALRR/ALRH	1
29	3	9	PT2013SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
30	3	10	PT2013SCRGENB10	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.

All 3 participating laboratories reported genotypes that were in full agreement with the assigned status of the reference blood samples (100% of agreement) (Table 2 and Table 3). All participating laboratories performed RT-PCR. In addition, LAB1 also performed sequencing as a confirmation method.

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)

Progenus s.a. (Gembloux, Belgium)

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