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172-PT

PROFICIENCY TESTING 2012

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: 02 APRIL 2012

DATE REPORT: 07 JUNE 2012

I. Introduction

Details relevant to the proficiency test 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'.

II. Aim

This proficiency test focused on genotype identification for the detection of genetically linked susceptibility to scrapie (SCR) in blood of sheep origin and aimed to assess the analytical accuracy of the tests conducted by the participants.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this proficiency test, predefined reference blood samples must be tested by means of RT-PCR. The procedures for the RT-PCR tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Ten reference blood samples of sheep origin, coded 'PT2012SCRGENB1', 'PT2012SCRGENB2', 'PT2012SCRGENB3', 'PT2012SCRGENB4', 'PT2012SCRGENB5', 'PT2012SCRGENB6', 'PT2012SCRGENB7', 'PT2012SCRGENB8', 'PT2012SCRGENB9' and 'PT2012SCRGENB10', were used. In total, 40 aliquots, prepared by the reference laboratory for SCR of the Veterinary and Agrochemical Research Center (CODA-CERVA), were distributed to the participating laboratories. Each participant was given one aliquot of each reference blood sample. The positions of the reference blood samples in the sent blocks were randomized for each participant (Table 2).

For each reference blood sample, a certificate containing the assigned genotype was made by the reference laboratory for SCR of CODA-CERVA (status of the sample = 'golden standard'). The assigned genotypes were obtained by testing each reference blood sample once before the proficiency test (pre-verification) by two different accredited assays (RT-PCR, DGGE-RFLP), hereby obtaining each time the same result. Consequently, these reference blood samples were considered as reliable samples to use for the purpose of this proficiency test. In addition, the reference blood samples were also tested once after the proficiency test in order to confirm their stability and status (post-verification).

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* (if the genotype was correctly identified) or *failure* (if the genotype was 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 all ten reference blood samples used in this proficiency test.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if at least 90% of the reference blood samples are analysed correctly, i.e. when the reported result corresponds with the status assigned by the reference laboratory for SCR of CODA-CERVA.

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 the CODA-CERVA.

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

The ten reference blood samples were sent on 2nd of April 2012 to each of the four participating laboratories (40 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 3rd (LAB 3) and 4th (LAB 1, LAB 2) of April. LAB 4 did not provide a starting date at which analysis was carried out.

IV.2. Dates at which results were returned to the CVD-ERA

Results from the participating laboratories have been received on 6th (LAB 2), 8th (LAB 4) and 10th (LAB 1 and LAB 3) of April 2012.

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

LAB 1, LAB 2 and LAB 3 reached 100% of agreement for genotype identification for the detection of genetically linked susceptibility to scrapie in reference blood samples. LAB 4 identified 90% of the genotypes correctly (Table 1).

Table 1. Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference blood samples assigned by the reference laboratory for SCR of CODA-CERVA. All participating laboratories received 10 reference blood samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	**2**	**3**	**4**
failure	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	9 (90.0)

IV.4.2. Variability among participating laboratories

No variability between LAB 1, LAB 2 and LAB 3 could be observed since these participants identified all reference blood samples correctly. LAB 4 misidentified sample PT2012SCRGENB8 (ALR**Q**/VLR**R** instead of ALR**R**/VLR**Q**). For each participating laboratory, the obtained responses for the reference blood samples are shown in Table 2.

Table 2. 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 the reference laboratory for SCR of the CODA-CERVA (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012SCRGENB1	ALRQ/ALRH	ALRQ/ALRH	1
2	1	2	PT2012SCRGENB2	ALRR/ALRR	ALRR/ALRR	1
3	1	3	PT2012SCRGENB3	ALRR/ALHQ	ALRR/ALHQ	1
4	1	4	PT2012SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
5	1	5	PT2012SCRGENB5	ALRR/ALRH	ALRR/ALRH	1
6	1	6	PT2012SCRGENB6	ALRR/ALRQ	ALRR/ALRQ	1
7	1	7	PT2012SCRGENB7	ALRR/ALRR	ALRR/ALRR	1
8	1	8	PT2012SCRGENB8	ALRR/VLRQ	ALRR/VLRQ	1
9	1	9	PT2012SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
10	1	10	PT2012SCRGENB10	VLRQ/VLRQ	VLRQ/VLRQ	1
11	2	1	PT2012SCRGENB3	ALRR/ALHQ	ALRR/ALHQ	1
12	2	2	PT2012SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
13	2	3	PT2012SCRGENB5	ALRR/ALRH	ALRR/ALRH	1
14	2	4	PT2012SCRGENB6	ALRR/ALRQ	ALRR/ALRQ	1
15	2	5	PT2012SCRGENB7	ALRR/ALRR	ALRR/ALRR	1
16	2	6	PT2012SCRGENB8	ALRR/VLRQ	ALRR/VLRQ	1
17	2	7	PT2012SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
18	2	8	PT2012SCRGENB10	VLRQ/VLRQ	VLRQ/VLRQ	1
19	2	9	PT2012SCRGENB1	ALRQ/ALRH	ALRQ/ALRH	1
20	2	10	PT2012SCRGENB2	ALRR/ALRR	ALRR/ALRR	1
21	3	1	PT2012SCRGENB5	ALRR/ALRH	ALRR/ALRH	1
22	3	2	PT2012SCRGENB6	ALRR/ALRQ	ALRR/ALRQ	1
23	3	3	PT2012SCRGENB7	ALRR/ALRR	ALRR/ALRR	1
24	3	4	PT2012SCRGENB8	ALRR/VLRQ	ALRR/VLRQ	1
25	3	5	PT2012SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
26	3	6	PT2012SCRGENB10	VLRQ/VLRQ	VLRQ/VLRQ	1
27	3	7	PT2012SCRGENB1	ALRQ/ALRH	ALRH/ALRQ	1
28	3	8	PT2012SCRGENB2	ALRR/ALRR	ALRR/ALRR	1
29	3	9	PT2012SCRGENB3	ALRR/ALHQ	ALRR/ALHQ	1
30	3	10	PT2012SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1

(Table 2 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	4	1	PT2012SCRGENB1	ALRQ/ALRH	ALRQ/ALRH	1
32	4	2	PT2012SCRGENB2	ALRR/ALRR	ALRR/ALRR	1
33	4	3	PT2012SCRGENB3	ALRR/ALHQ	ALRR/ALHQ	1
34	4	4	PT2012SCRGENB4	ALRH/VLRQ	ALRH/VLRQ	1
35	4	5	PT2012SCRGENB5	ALRR/ALRH	ALRR/ALRH	1
36	4	6	PT2012SCRGENB6	ALRR/ALRQ	ALRR/ALRQ	1
37	4	7	PT2012SCRGENB7	ALRR/ALRR	ALRR/ALRR	1
38	4	8	PT2012SCRGENB8	**ALRR/VLRQ**	**ALRQ/VLRR**	**0**
39	4	9	PT2012SCRGENB9	ALRQ/VLRQ	ALRQ/VLRQ	1
40	4	10	PT2012SCRGENB10	VLRQ/VLRQ	VLRQ/VLRQ	1

V. Discussion

The purpose of this proficiency test was to assess the performance of the participating laboratories when analyzing reference blood samples from sheep by RT-PCR in order to identify genotypes genetically linked to susceptibility to scrapie.

Data obtained in this proficiency test showed that three out of four participating laboratories provided responses that were in full agreement with the true status of the reference blood samples. One participating laboratory (LAB 4) reached 90% of agreement with the true status of the reference blood samples. This participant reported for sample PT2012SCRGENB8 a different genotype than the assigned genotype.

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 the reference laboratory for SCR of the CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance.

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), NRL for SCR (Ukkel, Belgium)

Progenus (Belgium)

Quality Partner SA (Belgium)