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172-PT

PROFICIENCY TESTING 2015

Bovine Viral Diarrhea Virus (BVDV)

Detection of BVDV-specific antigens in bovine Ear notch samples by Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR) and/or Enzyme Linked Immunosorbent Assay (ELISA)

CODA-CERVA-UCCLE

DATE BEGIN PT: 26 OCTOBER 2015

DATE REPORT: 15 JANUARY 2016

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 the absence or presence of BVDV-specific antigens in bovine ear-notch samples by RT-qPCR and/or antigen ELISA.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference ear-notch samples must be tested by means of a BVDV RT-qPCR and/or a BVDV antigen ELISA. The procedures for these tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Replicates of 5 reference ear-notch samples of bovine origin, either free from detectable BVDV-specific antigens (n=2; coded 'PT2015BVDVIRNE1' and 'PT2015BVDVIRNE2'') or containing detectable BVDV-specific antigens (n=3; coded 'PT2015BVDVIRPE1', 'PT2015BVDVIRPE2' and PT2015BVDVIRPE3), were used.
In total, 140 aliquots of reference ear-notch samples were distributed to 10 participating laboratories. LAB1, LAB2, LAB3 and LAB4 received 20 aliquots of ear-notch samples to performe BVDV RT-qPCR and BVDV antigen ELISA. LAB5, LAB6, LAB7, LAB8, LAB9 and LAB10 received 10 aliquots of ear-notch samples to performe BVDV RT-qPCR and/or BVDV antigen ELISA (Table 4 and Table 5).

For each reference ear-notch sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference ear-notch samples was based on (i) the historical background of the animals and (ii) the results obtained by an in-house developed BVDV RT-qPCR assays and the BVDV antigen test kit/serum plus ELISA from IDEXX.

The reference ear-notch samples PT2015BVDVIRNE1 and PT2015BVDVIRNE2 were obtained from 2 different BVDV-free animals. The reference ear-notch samples PT2015BVDVIRPE1, PT2015BVDVIRPE2 and PT2015BVDVIRPE3 were field samples obtained from animals that were classified as immunotolerant persistently BVDV-infected (IPI) animals, all carrying a BVDV type 1 strain.

For each reference ear-notch sample, the same qualitative result was obtained with an in-house developed BVDV RT-qPCR assays and the BVDV antigen ELISA kit from IDEXX.

After aliquoting the different ear-notch samples, a homogeneity check was performed on 3 aliquots of each reference ear notch sample using an in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX, hereby obtaining the same qualitative result for all aliquots of the same reference ear notch sample. Consequently, all reference ear-notch samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of BVDV-specific antigens in bovine ear notch samples. In addition, all reference ear notch samples were tested once after the PT in order to confirm their stability and status (post-verification) using the in-house developed BVDV RT-qPCR and the BVDV antigen ELISA kit from IDEXX.

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 (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or failure when the reported result does not match with the assigned status (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive, non-interpretable result when the reference sample is truly negative or positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* for each of the 10 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 10 aliquots of 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 CODA-CERVA-Uccle.

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

LAB1 and LAB3 participated in the PT for both RT-qPCR and Antigen ELISA. LAB2 and LAB4 had been registered for both RT-qPCR and Antigen ELISA, but gave only results respectively for Antigen ELISA and RT-qPCR. LAB5 and LAB6 participated in the PT for Antigen ELISA. LAB7 had been registered only for RT-qPCR but gave results for both RT-qPCR and Antigen ELISA. LAB8, LAB9 and LAB10 participated in the PT for RT-qPCR.
The reference ear-notch samples were sent frozen (dry ice) to each of the participating laboratories by national or international courier on 26th of October 2015. LAB1, LAB2, LAB3, LAB4, LAB6, LAB9 and LAB10 acknowledged receipt of the samples on the same day, whereas the other laboratories received the samples on 27th of October 2015. LAB5 announced at the reception of the samples that the labels of identification had fallen for several samples. A new set of reference ear-notch samples were sent frozen (dry ice) to them on 4th of November 2015. LAB5 received the samples on 5th of November 2015.
Analyses were performed between 28th of October and 17th of November 2015 (Table 1).

IV.2. Dates at which results were returned to the CODA-CERVA-Uccle

Results were submitted to the CODA-CERVA-Uccle between 30th of October and 18th of November 2015 (Table 1). All participants hereby respected the deadline of 20th of November 2015 for submission of the results.

Table 1. Overview of the laboratories that participated with relation to starting date and submission of results towards the CODA-CERVA-Uccle for the different assays

Participating laboratory	Reference samples received	Start of analysis ELISA	Start of analysis RT-qPCR	Submission of the results (Excel file)
LAB1	26/10/2015	28/10/2015	27/10/2015	13/11/2015
LAB2	26/10/2015	02/11/2015	NR	18/11/2015
LAB3	26/10/2015	12/11/2015	26-27/10/2015	16/11/2015
LAB4	26/10/2015	NR	5/11/2015	06/11/2015
LAB5	27/10/2015 First sending 5/11/2015 Second sending	13/11/2015	NA	17/11/2015
LAB6	26/10/2015	29/10/2015	NA	30/10/2015
LAB7	27/10/2015	02/11/2015	28/10/2015	02/11/2015
LAB8	27/10/2015	NA	17/11/2015	19/11/2015
LAB9	26/10/2015	NA	27/10/2015	04/11/2015
LAB10	26/10/2015	NA	30/10/2015	17/11/2015

Legend: NA = not applicable; NR = no results

IV.3. Compliance with the procedure

LAB1, LAB2, LAB3, LAB4, LAB6, LAB7, LAB8, LAB9 and LAB10 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:

(i) For the detection of BVDV-specific antigens by **RT-qPCR** (Table 2): all participating laboratories (LAB1, LAB3, LAB4, LAB7, LAB8, LAB9 and LAB10) provided qualitative results that were in full agreement with the true status of the reference ear-notch samples (100% of agreement).

(ii) For the detection of BVDV-specific antigens by **antigen ELISA** (Table 3): all participating laboratories (LAB1, LAB2, LAB3, LAB5, LAB6 and LAB7) provided qualitative results that were in full agreement with the true status of the reference ear-notch samples (100% of agreement).

Table 2. **RT-qPCR Ear-Notch:** Agreement between results generated by the participating laboratories (LABNR) and the status of the reference ear notch samples assigned by the BVDV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 10 aliquots of reference ear-notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR						
	1	**3**	**4**	**7**	**8**	**9**	**10**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

Table 3. **ELISA Ear-Notch**: Agreement between results generated by the participating laboratories (LABNR) and the status of the reference ear notch samples assigned by the BVDV reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 10 aliquots of reference ear-notch samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR					
	1	**2**	**3**	**5**	**6**	**7**
failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
success	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

(i) For the detection of BVDV-specific antigens by **RT-qPCR** no variability between the participating laboratories could be observed since all participants correctly identified all reference ear-notch samples.

(ii) For the detection of BVDV-specific antigens by **antigen ELISA** no variability between the participating laboratories could be observed since all participants correctly identified all reference ear-notch samples.

For each participating laboratory, the obtained results and the assigned statuses for the reference ear-notch samples are shown in Table 4 for RT-qPCR and in Table 5 for antigen ELISA.

Table 4. RT-qPCR : The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference ear-notch samples (SAMPLE), the positions (numbers) of the reference ear notch samples as provided for the laboratories (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BVDVIRPE1	POS	POS	1
2	1	2	PT2015BVDVIRNE2	NEG	NEG	1
3	1	3	PT2015BVDVIRPE1	POS	POS	1
4	1	4	PT2015BVDVIRPE2	POS	POS	1
5	1	5	PT2015BVDVIRNE1	NEG	NEG	1
6	1	6	PT2015BVDVIRPE3	POS	POS	1
7	1	7	PT2015BVDVIRPE2	POS	POS	1
8	1	8	PT2015BVDVIRPE3	POS	POS	1
9	1	9	PT2015BVDVIRNE2	NEG	NEG	1
10	1	10	PT2015BVDVIRNE1	NEG	NEG	1
11	3	1	PT2015BVDVIRPE1	POS	POS	1
12	3	2	PT2015BVDVIRNE2	NEG	NEG	1
13	3	3	PT2015BVDVIRPE1	POS	POS	1
14	3	4	PT2015BVDVIRPE2	POS	POS	1
15	3	5	PT2015BVDVIRNE1	NEG	NEG	1
16	3	6	PT2015BVDVIRPE3	POS	POS	1
17	3	7	PT2015BVDVIRPE2	POS	POS	1
18	3	8	PT2015BVDVIRPE3	POS	POS	1
19	3	9	PT2015BVDVIRNE2	NEG	NEG	1
20	3	10	PT2015BVDVIRNE1	NEG	NEG	1

Table 4 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	4	1	PT2015BVDVIRPE2	POS	POS	1
22	4	2	PT2015BVDVIRNE1	NEG	NEG	1
23	4	3	PT2015BVDVIRPE3	POS	POS	1
24	4	4	PT2015BVDVIRNE2	NEG	NEG	1
25	4	5	PT2015BVDVIRPE1	POS	POS	1
26	4	6	PT2015BVDVIRNE2	NEG	NEG	1
27	4	7	PT2015BVDVIRPE3	POS	POS	1
28	4	8	PT2015BVDVIRNE1	NEG	NEG	1
29	4	9	PT2015BVDVIRPE2	POS	POS	1
30	4	10	PT2015BVDVIRPE1	POS	POS	1
31	7	1	PT2015BVDVIRPE1	POS	POS	1
32	7	2	PT2015BVDVIRNE2	NEG	NEG	1
33	7	3	PT2015BVDVIRPE1	POS	POS	1
34	7	4	PT2015BVDVIRPE2	POS	POS	1
35	7	5	PT2015BVDVIRNE1	NEG	NEG	1
36	7	6	PT2015BVDVIRPE3	POS	POS	1
37	7	7	PT2015BVDVIRPE2	POS	POS	1
38	7	8	PT2015BVDVIRPE3	POS	POS	1
39	7	9	PT2015BVDVIRNE2	NEG	NEG	1
40	7	10	PT2015BVDVIRNE1	NEG	NEG	1
41	8	1	PT2015BVDVIRPE2	POS	POS	1
42	8	2	PT2015BVDVIRNE1	NEG	NEG	1
43	8	3	PT2015BVDVIRPE3	POS	POS	1
44	8	4	PT2015BVDVIRNE2	NEG	NEG	1
45	8	5	PT2015BVDVIRPE1	POS	POS	1
46	8	6	PT2015BVDVIRNE2	NEG	NEG	1
47	8	7	PT2015BVDVIRPE3	POS	POS	1
48	8	8	PT2015BVDVIRNE1	NEG	NEG	1
49	8	9	PT2015BVDVIRPE2	POS	POS	1
50	8	10	PT2015BVDVIRPE1	POS	POS	1
51	9	1	PT2015BVDVIRPE1	POS	POS	1
52	9	2	PT2015BVDVIRNE2	NEG	NEG	1
53	9	3	PT2015BVDVIRPE1	POS	POS	1
54	9	4	PT2015BVDVIRPE2	POS	POS	1
55	9	5	PT2015BVDVIRNE1	NEG	NEG	1
56	9	6	PT2015BVDVIRPE3	POS	POS	1
57	9	7	PT2015BVDVIRPE2	POS	POS	1
58	9	8	PT2015BVDVIRPE3	POS	POS	1
59	9	9	PT2015BVDVIRNE2	NEG	NEG	1
60	9	10	PT2015BVDVIRNE1	NEG	NEG	1

Table 4 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	10	1	PT2015BVDVIRPE2	POS	POS	1
62	10	2	PT2015BVDVIRNE1	NEG	NEG	1
63	10	3	PT2015BVDVIRPE3	POS	POS	1
64	10	4	PT2015BVDVIRNE2	NEG	NEG	1
65	10	5	PT2015BVDVIRPE1	POS	POS	1
66	10	6	PT2015BVDVIRNE2	NEG	NEG	1
67	10	7	PT2015BVDVIRPE3	POS	POS	1
68	10	8	PT2015BVDVIRNE1	NEG	NEG	1
69	10	9	PT2015BVDVIRPE2	POS	POS	1
70	10	10	PT2015BVDVIRPE1	POS	POS	1

Table 5. Antigen ELISA: The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference ear-notch samples (SAMPLE), the positions of the reference ear-notch samples as provided for the laboratories (LABPOSIT), and the status assigned by the BVDV reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015BVDVIRNE1	NEG	NEG	1
2	1	2	PT2015BVDVIRPE1	POS	POS	1
3	1	3	PT2015BVDVIRPE3	POS	POS	1
4	1	4	PT2015BVDVIRPE2	POS	POS	1
5	1	5	PT2015BVDVIRNE1	NEG	NEG	1
6	1	6	PT2015BVDVIRPE3	POS	POS	1
7	1	7	PT2015BVDVIRNE2	NEG	NEG	1
8	1	8	PT2015BVDVIRPE1	POS	POS	1
9	1	9	PT2015BVDVIRPE2	POS	POS	1
10	1	10	PT2015BVDVIRNE2	NEG	NEG	1
11	2	1	PT2015BVDVIRNE2	NEG	NEG	1
12	2	2	PT2015BVDVIRPE2	POS	POS	1
13	2	3	PT2015BVDVIRNE1	NEG	NEG	1
14	2	4	PT2015BVDVIRPE1	POS	POS	1
15	2	5	PT2015BVDVIRNE2	NEG	NEG	1
16	2	6	PT2015BVDVIRPE3	POS	POS	1
17	2	7	PT2015BVDVIRPE2	POS	POS	1
18	2	8	PT2015BVDVIRPE3	POS	POS	1
19	2	9	PT2015BVDVIRNE1	NEG	NEG	1
20	2	10	PT2015BVDVIRPE1	POS	POS	1

Table 12 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2015BVDVIRNE1	NEG	NEG	1
22	3	2	PT2015BVDVIRPE1	POS	POS	1
23	3	3	PT2015BVDVIRPE3	POS	POS	1
24	3	4	PT2015BVDVIRPE2	POS	POS	1
25	3	5	PT2015BVDVIRNE1	NEG	NEG	1
26	3	6	PT2015BVDVIRPE3	POS	POS	1
27	3	7	PT2015BVDVIRNE2	NEG	NEG	1
28	3	8	PT2015BVDVIRPE1	POS	POS	1
29	3	9	PT2015BVDVIRPE2	POS	POS	1
30	3	10	PT2015BVDVIRNE2	NEG	NEG	1
31	5	1	PT2015BVDVIRNE1	NEG	NEG	1
32	5	2	PT2015BVDVIRPE1	POS	POS	1
33	5	3	PT2015BVDVIRPE3	POS	POS	1
34	5	4	PT2015BVDVIRPE2	POS	POS	1
35	5	5	PT2015BVDVIRNE1	NEG	NEG	1
36	5	6	PT2015BVDVIRPE3	POS	POS	1
37	5	7	PT2015BVDVIRNE2	NEG	NEG	1
38	5	8	PT2015BVDVIRPE1	POS	POS	1
39	5	9	PT2015BVDVIRPE2	POS	POS	1
40	5	10	PT2015BVDVIRNE2	NEG	NEG	1
41	6	1	PT2015BVDVIRNE2	NEG	NEG	1
42	6	2	PT2015BVDVIRPE2	POS	POS	1
43	6	3	PT2015BVDVIRNE1	NEG	NEG	1
44	6	4	PT2015BVDVIRPE1	POS	POS	1
45	6	5	PT2015BVDVIRNE2	NEG	NEG	1
46	6	6	PT2015BVDVIRPE3	POS	POS	1
47	6	7	PT2015BVDVIRPE2	POS	POS	1
48	6	8	PT2015BVDVIRPE3	POS	POS	1
49	6	9	PT2015BVDVIRNE1	NEG	NEG	1
50	6	10	PT2015BVDVIRPE1	POS	POS	1
51	7	1	PT2015BVDVIRPE1	POS	POS	1
52	7	2	PT2015BVDVIRNE2	NEG	NEG	1
53	7	3	PT2015BVDVIRPE1	POS	POS	1
54	7	4	PT2015BVDVIRPE2	POS	POS	1
55	7	5	PT2015BVDVIRNE1	NEG	NEG	1
56	7	6	PT2015BVDVIRPE3	POS	POS	1
57	7	7	PT2015BVDVIRPE2	POS	POS	1
58	7	8	PT2015BVDVIRPE3	POS	POS	1
59	7	9	PT2015BVDVIRNE2	NEG	NEG	1
60	7	10	PT2015BVDVIRNE1	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing individual reference ear notch samples of bovine origin for the detection of BVDV-specific antigens by RT-qPCR and/or antigen ELISA.

For the detection of BVDV-specific antigens by RT-qPCR, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement).
One participating laboratory (LAB3) used an in-house developed BVDV RT-qPCR, whereas the other participants used commercially available BVDV RT-qPCR kits from 2 different kit producers. LAB1, LAB7, LAB8, LAB9 and LAB10 used the LSI VetMAX BVD4ALL kit from Life Technologies (LAB1: batch BVD4ALL-006; all other laboratories: batch BVD4ALL-007). LAB4 used the virotype® BVDV RT-PCR kit from Qiagen (batch 251120615).

For the detection of BVDV-specific antigens by antigen ELISA, all participating laboratories provided qualitative results that were in full agreement with the true status of the reference samples (100% agreement).
All laboratories used different batches of the same BVDV antigen test kit/serum plus ELISA kit from IDEXX: batch E811 (LAB1), E951 (LAB2), D891 (LAB3), E931 (LAB5), E411 (LAB6), F171 (LAB7).

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 samples assigned by the CODA-CERVA-Uccle (see III.3.3.). Consequently, all participating laboratories achieved a satisfactory performance for the detection of BVDV-specific antigens in reference ear-notch samples by RT-qPCR and by antigen ELISA.

Coordinator proficiency tests

Katia Knapen

Appendix

Name of the participating laboratories

Animal and Plant Health Agency (APHA) (Surrey, United Kingdom)
Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)
Association Régionale de Santé et d'Identification Animales (ARSIA) (Mons, Belgium)
Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)
DNAlysis Maastricht B.V. (Maastricht, The Nederlands)
Irish Equine Centre : Virology Department (Kildare – Ireland)
Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)
Lavetan NV (Turnhout, Belgium)
Thermofisher Scientific -LSI (LSI) (Lissieu, France)
Veterinary and Agrochemical Research Center (CODA-CERVA), (Ukkel, Belgium)