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172-PT

PROFICIENCY TESTING 2015

SALMONELLA (SAL)

Isolation of Salmonella spp. from faeces

CODA-CERVA-UCCLE

DATE BEGIN PT: 23 NOVEMBER 2015

DATE REPORT: 26 FEBRUARY 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 *Salmonella* spp. in faeces.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined faecal samples must be analyzed by means of *Salmonella* isolation tests as described in ISO 6579, annex D. The procedures for the isolation tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Faeces collected from cattle were homogenized, aliquoted per 10g and stored in the freezer. Approximately 10% of the yet unfrozen aliquots were analyzed on different days for the presence of *Salmonella* spp. by the *Salmonella* reference laboratory of CODA-CERVA-Uccle, hereby following method ISO 6579 annex D. Since all tested aliquots were found negative for *Salmonella* spp., the collected faeces were considered as *Salmonella* negative and hence the remaining aliquots as suitable for the PT.

On 23th of November 2015 (start date of the PT), 100 aliquots of faecal samples were prepared and randomized, either for the PT (70 aliquots) or the verification tests that had to be performed by the *Salmonella* reference laboratory of CODA-CERVA-Uccle in parallel with the PT (30 aliquots):

- 38 aliquots (28 for the PT and 10 for the verification tests) were used as such and considered as negative faecal samples ('PT2015SALBACNO1')
- 31 aliquots (21 for the PT and 10 for the verification tests) were inoculated with 450 cfu *Salmonella* Typhimurium (antigenic formula O4,5,12:i:1,2) and were considered as strong positive faecal samples ('PT2015SALBACPO1').
- 31 aliquots (21 for the PT and 10 for the verification tests) were inoculated with 45 cfu *Salmonella* Typhimurium (antigenic formula O4,5,12:i:1,2) and were considered as weak positive faecal samples ('PT2015SALBACPO2').

In total, 70 aliquots of faecal samples were distributed to 7 participating laboratories. All participants were given 10 aliquots of faecal samples: 4 aliquots of the negative faecal sample PT2015SALBACNO1, 3 aliquots of the strong positive faecal sample PT2015SALBACPO1 and 3 aliquots of the weak positive faecal sample PT2015SALBACPO2.

For most PTs organized by the CODA-CERVA-Uccle, the PT samples can be made in bulk and subsequently aliquoted. In order to confirm the status of these PT samples and to check the homogeneity of the aliquoted samples, 10 aliquots of each PT sample are analyzed before the start of the PT. In contrast, for this PT bacteriology, the verification tests could only be performed on samples similar as those sent to the participants and in parallel with the PT (cfr. Manual for the participant, section III.1). Therefore, the *Salmonella* reference laboratory of CODA-CERVA-Uccle tested 5 aliquots of each category of faecal samples for the presence of *Salmonella* on both 23th (day 1) and 24th (day 2) of November 2015, in order to verify the status of the sent faecal samples.

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 the participating laboratories is expressed as the percentage of *success* for the 10 aliquots of faecal 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 faecal samples is : for the strong positive faecal samples : no mistakes allowed (100% of agreement) and for both the weak positive and the negative faecal samples : 1 mistake allowed.

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

The 10 aliquots of faecal samples were sent at 5±3°C to each of the 7 participating laboratories by national courier on 23th of November 2015 (70 aliquots in total). Analyses were started on 23th and 24th of November 2015 (Table 1).

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

Results from the participating laboratories have been received between 26th of November and 2^{d} of December 2015. (Table 1).

Table 1. Overview of the dates on which (i) the faecal samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)	Dated & signed hard copy of the results received
LAB1	23/11/2015	23/11/2015	02/12/2015	02/12/2015
LAB2	23/11/2015	23/11/2015	26/11/2015	26/11/2015
LAB3	23/11/2015	23/11/2015	01/12/2015	08/12/2015
LAB4	23/11/2015	23/11/2015	02/12/2015	08/12/2015
LAB5	23/11/2015	23/11/2015	01/12/2015	01/12/2015
LAB6	23/11/2015	23/11/2015	02/12/2015	02/12/2015
LAB7	23/11/2015	24/11/2015	02/12/2015	02/12/2015

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

Six out of seven participating laboratories (LAB1, LAB2, LAB3, LAB4, LAB6 and LAB7) provided qualitative results that were in full agreement with the assigned status of the faecal samples and hence achieved 100% of agreement. In contrast, LAB5 misclassified 4 faecal samples (60% of agreement) (Table 2).

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

Success while screening the samples (0 = Failure, 1 = Success)							
	Laboratories						
	1 (N=10)	**2 (N=10)**	**3 (N=10)**	**4 (N=10)**	**5 (N=10)**	**6 (N=10)**	**7 (N=10)**
0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)
1	10 (100%)	10 (100%)	10 (100%)	10 (100%)	6 (60%)	10 (100%)	10 (100%)

IV.4.2. Variability among participating laboratories

No variability between LAB1, LAB2, LAB3, LAB4, LAB6 and LAB7could be observed since these participants correctly identified all faecal samples. In contrast, LAB5 misclassified 2 out of 4 aliquots of the reference feacal sample PT2015SALBACNO1 (POS instead of NEG) and 2 out of 3 aliquots of the reference faecal sample PT2015SALBACPO2 (NEG instead of POS).

For each participating laboratory, the obtained results and the assigned statuses for the faecal samples are shown in Table 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the faecal samples (SAMPLE), the external identification of the faecal samples (LABPOSIT), and the status assigned by the *Salmonella* reference laboratory of CODA-CERVA-Uccle (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2015SALBACPO1	POS	POS	1
2	1	2	PT2015SALBACPO2	POS	POS	1
3	1	3	PT2015SALBACNO1	NEG	NEG	1
4	1	4	PT2015SALBACPO2	POS	POS	1
5	1	5	PT2015SALBACPO1	POS	POS	1
6	1	6	PT2015SALBACPO1	POS	POS	1
7	1	7	PT2015SALBACNO1	NEG	NEG	1
8	1	8	PT2015SALBACPO2	POS	POS	1
9	1	9	PT2015SALBACNO1	NEG	NEG	1
10	1	10	PT2015SALBACNO1	NEG	NEG	1
11	2	1	PT2015SALBACPO2	POS	POS	1
12	2	2	PT2015SALBACNO1	NEG	NEG	1
13	2	3	PT2015SALBACNO1	NEG	NEG	1
14	2	4	PT2015SALBACPO1	POS	POS	1
15	2	5	PT2015SALBACPO2	POS	POS	1
16	2	6	PT2015SALBACNO1	NEG	NEG	1
17	2	7	PT2015SALBACPO2	POS	POS	1
18	2	8	PT2015SALBACPO1	POS	POS	1
19	2	9	PT2015SALBACNO1	NEG	NEG	1
20	2	10	PT2015SALBACPO1	POS	POS	1
21	3	1	PT2015SALBACPO1	POS	POS	1
22	3	2	PT2015SALBACPO2	POS	POS	1
23	3	3	PT2015SALBACNO1	NEG	NEG	1
24	3	4	PT2015SALBACPO2	POS	POS	1
25	3	5	PT2015SALBACPO1	POS	POS	1
26	3	6	PT2015SALBACPO1	POS	POS	1
27	3	7	PT2015SALBACNO1	NEG	NEG	1
28	3	8	PT2015SALBACPO2	POS	POS	1
29	3	9	PT2015SALBACNO1	NEG	NEG	1
30	3	10	PT2015SALBACNO1	NEG	NEG	1

Table 3 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	4	1	PT2015SALBACPO2	POS	POS	1
32	4	2	PT2015SALBACNO1	NEG	NEG	1
33	4	3	PT2015SALBACNO1	NEG	NEG	1
34	4	4	PT2015SALBACPO1	POS	POS	1
35	4	5	PT2015SALBACPO2	POS	POS	1
36	4	6	PT2015SALBACNO1	NEG	NEG	1
37	4	7	PT2015SALBACPO2	POS	POS	1
38	4	8	PT2015SALBACPO1	POS	POS	1
39	4	9	PT2015SALBACNO1	NEG	NEG	1
40	4	10	PT2015SALBACPO1	POS	POS	1
41	5	1	PT2015SALBACPO1	POS	POS	1
42	5	2	PT2015SALBACPO2	POS	POS	1
43	5	3	PT2015SALBACNO1	**NEG**	**POS**	**0**
44	5	4	PT2015SALBACPO2	**POS**	**NEG**	**0**
45	5	5	PT2015SALBACPO1	POS	POS	1
46	5	6	PT2015SALBACPO1	POS	POS	1
47	5	7	PT2015SALBACNO1	NEG	NEG	1
48	5	8	PT2015SALBACPO2	**POS**	**NEG**	**0**
49	5	9	PT2015SALBACNO1	NEG	NEG	1
50	5	10	PT2015SALBACNO1	**NEG**	**POS**	**0**
51	6	1	PT2015SALBACPO2	POS	POS	1
52	6	2	PT2015SALBACNO1	NEG	NEG	1
53	6	3	PT2015SALBACNO1	NEG	NEG	1
54	6	4	PT2015SALBACPO1	POS	POS	1
55	6	5	PT2015SALBACPO2	POS	POS	1
56	6	6	PT2015SALBACNO1	NEG	NEG	1
57	6	7	PT2015SALBACPO2	POS	POS	1
58	6	8	PT2015SALBACPO1	POS	POS	1
59	6	9	PT2015SALBACNO1	NEG	NEG	1
60	6	10	PT2015SALBACPO1	POS	POS	1

Table 3 (Continued)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	7	1	PT2015SALBACPO1	POS	POS	1
62	7	2	PT2015SALBACPO2	POS	POS	1
63	7	3	PT2015SALBACNO1	NEG	NEG	1
64	7	4	PT2015SALBACPO2	POS	POS	1
65	7	5	PT2015SALBACPO1	POS	POS	1
66	7	6	PT2015SALBACPO1	POS	POS	1
67	7	7	PT2015SALBACNO1	NEG	NEG	1
68	7	8	PT2015SALBACPO2	POS	POS	1
69	7	9	PT2015SALBACNO1	NEG	NEG	1
70	7	10	PT2015SALBACNO1	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing faecal samples for the detection of *Salmonella* spp. by bacteriological isolation. Six out of the seven participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference feacal samples (100% of agreement). Hereby, culture media from different producers were used (Bio-Rad, Oxoid, Biomérieux, Biokar, Beckton-Dickinson). In contrast, LAB5 misclassified 4 aliquots (60% of agreement) of reference faecal samples.
The statistical analysis was done based on the "Labposit = Sample N° in the Excel file PT2015SALBAC" and the "Result = Result in the Excel file PT2015SALBAC" (table 3). In this case the LAB5 misclassified 4 aliquots (60% of agreement) of reference faecal samples. Nevertheless, it was observed that if the analyze was performed on the results based on the "Random N° in the Excel PT2015SALBAC file", the results for LAB5 would be complient.

LABNR	RANDOM N°	SAMPLE	STATUS	RESULT	SUCCESS
5	87	PT2015SALBACPO2	POS	POS	1
5	70	PT2015SALBACPO2	POS	POS	1
5	42	PT2015SALBACPO2	POS	POS	1
5	52	PT2015SALBACNO1	NEG	NEG	1
5	85	PT2015SALBACPO1	POS	POS	1
5	95	PT2015SALBACPO1	POS	POS	1
5	46	PT2015SALBACNO1	NEG	NEG	1
5	1	PT2015SALBACNO1	NEG	NEG	1
5	100	PT2015SALBACNO1	NEG	NEG	1
5	82	PT2015SALBACPO1	POS	POS	1

There was no problem with the « Sample N° » and « Random N° » of the other laboratories.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if no mistakes were made for the strong positive faecal samples, maximum 1 mistake for the weak positive and maximum 1 mistake for the negative faecal samples (see III.3.3.). Consequently, based on the « Random N° » all participants achieved a satisfactory performance for the isolation of *Salmonella* spp. from faeces. Based on the « Sample N° » LAB5 did not reach the required performance for the isolation of *Salmonella* spp. from faeces .

Coordinator proficiency tests
Katia Knapen

Appendix

Name of the participating laboratories

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

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

FLVVM (Melle, Belgium)

Laboratoire de Médecine Vétérinaire de l'Etat (LMVE) (Grand Duchy of Luxemburg)

Lavetan NV (Turnhout, Belgium)

LFSAGx (Gembloux, Belgium)

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