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172-PT

PROFICIENCY TESTING 2016

SALMONELLA (SAL)

Isolation of Salmonella sp. from organs

CODA-CERVA-UCCLE

DATE BEGIN PT: 10 OCTOBER 2016

DATE REPORT: 22 NOVEMBER 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. (*Salmonella* Pullorum and *Salmonella* Gallinarum) in organs (liver).

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined organ (liver) samples must be analyzed by means of *Salmonella* isolation tests as described in the instructions, provided by the PT provider, and the laboratory instructions. The procedures for the isolation tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

Different packages of liver (chicken) were bought in a department store (Delhaize) and were homogenized, aliquoted per 10 ± 1g and stored in the freezer. Two times twelve aliquots were analyzed on the 3th of October for the presence of *Salmonella* spp. by the *Salmonella* reference laboratory of CODA-CERVA-Uccle, hereby following the methods, described in the laboratory SOPs. Since all tested aliquots were found negative for the presence of *Salmonella* spp., the collected organs (liver) were considered as *Salmonella* negative and hence the remaining aliquots as suitable for the PT.

On the10th of October 2016 (start date of the PT), 60 aliquots of liver samples were prepared and randomized, either for the PT (30 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):

- 18 aliquots (12 for the PT and 6 for the verification tests) were used as such and considered as negative liver samples ('PT2016SALBACNO1')
- 12 aliquots (6 for the PT and 6 for the verification tests) were inoculated with a 10^{-1} dilution of *Salmonella* Pullorum and were considered as strong positive liver samples for *Salmonella* Pullorum ('PT2016SALBACPO1').
- 9 aliquots (3 for the PT and 6 for the verification tests) were inoculated with a 10^{-2} dilution of *Salmonella* Pullorum and were considered as weak positive liver samples for *Salmonella* Pullorum ('PT2016SALBACPO2').
- 12 aliquots (6 for the PT and 6 for the verification tests) were inoculated with a 10^{-1} dilution of *Salmonella* Gallinarum and were considered as strong positive liver samples for *Salmonella* Gallinarum ('PT2016SALBACPO3').
- 9 aliquots (3 for the PT and 6 for the verification tests) were inoculated with a 10^{-3} dilution of *Salmonella* Gallinarum and were considered as weak positive liver samples for *Salmonella* Gallinarum ('PT2016SALBACPO4').

In total, 30 aliquots of liver samples were distributed to 3 participating laboratories. All participants were given 10 aliquots of liver samples: 4 aliquots of the negative liver sample PT2016SALBACNO1, 1 aliquot of the weak positive liver samples PT2016SALBACPO2 (*Salmonella* Pullorum) and PT2016SALBACPO4 (*Salmonella* Gallinarum) and 2 aliquots of the strong positive liver samples PT2016SALBACPO1 (*Salmonella* Pullorum) and PT2016SALBACPO3 (*Salmonella* Gallinarum).
For this PT, the verification tests were performed in parallel on samples sent out the same day as the samples sent to the participants (cfr. Manual for the participant, section III.1). Therefore, the *Salmonella* reference laboratory of CODA-CERVA-Uccle tested 3 aliquots of each sample (PT2016SALBACNO1, PT2016SALBACPO1, PT2014SALBACPO2, PT2014SALBACPO3 and PT2014SALBACPO4) for the presence of *Salmonella* on both 10th (day 1) and 11th (day 2) of October 2016, in order to verify the status of the sent liver 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 liver samples used for this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the participant is able to classify all samples correctly (100% agreement) if the samples are strong positive (PT2016SALBACPO1, PT2014SALBACPO3). For the negative (PT2016SALBACNO1) and weak positive samples (PT2016SALBACPO2, PT2016SALBACPO4) only 1 misclassification is 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 liver samples were sent at 5±3°C to each of the 3 participating laboratories by national courier on 10th of October 2016 (30 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were started on 10th of October 2016 (Table 1).

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

Results from the participating laboratories have been received between 19th and 24th of October 2016. LAB2 hereby exceeded the deadline of 21th of October 2016 for submission of the results (Table 1).

Table 1. Overview of the dates on which (i) the liver 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)
LAB1	10/10/2016	10/10/2016	19/10/2016
LAB2	10/10/2016	10/10/2016	**24/10/2016**
LAB3	10/10/2016	10/10/2016	20/10/2016

IV.3. Compliance with the procedure

Except LAB3, 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

Qualitative data analysis showed that all participating laboratories provided qualitative results that were in full agreement with the assigned status of the liver samples (100% of agreement) (Table 2).

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

	LABNR		
	1	2	3
failure	0 (0%)	0 (0%)	0 (0%)
success	10 (100%)	10 (100%)	10 (100%)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between participating laboratories since all participants reached 100% of agreement for the detection of reference liver samples.

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

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the liver samples (SAMPLE), the external identification of the liver 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	PT2016SALBACPO1	POS	POS	1
2	1	2	PT2016SALBACPO3	POS	POS	1
3	1	3	PT2016SALBACNO1	NEG	NEG	1
4	1	4	PT2016SALBACPO1	POS	POS	1
5	1	5	PT2016SALBACNO1	NEG	NEG	1
6	1	6	PT2016SALBACPO4	POS	POS	1
7	1	7	PT2016SALBACPO3	POS	POS	1
8	1	8	PT2016SALBACNO1	NEG	NEG	1
9	1	9	PT2016SALBACPO2	POS	POS	1
10	1	10	PT2016SALBACNO1	NEG	NEG	1
11	2	1	PT2016SALBACNO1	NEG	NEG	1
12	2	2	PT2016SALBACPO1	POS	POS	1
13	2	3	PT2016SALBACNO1	NEG	NEG	1
14	2	4	PT2016SALBACPO4	POS	POS	1
15	2	5	PT2016SALBACNO1	NEG	NEG	1
16	2	6	PT2016SALBACPO3	POS	POS	1
17	2	7	PT2016SALBACPO1	POS	POS	1
18	2	8	PT2016SALBACNO1	NEG	NEG	1
19	2	9	PT2016SALBACPO3	POS	POS	1
20	2	10	PT2016SALBACPO2	POS	POS	1

(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
21	3	1	PT2016SALBACPO1	POS	POS	1
22	3	2	PT2016SALBACPO3	POS	POS	1
23	3	3	PT2016SALBACNO1	NEG	NEG	1
24	3	4	PT2016SALBACPO1	POS	POS	1
25	3	5	PT2016SALBACNO1	NEG	NEG	1
26	3	6	PT2016SALBACPO4	POS	POS	1
27	3	7	PT2016SALBACPO3	POS	POS	1
28	3	8	PT2016SALBACNO1	NEG	NEG	1
29	3	9	PT2016SALBACPO2	POS	POS	1
30	3	10	PT2016SALBACNO1	NEG	NEG	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing organ (liver) samples for the detection of *Salmonella spp. (Salmonella Pullorum and Salmonella Gallinarum)* by bacteriological isolation.

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference liver samples (100% of agreement) (Table 2 and Table 3).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if, with relation to the status of the liver samples assigned by the *Salmonella* reference laboratory of CODA-CERVA-Uccle, no mistakes were made for the strong positive liver samples and maximum 1 mistake for both the weak positive and the negative liver samples (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the isolation of *Salmonella* spp. from liver.

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)

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